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Technology Transfer

Emerald Ash Borer

2014 Emerald Ash Borer National Research and Technology Development Meeting

October 15 & 16, 2014
Wooster, Ohio

Compiled by James Buck, Gregory Parra, David Lance, Richard Reardon, and Denise Binion

Forest Service
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2014 Emerald Ash Borer National Research and Technology Development Meeting

October 15 & 16, 2014

Ohio Agricultural Research and Development Center
Wooster, Ohio

Sponsored by

The Ohio State University and
the United States Department of Agriculture
Animal and Plant Health Inspection Service

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FOREWARD

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is an invasive species that has shaped, and will continue to shape, the composition and richness of natural and urban forests in North America. All species of North American *Fraxinus* (ash) appear to be susceptible to attack by this beetle, and all succumb and die within a few years of colonization. Like chestnut blight (*Cryphonectria parasitica*) and Dutch elm disease (*Ophiostoma ulmi*), EAB has the potential to effectively eliminate entire tree species from our forests. In the case of EAB, it could potentially eliminate all of the species in an entire genus.

EAB was first discovered killing ash trees in 2002 near Detroit, MI, and results of subsequent dendrochronological studies suggest that the pest had been introduced at least 8 years earlier (Siegert et al., Diversity and Distributions 7, 847 [2014]). The insect spread rapidly after its introduction and has been found in most states of the eastern U.S., south to Louisiana and Georgia and west as far as Colorado. It also occurs in Ontario and Quebec. The insect is an active flier, but longer-range spread has resulted from humans moving EAB-infested ash wood and nursery stock. One major thrust of the current management approach of the USDA and affected states is to limit human-assisted spread of EAB through an aggressive outreach program and regulations to control movement of ash trees and wood. The program believes this effort has greatly reduced commercial movement of infested wood, and modeling studies suggest that this has indeed limited spread. However, movement of host material for personal use, especially as firewood, remains a threat as it is difficult to regulate.

This volume contains abstracts from papers presented at a meeting that took place October 15-16, 2014, in Wooster, OH. The studies described herein were aimed at the broad goal of improving our ability to manage EAB populations. The focus of this compendium is work conducted from 2012 through 2014, although some of the abstracts describe work across a broader time frame. These abstracts detail work to improve our basic understanding of the insect and its ecology in North America, efforts to enhance our tools for containing, monitoring, and controlling populations of the pest, and development and evaluation of strategies to use these tools in management scenarios ranging from a single tree to national in scope. Improving methods for detecting EAB populations has continued to be a focus, and studies here describe improvements in traps and trapping methods as well as the development of risk-based algorithms that have improved the efficiency and effectiveness of the overall EAB monitoring system. In addition, two areas of research are prominent in these abstracts and will likely receive continuing effort into the future: biological control of EAB and host-plant resistance.

Biological control has become a major focus of the Emerald Ash Borer Program of USDA and its cooperators. Several years ago, the USDA-APHIS invested in the construction of a facility to produce EAB parasitoids. Currently, three species of parasitic wasps (two that attack EAB larvae and one that attacks the eggs) are being reared in this facility, and a fourth is in the final stages of evaluation. These species had previously been evaluated for such factors as host specificity and ability to attack EAB and reproduce in the field. Operational releases of these insects began in 2010 as part of a 5-year implementation strategy. Ongoing research and development efforts described here include studies on the biology of these insects in the field and assessments of their effectiveness as biological control agents.

The study of host resistance to EAB was initiated in the 2000's and was subsequently the focus of an ex panded 3-year research program starting in 2011. That expanded effort has borne fruit, and the abstracts here describe a number of the resulting projects in such areas as determining the relative resistance of varI-ous North American and Asian ash species, characterizing the chemical and genetic bases of resistance, and identifying any indigenous resistance in North American ash. In addition, the discovery of the first non-ash to act as an EAB host in North America is reported (white fringetree, *Chionanthus virginicus*). The ultimate goal of this work is to facilitate the development of EAB-resistant ash varieties that are wholly, or almost wholly, comprised of North American gene stock.

The EAB Research and Technology Development Meetings were originally held annually but have been decreasing in frequency – the meeting previous to this one was held in 2011. In addition, this meeting was smaller than most of the more recent versions in numbers of both attendees and presentations. In part, this reflects that the research and development effort has been successful in answering some key questions; however, levels of research also were affected by reductions in Federal funding for emerald ash borer pro-grams, especially from Fiscal Year 2012 onward. Host resistance studies, one area with funding that carried through the period of 2011 to 2014, has more recently been hit with lapses in funding as well.

Early in Fiscal Year 2015, the APHIS-PPQ Emerald Ash Borer Program took on a self-evaluation beginning with its basic goals. As noted above, reducing the spread of the pest has been, and remains, a priority. However, as the insect continues to fill out its projected range in North America, the program envi-sions transitioning from regulatory to a broader management mode, with an overall goal of maintaining ash as a viable component in North American ecosystems. The program believes two key components are likely needed to achieve this goal in a sustainable manner: effective biological control and ash trees that exhibit greater resistance to EAB than do typical North American ash. Bringing this vision to life will require a greater level of resources, both programmatically and for developmental projects, than have been available in recent years, and APHIS Plant Protection and Quarantine is pursuing options that have the potential to increase the funding available to the EAB program in upcoming years. The future of ash in North Ameri-ca's natural and urban forests may depend on this effort.

CONTENTS

PROGRAM UPDATES

EAB IN CANADA: LATEST UPDATES IN TERMS OF REGULATION AND BIOLOGICAL CONTROL RESEARCH WITH ENTOMOPATHOGEN FUNGI

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ABSTRACT

As Canada's National Plant Protection Organization, the Canadian Food Inspection Agency (CFIA) has the mandate to protect non-infested areas of Canada. Given that the emerald ash borer (Agrilus planipennis Fairmaire, Coleoptera: Buprestidae) (EAB) was detected in multiple locations outside of the regulated areas during the 2013 survey season, the CFIA decided to expand and consolidate the regulated area to include highway corridors, which are the primary pathways of pest-spread (Figure 1). This approach, implemented on April 1, 2014, allows to better protect parts of Ontario and Québec not yet infested by this pest as well as other parts of Canada. Regulated articles can move within the regulated area but cannot exit the area without written permission of the CFIA.

There are a number of reasons that led the CFIA to move in this direction. Although detection tools have improved over the years, EAB remains cryptic and difficult to detect at low population densities. Detections are usually made 3 to 4 years after establishment in an area, which implies that EAB had likely already spread beyond the detection boundary. Furthermore, the new approach allows the CFIA to focus surveillance and management efforts at the perimeter in order to better monitor the spread of the pest. The CFIA oversees product movement out of the regulated area, provides electronic communication materials and supports research on EAB management tools. The CFIA also fully supports and encourages stakeholder engagement and implementation of by-laws as well as outreach communications to encourage the public to do not move regulated products within the regulated area and buy and burn firewood locally.

In 2014, the CFIA continued to conduct detection surveys outside of regulated areas across Canada, using green sticky prism traps baited with Z-3-hexenol and lactone. Survey strategies included both trapping and visual surveys at various locations, including urban centres, holiday destinations (such as provincial parks and seasonal campgrounds), rest stops along major transportation corridors, new subdivisions and other areas with recently planted ash nursery stock, areas identified by the public and reported as suspects through

public inquiries and areas with ash decline.

Approximately 470 traps were deployed nationally by also did trapping for EAB and all efforts were coordinated to ensure maximum coverage. Only two traps were positives for EAB outside of the regulated area in 2014, both in the municipality of Notre-Dame-du-Laus, in Quebec. The traps had been installed and checked by Quebec's Ministry of Natural Resources. The CFIA is currently working to revise the regulated area to include these finds.

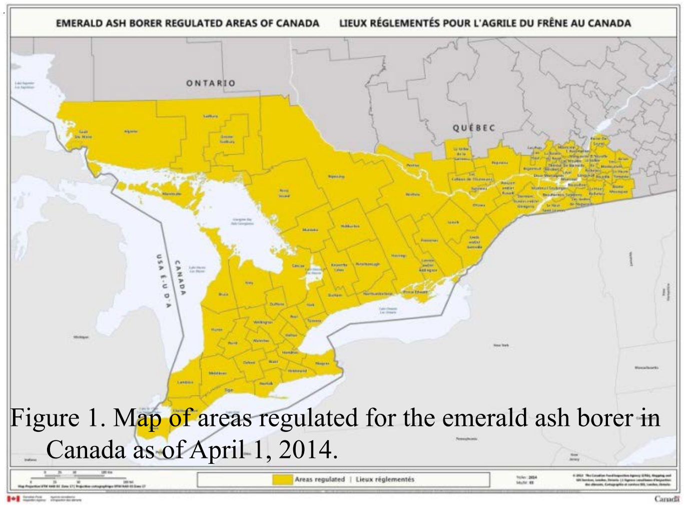

Figure 1. Map of areas regulated for the emerald ash borer in Canada as of April 1, 2014.

In addition to trapping, the CFIA is doing as much outreach as possible. Different communication products have been developed for distribution, some of which are available of the CFIA's website at www.inspection.gc.ca/pests under 'Emerald Ash Borer'.

Biological control with entomopathogen fungi

Biological controls of EAB represent an environmentally acceptable tool to respond to EAB ash tree territory invasion. The apparent low contribution of our native predators and pathogens partly explain the increase of local EAB populations in our susceptible ash tree species. Introducing a fungal disease in the EAB population could contribute to population mortality

Considering that EAB adults feed on canopy top tree foliage, they are not easily seen and because larvae develop under the bark, symptoms are initially hard to detect. Even with the best available traps and lures, this insect can be easily missed. The autodissemination strategy is to attract the EAB adult in an insect trap where it will be contaminated with the fungus, then fly off and expecting it can find its sexual partners and transmit a lethal infection before its death. Moreover, because EAB perform multiple mating, this behaviour could help to spread the disease in the local EAB population.

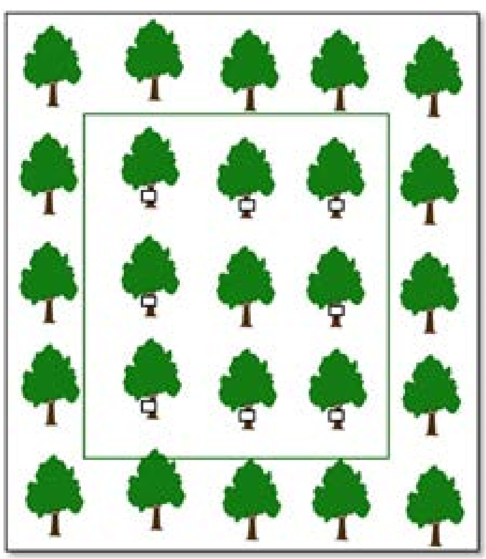

Figure 2. In 2012, one of the 16 blocks (8 blocs in two plantations) with an ACD hang on the central tree surrounded by green prism traps and Pestic coated plastic wrap fixed on the bole of 9 trees (with boxes) per bloc.

An autocontamination device (ACD) modelled after a black multi-funnel Lindgren trap for bark beetles was adapted for EAB. The ACD main role is to protect the infectious conidia of a virulent *Beauveria bassiana* (Balsamo) Vuillemin isolate from deleterious UV and rain. Insects are attracted by the green Lindgren trap coated with Fluon and baited with 3Z-hexenol (Synergy Semiochemicals Corp., Burnaby, BC) (2012-2014) and 3-Z Lactone (2014) (P. Silk Lab RNCan). The attracted EAB fall in the trap bottom part where it has to cross a fungus mat covered with conidia in order to escape. Previous work demonstrated that trapped beetles obtain a lethal dose of conidia and die 5 days after initial contamination (Lyons et al. 2012).

Since our previous works, field tests were performed to demon-

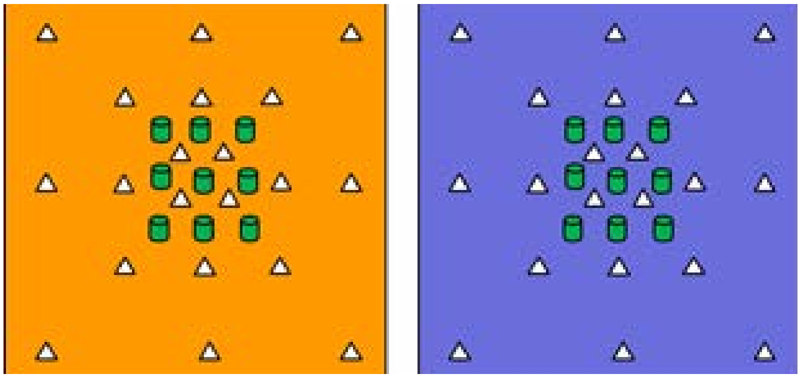

Figure 3. In 2013, two blocks of 9 autocontamination traps (green cylinders) regularly surrounded with 21 green prism traps in the Sarnia (Ont.) plantation.

strate the autocontamination concept feasibility with green Lindgren traps and the autocontamination device (ACD) exposed to natural outdoor conditions. During three consecutive years, different experimental designs were used based on the size and shape of the plantations and knowledge accumulated on how toimprove the fungal dissemination process. In 2012 and 2013, we conducted tests in heavily infested young ash tree plantations located near Sarnia (Ontario, Canada). In 2014, we run our test in a 20 year-old plantation located near London (Ontario, Canada) where EAB was at an endemic level.

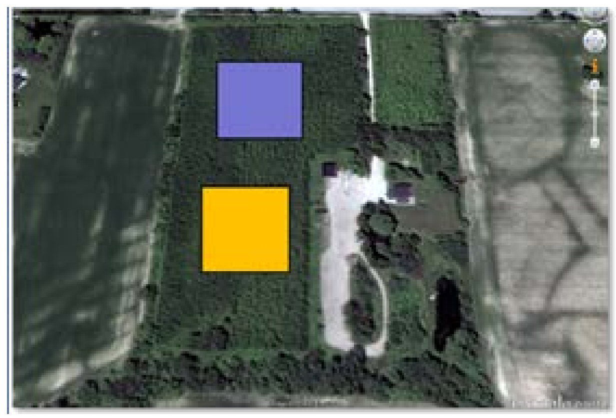

Figure 4. Blocks localisation in the Sarnia (Ontario) plantation in 2013.

During 3 years, EAB adults were collected with sticky traps set in the close vicinity of the autocontamination traps. EAB adults were removed from the sticky traps daily (2012) or weekly (2013) to determine if they were contaminated with the *Beauveria bassiana* isolate. Sticky trap catches demonstrated the autocontamination and dispersal of contaminated EAB adults. In 2012, when one Lindgren trap was used per block (Figure 2) we evaluated that 8% of captured EAB were infected/contaminated with the introduced fungus as confirmed by molecular tools (Johny and Kyei-Poku 2014). In 2013, 9 Lindgren traps were regularly spread but pooled in the centre of 2 blocs in a plantation to create an "inoculation center". Prism traps were spread around the Lindgren traps to monitor insect contamination and insect dispersal (Figure 3, 4). This design resulted in a much higher proportion of EAB (51%) captured on prism traps that were Bb positive. However, in 2014, under endemic population level in an older ash plantation conditions (Figure 5), even with 21 Lindgren autocontamination traps used again as "inoculation centre", few EAB were caught on the 44 sticky prism traps and less than 1% of EAB collected were contaminated with the *Beauveria bassiana* isolate.

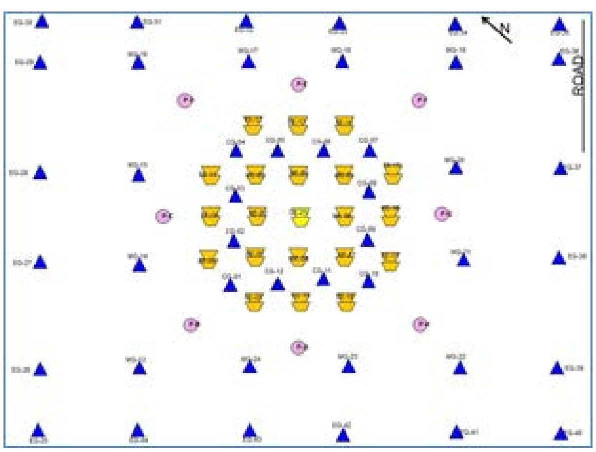

Figure 5. Lindgren trap (yellow cones) and prism traps (blue triangle) set up in 2014 in an ash plantation near London (Ontario). In purple, traps to monitor flight.

These results will help to develop the autocontamination strategy using entomopathogen fungi according to the EAB biology and behavior. Because the EAB is a serious threat to ash trees in cities, further tests should be conducted under city street conditions to document the fungal dissemination process. Added to native parasites and predators, classical biological control with parasitoids, chemical pesticides, SLAM strategy and regulation, all these tools could eventually help to reduce the threshold of EAB to a more acceptable level.

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HOST INTERACTIONS

USING WATER- AND NUTRIENT-STRESS PHENOTYPES TO IDENTIFY ASH BIOMARKERS OF RESISTANCE TO EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB) is an alien invasive, wood-boring beetle that is devastating North American ash (*Fraxinus* spp.) populations in urban and natural forest settings. A critical part of landscape-scale, long-term management will be deployment of host resistance, which has not yet been characterized in ash. It is well known that tree defense physiology is significantly affected by resource availability or hormonal treatment. In principle, such treatments can therefore be used to characterize resistance mechanisms. Hence, two ash cultivars, previously identified as constitutively resistant and susceptible to EAB, were subjected to different levels of water-, nutrient-, and phytohormone-stress to expand observable resistance phenotypes. Trees were challenged with EAB egg inoculations to control for oviposition preference, and resistance was assessed using larval growth and survival after 70 days.

Significant decreases in larval growth and survival in Manchurian vs. white ash in this study confirm the importance of direct phloem-based defenses in determining EAB resistance. Significant decreases in growth and survival of larvae feeding on trees pre-treated with methyl jasmonate are consistent with previous studies and demonstrate at least partial inducibility of effective defenses in coevolved and naïve ash species.

The biochemical bases of inter- and intraspecific variation in resistance observed in this study are being explored through comparative transcriptomics and metabolite profiling. Traits identified in this study may be used as biomarkers for selective breeding. The results will also contribute to the understanding of coevolved and naïve tree-insect interactions under changing resource conditions, which are expected to occur more frequently under accepted scenarios of climate change.

MECHANISMS OF ASH RESISTANCE TO EMERALD ASH BORER: PROGRESS AND GAPS

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ABSTRACT

The emerald ash borer (EAB) invasion of North America has caused widespread mortality of native ash, and is threatening the native ash resources. As a consequence of its devastating impact, EAB has caught the attention of the scientific community, and several studies have focused on different aspects of the biology and behavior of this pest, including its interaction with host defenses. Here we present a review of the published literature on mechanisms of ash resistance to EAB, the understanding of which, despite starting from a *tabula rasa*, has achieved significant progress in the last few years.

The native North American species white, green and black ash, which did not coevolve with *Agriuls planipennis*, are highly susceptible to the beetle. In contrast, Asian ash species, such as Manchurian ash, which share a coevolutionary history with EAB, are more resistant, and appear to be susceptible only when stressed.

Recently it has been shown that resistant Manchurian ash is less preferred as a host for oviposition compared to susceptible North American species, which confirms the importance of antixenosis mechanisms in interspecific variation of ash resistance to EAB. Decreased performance of EAB larvae on Manchurian ash compared to black ash confirms that antibiosis mechanisms are also important, and studies published to date suggest that its constitutive bark defense system plays a major role, including higher constitutive levels of lignans, a faster browning reaction, higher expression of four putative defensive proteins, and higher levels of the amino acid proline and the monoamine tyramine. Larval feeding induces the accumulation of the lignan pinoresinol A, which suggests that induced responses may also be involved. However, no biochemical responses of Manchurian ash to exogenous application of methyl jasmonate (MeJA) (a key defense phytohormone) were detected in a recent study. Drought stress increases susceptibility of Manchurian ash to EAB, but has no effect on its bark phenolic content. This suggests that phenolics are perhaps not as significant in the intraspecific variation of resistance resulting from stress as previously hypothesized, although this could also depend on how quickly and strongly phenolic compounds of healthy Manchurian ash re-

spond to browning reactions and enzymes relative to those of drought stress trees.

In contrast, white and green ash, which have no evolutionary history with EAB, have been shown to become more resistant after induction with MeJA. This phenotypic variation has been mainly associated with increased concentrations of verbascoside, which was confirmed to have a detrimental impact on EAB larvae *in vitro*. Accumulation of lignin and higher trypsin inhibitor activity were associated with this phenotype as well, and proxies of these traits were also found to have a detrimental impact on EAB larvae *in vitro*. This suggests that white and green ash possess the genetic potential for inducible resistance. However, both species experience very high mortality in the field, suggesting that they are ultimately unable to mount an effective resistance response against EAB. Timing of induction might perhaps be a crucial factor in the phenotypic outcome.

Black ash, despite being phylogenetically closely related to resistant Manchurian ash, is highly susceptible to EAB. Its phenolic profile is highly similar to that of Manchurian ash, but its browning reaction is not as strong, suggesting that phenolics may not be as strongly oxidized when consumed. Application of MeJA also induced increased resistance of black ash to EAB, and this response was associated with higher activity of trypsin inhibitors.

Lastly, blue ash, which is more phylogenetically distantly related to the other North American species tested, as well as to Manchurian ash, had a distinct bark phenolic profile that was particularly rich in the hydroxycoumarin esculin, which might in part explain the higher EAB resistance of this species relative to other North American ash species evaluated to date.

WHITE ASH – IS EAB ALWAYS A DEATH SENTENCE?

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ABSTRACT

White ash (*Fraxinus americana*) is native to at least 34 states, tolerates a range of soil and site conditions and is one of the most widely distributed ash species in the eastern US. White ash dominates some forest sites but is often found in mixed stands with other hardwood species. It is a common landscape species, even in states where it is not native, and white ash timber is highly valued. White ash is assigned to the Melloides section of the *Fraxinus* phylogenetic tree, along with eight other North American ash species and the tropical *F. uhdei* (Wallender 2008).

Three common ash species, green ash (*F. pennsylvanica*), black ash (*F. nigra*) and white ash, have been severely impacted by emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) in areas of southeast Michigan and Ohio (Herms and McCullough 2014). Mortality rates approaching 100% were recorded in plots established in large forested parks (Klooster et al. 2013, Knight et al. 2013). An occasional surviving tree, sometimes referred to as a "lingering ash" are sometimes observed in areas where virtually all other ash trees have been killed. Such trees may simply have escaped EAB colonization or may demonstrate putative resistance to EAB (Klooster et al. 2012).

Other studies have evaluated EAB impacts in areas where two ash species co-occur. In two wooded areas with a mix of white ash and blue ash (*F. quadrangulata*), all or nearly all white ash trees were killed by EAB, while 60-70% of the blue ash survived the EAB invasion and remain healthy (Tanis and McCullough 2012). However, white ash is clearly a less preferred host for EAB than green ash. In three urban areas with a mix of similarly-sized green and white ash landscape trees, green ash trees were attacked earlier, at higher densities and succumbed sooner than white ash trees (Anulewicz et al. 2007). In a research plantation with alternating green ash and white ash trees, larval density on green ash trees was at least 5-fold greater than on the white ash trees (Limback 2010).

Foliar terpenes differed between green and white ash in the plantation. Several foliar terpene compounds associated with highly attractive girdled ash were higher in green ash than in white ash (Limback 2010). In a more recent plantation study, randomized blocks of green, white, black, blue and Manchurian ash (*F. mandshurica*), an Asian species, were exposed to a high density EAB population during the summer, then debarked in fall. Green and black ash trees were heavily colonized and killed, while few galleries were found on any of the blue ash or Manchurian ash. Larval density on white ash was highly variable; some trees were killed but others had very few galleries (Tanis and McCullough 2015).

While there are many forested sites where all or nearly all white ash trees have been killed by EAB, we continue to note stands where an unexpectedly high proportion of white ash are alive, despite 6-10 years of EAB presence. These are not "lingering ash" but rather areas where the majority of white ash are not only alive but appear to be relatively healthy.

In 2014, we identified more than 40 large forested parks or wildlife areas in southeast and central Michigan where we know EAB has been present for several years. Each location was scouted and we determined 28 of the sites had one or areas with abundant white ash. We established a 1.5 km radius around a center point in each of the 28 sites and delineated polygons by land use category and cover type. Variable radius plots were surveyed in each polygon and additional fixed radius plots were established in the white ash area. We recorded species, size and canopy condition of ash and other overstory trees. Surface area of live and dead white ash phloem was calculated following methods of McCullough and Siegert (2007).

Preliminary results show that most overstory trees, including ash, in these second growth stands are ≤ 15 cm DBH. White ash mortality in 2014 ranged from 0 to 100% across the 28 sites. Small trees generally had higher survival rates than large trees (>25 cm), but in 20 of the 28 sites, at least 50% of the white ash phloem remains alive. More intensive surveys of white ash overstory trees and regeneration in these sites are planned for 2015. Site, land use and related variables that may be associated with relatively high, or conversely, very low, white ash survival will be further evaluated using a GIS and spatial analyses. Results from this project may have practical implications for resource managers. Detection efforts, for example, should focus on green ash or black ash trees, rather than white ash or blue ash, which are consistently less preferred hosts of EAB. Identification of specific traits associated with high or very low white ash survival would be helpful in prioritizing areas for timber or pre-salvage harvest.

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WHITE FRINGETREE, *CHIONANTHUS VIRGINICUS*, AS A NOVEL LARVAL HOST FOR EMERALD ASH BORER

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ABSTRACT

Emerald ash borer is an invasive Asian pest of ash species in North America. All North American species of ash tested so far are susceptible to it, but there are no published reports of this insect developing fully in non-ash hosts in the field in North America. I report here evidence that EAB can attack and complete development in white fringetree, *Chionanthus virginicus* L., a species native to the southeastern U.S. that is also planted ornamentally. Four of 20 mature ornamentally-planted white fringetrees examined in the Dayton-Springfield, Ohio area in the summer and fall of 2014 showed external symptoms of emerald ash borer attack, including the presence of adult exit holes from the current and past years, canopy dieback, bark splitting and other deformities. Removal of bark from one of these trees yielded evidence of at least three generations of usage by emerald ash borer larvae, several actively feeding live larvae, and a dead adult confirmed as emerald ash borer. These findings indicate that emerald ash borer adults are capable of detecting and ovipositing on white fringetree in the field, larvae are able to feed and survive to the adult stage, and that adults can emerge from this tree. In turn, while white fringetree appears to have a strong wound healing response, attacked stems and heavily attacked trees show substantial dieback. These findings suggest that white fringetree is an acceptable alternative host for emerald ash borer in the field, and that the vigor and survival of this species may be threatened by emerald ash borer. These findings also indicate that wild and ornamental relatives of white fringetree are worthy of further scrutiny as potential hosts for emerald ash borer.

TRAPPING AND CHEMICAL CONTROL

INNOVATION UNDER PRESSURE: NEW TOOLS & METHODS IN SYSTEMIC INJECTION

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ABSTRACT

Recent research has demonstrated that systemic tree injection with efficacious insecticides provides a useful tool in protection of ash trees (Herms et al., 2014). In this area, technology has evolved to meet the demands of destructive pests such as Emerald ash borer (EAB) and the need to protect trees. Presented are (1) new innovations in tree injection methods, (2) a discussion of emamectin benzoate chemistry and Tree-äge® formulation, and (3) recent updates in emerald ash borer field studies.

Figure 1. The QUIK-jet Air, a new tool for systemic tree injection using air-assist technology.

Tree Injection Tools and Method of Treatment

Two basic methods are used to inject or infuse trees, these are: (1) microinjection and (2) Micro-Infusion®. Microinjection is defined as the administration of relatively low volumes (5-10 milliliters) of fluid into the trees vascular system, applying moderate pressure (up to 200 psi), and is generally delivered quickly, taking a few minutes (~2-5 min. per tree). The Micro-Infusion method delivers higher volumes (up to 600 ml / Tree I.V. bottle), at lower pressure (up to 60 psi), and generally requires more time to apply (~60 min. per tree). An example of a Micro-Infusion tool is the Arborjet Tree I.V. This is a useful tool when formulations require dilution with water. Tree dosages are typically calculated on the basis of tree diameter. These may be reported as a rate (e.g.,, milliliters per diameter inch) or as a dosage per tree (milliliters per tree). Always refer to the label to determine the correct tree dosage. In a typical Micro-Infusion application, the method is (1) determine dose, (2) measure and add to bottle, (3) pressurize bottle using the pressure pump and (4) purge the lines of air. To administer the application, locate injection sites at the base of the tree; insert the Arborplug® into the small pre-drilled hole. The injector needle pierces the internal Arborplug septum to deliver the dose. Micro-injection tools include the QUIK-jet, Air Hydraulic and new technology, the QUIK-jet Air. QUIK-jet Air was designed to (1) deliver a measured dose into the tree, to (2) deliver the dose efficiently, and (3) provides the means to start, meter and stop the injection. The device uses compressed air assist technology. It features a 5 cc capacity barrel, on/off switch, and quick disconnect (QD) injector needles. The clear barrel is designed to make the application visible. Barrel loading is adjustable: from 1 to 5 milliliters. An illustration of the device appears in Figure 1.

Drilling Method for Proper Arborplug® Depth: The Arborplug was designed to (1) prevent backflow of fluid, (2) reduce exposure of chemistry to the environment; (3) facilitate systemic uptake, (4) protect the lateral cambium from the injected chemistry, and (5) provide a surface for injection site closure. To install correctly, the Arborplug requires that it is inserted into sapwood, the vascular tissue that moves water and solutes in the tree. The method of drilling to access this tissue is as follows. Use a cordless electric drill (typ., 18 or 24 V) and clean, sharp high helical bit. Squeeze the trigger, but apply no pressure, the bit will cut easily through the bark but "lands" at the denser sapwood: this is the depth at which the Arborplug will be countersunk. Next apply pressure to the drill to cut 5/8" (15 mm) into the sapwood: this is the depth to accommodate the Arborplug in the sapwood. Improper (shallow) installation into the bark may displace bark from the sapwood, cause cambial injury, bark cracking and increase the probability of leakage from the injection site.

Emamectin benzoate and Tree-äge®

Emamectin benzoate (EB) is a complex glycoside, and occurs as a mix of $C_{55}H_{79}NO_{15}$ (B1b, minor) and $C_{56}H_{81}NO_{15}$ (B1a, major), and molecular weights of 993 to 1007, resp. It is a semi-synthetic derived from abamectin through a chemical process discovered in 1984. Abamectin is the fermentation by-product of a naturally occurring soil organism, *Streptomyces avermitilis.* EB is extremely potent against Lepidoptera (LC_{90}'s ~2-20 ppb's) (Jansson et al., 1996), nematodes (LC_{95}~30 ppbs) (Takai et al, 2003), and certain species of Coleoptera, including *Agrilus planipennis* (McCullough et al., 2007) and *Ips* spp (Grosman and Upton, 2006). EB MOA: This molecule acts if swallowed and has some contact action, inhibits muscle contraction, causing a continuous flow of chlorine ions in the GABA and H-Glutamate receptor sites which prevents insect muscle contraction. Tree-äge is 4% [wt/wt] emamectin benzoate MEC (Syngenta Crop Protection, LLC Greensboro, NC) insecticide formulation applied by injection for two year control of listed arthropod pests in deciduous and coniferous trees and palms.

EAB Studies Updates

Reported here are three EAB studies which were initiated since 2008 using Tree-äge in various Midwestern states.

Batavia Illinois 2008-2012: The earliest was initiated in Batavia, Illinois with Dr. Frederic Miller. Trees ranged from 12-23" (30-58 cm) DBH, and treated at rates of 0.2 to 0.3 GAI /2.5 cm DBH by micro-injection or 0.4 to 0.6 GAI /2.5 cm DBH by micro-infusion. After 4 years, the untreated trees exhibited significant (56%) canopy dieback compared to the Tree-äge treatments, all of which remained healthy. The data is presented in Table 1.

Table 1. Batavia Illinois EAB Study 2008-2012

			mean percent dieback-canopy thinning				
Treatment	**Rate**	**inch DBH**	**2008**	**2009**	**2010**	**2011**	**2012**
Quik-jet	0.2	12-17″	7a	11a	12a	15a	5a
Quik-jet	0.3	18-23″	8a	14a	12a	15a	5a
Tree IV	0.4	12-17″	12a	7a	14a	16a	5a
Tree IV	0.6	18-23″	6a	14a	6a	9a	5a
UTC	0	12-23″	11a	14a	11a	30b	56b
			NS	NS	NS		

Elyria, Ohio 2010-2014: In 2010, we initiated an EAB study where Tree-äge was injected once every 2 years. In addition, an experimental formulation, XCLr8 was injected once. Canopy decline symptoms were evaluated by Dr. Daniel A. Herms, OSU. Year 4 data is presented in Figure 2. The 40% reference line was established (Smitley et al., 2008), where tree decline symptoms are regarded as extensive and trees as unlikely to recover. In 2014, the Tree-äge trees remained healthy (avg., 13% thinning) compared to 58% dieback in the untreated trees.

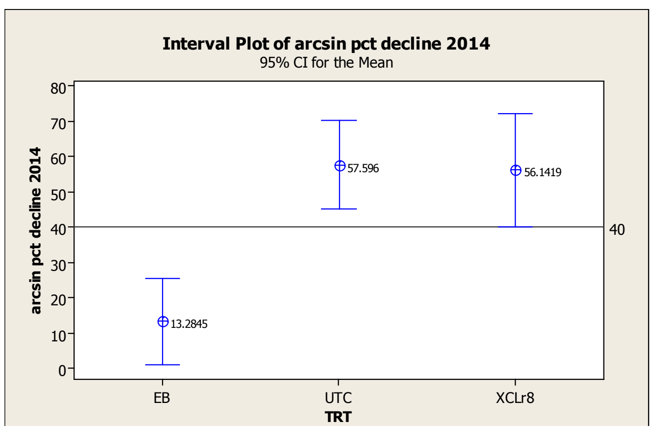

Figure 2. Arcsine transformed percent decline in ash canopy in year 4. The Tree-age (EB) treated trees remained healthy compared to the untreated check trees (UTC) or XCLr8 treatments. The 40% reference line (Smitley et al., 2008) is the point at which trees are unlikely to recover.

Louisville Metro Parks EAB Study (2011-2016): In this study, 25 to 45” (63 to 113 cm) DBH ash were treated with Tree-age in 2011. The 2.5 and 5 ml /2.5 cm DBH rates were administered by microinjection, and the 10 and 15 ml rates were administered by Tree I.V. The rating scale used in the study is a 5 point rating, where 1 is healthy and 5 is dead. These correlate to canopy thinning percentages, in 20% increments and presented in Table 2. In 2014 the untreated trees were noticeably in decline, whereas the Tree-age treatments remained healthy (Figure 3).

Table 2 Rating Scale based on severity of percent canopy thinning, in 20% increments.

Canopy Condition	% Canopy Thinning	Rating Scale
Full	20%	1
Thinning	40%	2
Dieback	60%	3
Decline	80%	4
Dead	100%	5

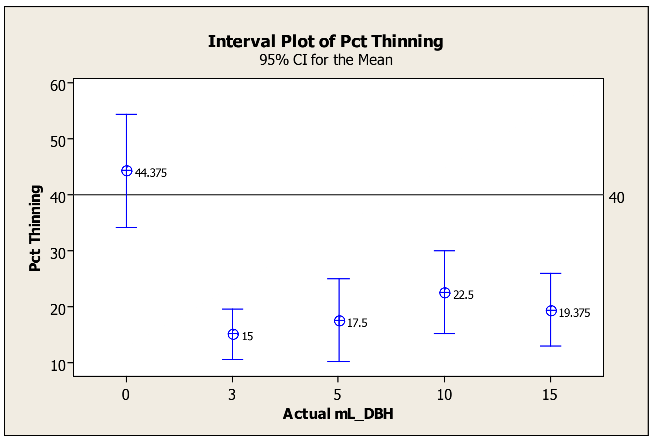

Figure 3. Canopy thinning in ash trees treated with 0 milliliters (UTC) or Tree-age from 2.5 to 15 ml per 2.5 cm dbh, three years after the study was initiated. The canopy ratings in this large DBH tree study show statistical differences in the untreated (0) and treated (3, 5, 10 and 15 ml per 2.5 cm DBH). No Tree-age treated tree differed statistically from each other.

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EFFICACY OF A NOVEL EMAMECTIN BENZOATE FORMULATION FOR EMERALD ASH BORER CONTROL

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ABSTRACT

Currently, the most effective insecticide tool for control of invasive species Emerald Ash Borer (EAB), *Agrilus planipennis*, on ash trees is emamectin benzoate. Arborists, applicators and scientists noted, when working with formerly the only available 4% emamectin benzoate formulation for tree injection, Arborjet's TREE-age®, that they were challenged by its restricted use label and the high variability and amount of time for insecticide uptake. The purpose of developing a novel formulation, ArborMectin™, was to create a 4% emamectin benzoate General Use pesticide with a consistent uptake speed that is effective for Emerald Ash Borer control. The research objectives were to 1) determine the efficacy of ArborMectin™ on EAB and 2) to evaluate the speed and variability of product uptake for ArborMectin™.

It was hypothesized that there is no significant difference between ArborMectin™ and Tree-age® for EAB control. To evaluate, the Canopy Thinning and Dieback Rating Scale, developed by Dr. David Smitely at Michigan State University (http://www.emeraldashborer.info/treatment.cfm#sthash.53HPxX9i.dpbs) was utilized across all studies. All applications were completed through Arborjet's TREE I.V. MICRO INFUSION® equipment. Four efficacy studies were conducted to evaluate ArborMectin™ across multiple tree size classes and insect pressures.

Rainbow Scientific contracted Ohio State University entomologist Dr. Dan Herms to evaluate ArborMectin™, TREE-age® and Xytect™ (imidacloprid) insecticide treatments on small (6" DBH) green ash trees (*Fraxinus pennsylvanica*) for Emerald Ash Borer control. Treatments were applied once in the spring of 2012 and evaluations consisting of canopy ratings yearly, exit hole counts yearly and bark pealing and counting EAB larval galleries in August 2014. Exit hole counts were unreliable due to visual difficulty and canopy ratings were indistinguishable due to initial low insect pressure. Untreated control trees averaged 55 larval galleries per tree, and Xytect™ treatments averaged higher than control trees and ArborMectin™ and TREE-Age® averaged 3.5 galleries per tree, statistically equivalent.

Rainbow Scientific partnered with the Village of Mt Prospect, IL and The Morton Arboretum to evaluate ArborMectin™, Transtect™ (dinotefuran) and Xytect™ (imidacloprid) insecticide treatments on large (>15" DBH) green ash trees for emerald ash borer pressure utilizing the Smitley Canopy Thinning and Dieback rating scale. Treatment applications occurred in 2012 and in 2014, with evaluations conducted annually in early spring and summer. Untreated control trees averaged 72% dieback, Transtect™ averaged 52% dieback, Xytect™ averaged 22% dieback, and ArborMectin™ averaged 14% canopy dieback in 2014.

Rainbow Scientific partnered with Bartlett Tree Experts to evaluate ArborMectin™ and TREE-age® insecticide treatments on large (22" DBH) green ash trees for emerald ash borer pressure utilizing Smitley Canopy Thinning and Dieback rating scale. Treatment applications occurred in 2011 and in 2014 and visual evaluations were conducted annually, early spring and summer. The Untreated control trees averaged 64.4%

dieback while TREE-Age® and ArborMectin™ performing equivalently, averaging 12.0% canopy dieback.

Rainbow Scientific contracted Purdue University entomologist Dr. Cliff Sadof to evaluate ArborMectin™ and TREE-age® insecticide treatments on large (30" DBH) green ash trees for emerald ash borer pressure by utilizing Smitley Canopy Thinning and Dieback rating scale. Treatments were applied once in 2013 and visual evaluations are conducted annually in fall and spring. Preliminary results from Site 1 show untreated control trees averaged 27.7% canopy thinning in 2014, TREE-age® trees averaged 8.57% thinning and ArborMectin™ trees averaged 2.5% thinning with 5 replicates. At Site 2, untreated control trees averaged 21.0% canopy thinning in 2014, TREE-age® trees averaged 9.0% thinning and ArborMectin™ trees averaged 7.0% thinning with 12 replicates. Evaluations will continue over the next 2 years as untreated trees reach mortality.

It was hypothesized that there is no significant difference between ArborMectin™ and TREE-age® in insecticide uptake speed and variability. This hypothesis was tested on two sets of equipment, Arborjet's TREE I.V. MICRO INFUSION® system and Rainbow Treecare Scientific Advancements' Q-Connect plug-less injection system. Three studies were set up in July 2014 to evaluate insecticide uptake and variability. Each utilized 10 replications per treatment in a Randomized Block design. To determine uptake speed, a stop watch was started when the valve allowing insecticide flow was opened and the watch was stopped when the product completely cleared the lines.

In Kansas City, MO Rainbow Scientific collaborated with Davey Tree to determine uptake speed on 9" DBH white ash trees (*Fraxinus americana*). This study utilized the Q-Connect injection system with ArborMectin™ and TREE-age®. ArborMectin™ was found to have 28% faster uptake than TREE-age® with one fourth the variability in uptake speed. In Plymouth, MN the experiment was repeated on green ash trees averaging 19.5" in diameter. The TREE I.V. MICRO INFUSION® system was used in addition to the Q-Connect. In large trees, the ArborMectin™ was found to have 62% and 58% faster uptake than TREE-age® on systems respectively with one third the variability in uptake speed. Statistically significant differences were found between formulations across all treatments.

Additionally, in Plymouth, MN an experimental design was implemented where two Q-Connect systems were attached to a single large (19.5" in diameter) green ash tree. This was repeated for 10 trees. The total number of injection ports recommended per tree (DBH / 2) was maintained by taking the total number of recommended ports divided by two on each system. Each system alternated port location and half the dose of emamectin benzoate were placed within each system. This experimental design addresses potential variability in prior studies caused by the individual tree. To determine uptake speed, a stop watch was started when the two valves allowing insecticide flows were opened simultaneously, time was noted when product completely cleared the first system's lines and the watch was stopped when the product completely cleared the second system's lines. ArborMectin™ was found to have 68% faster uptake than TREE-age® on each tree with one fourth the variability in uptake speed.

The successful development of ArborMectin™ has major implications for applicators and municipalities. ArborMectin™ was found to be statistically equivalent to TREE-age® in terms of efficacy for emerald ash borer control. ArborMectin™'s CAUTION EPA signal word and general use label does not negatively impact insect control. Additionally, in most states, applicators do not need to hold a pesticide applicators license to apply ArborMectin™ for personal use. The 28% to 62% increase in product uptake speed will allow applicators to treat and protect ash trees more efficiently. This efficiency leads to more cost effective applications allowing more ash trees to be protected from Emerald Ash Borer.

INTEGRATED MANAGEMENT OF EAB IN A MID-SIZE ILLINOIS COMMUNITY: A MODEL FOR EFFECTIVE AND RESPONSIBLE EAB MANAGEMENT INTEGRATING INVASIVE PEST BIOLOGY, ECONOMICS, AND COMMUNITY LEADERSHIP

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ABSTRACT

The emerald ash borer, *Agrilus plannipennis* Farimaire continues to spread across the United States killing millions of ash trees in its path. Since arriving in Illinois in 2006, the EAB has spread throughout the state causing many communities to come to grips with the reality of massive ash mortality and destruction of the urban forest. Current management options include a spectrum including doing nothing (i.e. let all of the ash trees die and then replace them), remove all ash trees and replant, treat certain high value trees with insecticides and let the rest succumb to EAB, or attempt to save a large portion of the ash trees by treating them with protective systemic chemicals.

In order to make the proper management decision, communities must have an up to date tree inventory, know their level of risk from EAB, have strong community support, and have dedicated a EAB management plan. Depending on the proportion of ash (*Fraxinus* spp.) trees in a given urban forest community, some areas will suffer more from EAB than others. Regardless of the economics, human resources, and urban forestry expertise, decision-makers wrestling with an EAB infestation need to understand unprotected ash trees will die from EAB and the management will either function at the mercy of the insect or have some level of control.

Using the City of Naperville, Illinois EAB management plan as a model, we will describe a viable and working model for a mid-sized community with a high proportion of publicly owned ash trees including the following: 1) a history of EAB in the Naperville, Illinois community, 2) decision making process for developing the EAB management plan, 3) how the management plan is working after three years, and 4) future projections on how the management plan will evolve over the next decade.

The city of Naperville, Illinois has a population of approximately 145,000 residents, encompasses a land area of 39 mi^2, covers two Illinois counties, and is located about 30 miles west of Chicago. The community includes well educated residents (97% are high school graduates and 66% are college educated), 77% are homeowners, and a number of major industries call Naperville home.

The emerald ash borer (EAB) was first discovered in southwest Naperville in June, 2008. Immediately, the city implemented a containment strategy and spot treatments began. By 2009, EAB had spread to

northwest Naperville. Spot treatments continued and in 2010, the Valent sponsored Legacy Tree Program (LTP) began with 200 trees being treated. By 2011, EAB had spread throughout the city. With 17,000 city owned ash trees (approximately 30% of the urban tree cover) and the vast majority (68%) being green ash (*F. pennslyvanica*) in the EAB cross-hairs, it was imperative a decision was required on how to manage for this invasive and destructive insect pest. The city leadership considered two options, 1) remove and replace all public ash trees over a period of six (6) years or 2) treat all ash trees that warranted protection (i.e. good investment) and remove and replace as needed. After careful deliberation, considering aesthetics, monetary and economic benefits, and cost effectiveness, the city elected to go with option #2 (treat and remove and replace as needed). In conjunction with the above decision-making process, the city hosted a series of town hall meetings spear-headed by homeowner associations, featuring community leaders, professional arborists, researchers, and industry representatives. The town hall meetings were well attended and there was substantial community support for the proposed EAB management plan.

Beginning in 2012, the city council appropriated $350,000 for insecticide treatments and $975,000 for removal and replacement of ash trees. In addition, prior to initiating treatments, all city trees were visually assessed and determined whether treatment was cost effective. Comprehensive parkway tree treatments began in April, 2012 and by July, 16,000 trees had been treated. Condition surveys were completed by August using a scale of 1 to 4 with 1 = good; 2 = fair; 3 = marginal; and 4 = poor was used. Approximately, 1,000 trees were "culled" and not treated. However, individual homeowners did have the prerogative to treat their parkway treat at their own expense if they chose. The three (3) insecticides selected for treatments were annual treatments of imidacloprid, dinotefuran, and treatments of emamectin benzoate every other year.

Trees not responding to treatment or were not cost effective to treat were removed. Thirteen (13) trees were removed in 2008, peaking at 759 in 2013. To date, 150 trees have been removed in 2014. At the same time, 367 trees were treated in 2008 reaching a high of 15,972 in 2012 and leveling off at approximately 13,000 in both 2013 and 2014. Of the 12,812 public trees treated in 2014, 98% of them (12,524) were treated by outside contractors. Of the nearly 13,000 treated trees, 8,845 were treated with imidacloprid (69%), 3,265 (25%) with emamectin benzoate, 702 (5%) and 200 (2%) as part of the Valent LTP. The percent ash tree in the Naperville urban forest has decreased by 5% (27% to 22%) since treatments began in 2008.

After completing the third year of the treatment program, how is it working? Using the annual tree assessments as a measure of tree health and insecticide efficacy, the overall health (good and fair) of the entire ash population for all treatments declined by 10% from 88% (2012) to 78% (2014) for trees rated good and fair. Overall tree health (good and fair) by treatment for this same period reflects trees treated with imidacloprid has declined by 7% (93% to 86%), but remained steady 93% (2012) versus 92% (2014) for emamectin benzoate-treated trees. Trees treated with dinotefuran showed 5% drop in overall tree health (92% to 87%) and an 8% increase in tree health (91% to 98%) for trees in the Valent LTP. Tree health for treated green ash (*F. pennsylvanica*) and white ash (*F. americana*) trees are comparable with a 5% (88% to 93%) increase, and a 2% decrease (92% to 90%), respectively. Evaluating the efficacy of individual products (imidacloprid, dinotefuran, and emamectin benzoate), good and fair rated trees have demonstrated an 84% to 95% likelihood of recover compared to imidacloprid-treated trees with 77% to 63% likelihood, and a 73% to 63% likelihood of recovery for dinotefuran-treated trees, respectively. Overall, after three (3) years of treatment, the vast majority of city-owned ash trees are in good to fair condition.

Examining the economics of the Naperville EAB management plan reveals that the removal-replacement plan (Option #1) would have cost the city a total of approximately $12 million over the course of nine (9) years (2008 – 2017) requiring removing 2,800 trees per year for six (6) years at a cost of $1.7 million, and a loss of 100% of all publically owned ash trees or approximately 30% of the city's tree cover. In contrast, implementing option #2, treating ash trees and removing and replacing as needed, the city is preserving 65% of the ash trees, has spread out removal costs with no more than 1,000 trees removed per year, at a cost of $850,000 per year. Based on the current trends and costs, it is estimated a reduced treatment regimen could be maintained until 2028 before treatment costs would reach removal and replacement costs (option #1).

Using the EAB ash tree death curve developed by Dr. Dan Herms, Ohio State University, and using field observations of private ash tree mortality, the city as a whole appears to be on the steepest portion of the curve or area of most rapid ash tree decline. At this rate, it is estimated greater than 50% of untreated ash trees will be dead by the end of the 2014 field season and all untreated private ash trees will be dead by 2017. In addition, treated city ash trees appear to be "beating" the death curve with removals less than untreated trees (759 trees removed in 2013 and to date, 150 trees removed in 2014). Treatment reduction may be reduced and/or terminated by as early as 2018. The current cost/benefit scenario maintains a current treatment regimen through 2020 with city ash tree deaths returning to a normal mortality rate (1%) by 2020. At this point in the EAB management timeline, the city leadership intends to "stay the course".

The above projections are based on our current knowledge of EAB behavior, consultation with other entomologists, arborists, and industry professionals. There are still a number of unknowns waiting to reveal themselves. For example, how do large groups of untreated trees affect the death curve? How will untreated private trees impact the number of years required for treating public trees and will high number of untreated private trees "stretch out" the death curve? Work by McCullough and Mercader (2011) seems to indicate untreated private trees will have minimal effect on the ash tree death curve. Hopefully, the Naperville EAB management program will confirm these findings providing viable and cost effective management options for mid-sized communities yet to experience the potential devastation of the EAB.

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STRATEGIES AND TACTICS FOR EAB MANAGEMENT AND ASH CONSERVATION IN THE URBAN FOREST

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ABSTRACT

Research has shown that insecticides provide an efficacious and cost-effective tool for conserving ash in urban forests as emerald ash borer (EAB) continues to spread. Multiyear studies have been conducted to evaluate the effectiveness of systemic insecticides for protecting large caliper ash street trees from emerald ash borer in the face of extremely high pest pressure. Specific objectives were to: (1) evaluate efficacy of different rates of imidacloprid (Merit and Xytect) soil drenches applied annually in spring or fall, (2) to determine the number of years of control provided by different rates of a single emamectin benzoate (Tree-äge) trunk-injection, (3) to evaluate in head-to-head comparisons various products commonly used by arborists to control EAB, and (4) to evaluate the effectiveness of emamectin benzoate (Tree-äge) trunk-injections for protecting very large (32-48 inch DBH) ash trees.

Objective 1: Efficacy of imidacloprid soil drenches applied in spring or fall. The following imidacloprid soil drench treatments were applied from 2006-2014 to a green ash (*Fraxinus pennsylvanica*) street tree planting ranging in size from 11-21 inches DBH (mean = 15 in)when the study was initiated:

1. untreated control
2. imidacloprid, 1.42 g ai / inch DBH applied in May
3. imidacloprid, 1.42 g ai / inch DBH, applied in October
4. imidacloprid, 2.84 g ai / inch DBH, applied in May
5. imidacloprid, 2.84 g ai / inch DBH, applied in October

No trees showed visible symptoms of canopy decline in 2006 when treatments began. Untreated trees began to decline in 2008 and by 2010, all were dead. Trees treated in spring or fall with the high rate of imidacloprid, or in spring with low or high rate of imidacloprid, have maintained healthy canopies, with percent canopy thinning less than 5% in all cases in 2014. Trees treated in the fall with the low rate (1.42 g ai/inch dbh) exhibited much greater canopy decline (averaging 40-60%) and all were removed by the city forester in 2011 after being deemed hazards. These results suggest that when applied annual applications of imidacloprid soil drenches can effectively protect trees in the 15-20 inch DBH size class even under intense pest pressure, and at lower rates that spring applications are more effective than fall applications. Studies continue to determine if trees can be effectively protected with less frequent treatments now that the EAB population has dropped to very low levels.

Objective 2: Duration of efficacy of emamectin benzoate (Tree-äge**) trunk-injections.** The following rates of emamectin benzoate (Tree-äge) were applied as trunk injections using the Arborjet Viper Tree

IV on 14 June 2006 to a green ash street tree planting ranging in size from 13-24 in DBH (mean = 21 in): 0.1, 0.2, 0.4, and 0.8 g ai/in DBH. Pest pressure was intense at the study site and canopy decline of untreated trees averaged 53% and 96% in 2008 and 2009 respectively, and all untreated trees had died by 2010. All rates provided excellent control through two years (less than 10% canopy decline in 2008). However, in the third year, canopy decline increased significantly in all treatments, indicating that control was beginning to relax. In 2009, canopy decline averaged 25%, 55%, 60%, and 13% in the 0.1, 0.2, 0.4, and 0.8 g ai/in DBH treatments, respectfully. These results indicate that trees in the 20-25 inch DBH size class can be protected effectively for two years by a single application of emamectin benzoate. However, under intense pest pressure, treatment efficacy begins to decline in the third year. No evidence of phytotoxicity was observed even at the highest rate.

Objective 3: Comparison of efficacy of products commonly used by arborists to control EAB. The following treatments were applied on 11 June 2010 and 9 June 2011 to green ash (*Fraxinus pennsylvanica*) in parking lot planting in Columbus, Ohio. Treatments were evaluated on 17 August 2011 and 29 May 2012. Trees ranged in size from 8-11 inches DBH when the study was initiated:

1. Merit (imidacloprid) 75WP, 1.42 g ai / inch DBH, applied as a soil drench.
2. Pointer (imidacloprid), 2 ml / 4 in circumference, applied with the Wedgle Direct Inject Tree Injection Unit.
3. Tree-äge (emamectin benzoate), 0.2 g ai / in, applied with the Arborjet Tree I.V.

Trees averaged 20% canopy thinning at the time treatments were applied, but only one tree in the planting that was not used in the study showed any signs of EAB infestation (a single adult emergence hole). In August 2011, canopy thinning of untreated trees averaged 42%, while canopy thinning of trees treated with Merit, Pointer, and Tree-äge, and averaged 32, 36, and 22%, respectively. These differences were not statistically significant. On 29 May 2012, canopy thinning of trees treated with Merit (36%) and Tree-äge (30%) was significantly less than thinning of untreated trees (72%). Trees treated with Pointer averaged 64% canopy thinning, which did not differ from the untreated controls.

Objective 4: Efficacy of emamectin benzoate (Tree-äge**) trunk-injections for protecting very large ash trees**. In 2010, a study was initiated in northern Ohio to evaluate the efficacy of emamectin benzoate (Tree-äge) trunk-injections at two rates (either 5 or 10 ml / inch DBH, 10 replicate trees per rate) for protecting ash trees ranging in DBH from 32 to 47 inches Trees were injected in 2010, 2012, and 2014. In 2010, most trees showed some signs of EAB attack (woodpecker strikes and bark splits that exposed galleries in branches), and percentage canopy dieback of trees receiving the 5 and 10 ml rate averaged 10 and 11%, respectively. In August 2014, percentage canopy dieback averaged 1.3% for both rates. Pest pressure was severe in the study area in 2014, with most untreated trees experiencing severe decline, or had died and been removed.

THE *IN VITRO* METABOLISM OF OLEUROPEIN IN EXTRACTS OF ASH PHLOEM AND EAB LARVAE: A POSSIBLE RESISTANCE MECHANISM IN MANCHURIAN ASH?

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ABSTRACT

Oleuropein is a coumarin-like secoiridoid glycoside with exocyclic 8,9-olefinic functionality, a structure common and specific to many members of Oleaceae. This compound is responsible for the majority of the phenolic mass in fruits and leaves in olive trees, has demonstrated antimicrobial activity, and has been shown to be a potent anti-herbivore defense in privet (*Ligustrum obtusifolium*). However, the anti-herbivore effects of oleuropein in privet are not related to the concentration of oleuropein in tissues, but rather the activity of enzymes responsible for metabolizing oleuropein into a potent protein cross-linking/denaturing compound (Konno et al. 1999). Concentrations of oleuropein are also very similar in North American black ash (*Fraxinus nigra*) and Manchurian ash (*F. mandshurica*), which has coevolved with emerald ash borer in Asia. The two species are phylogenetically closely-related but differ dramatically in their resistance to EAB, with black ash much more susceptible. Our objective was to explore differential metabolization of oleuropein by these two species and attempt to address the putative role of oleuropein in interspecific variation in resistance of ash to emerald ash borer.

Phloem tissue from black and Manchurian ash were harvested from trees in a common garden at The Ohio State University's Ohio Agricultural Research and Development Center in Wooster, OH. Proteins were subsequently extracted, and the activities of β-glucosidases, polyphenol oxidases, and peroxidases were quantified spectrophotometrically using standard substrates and purified oleuropein. All enzyme activities were significantly higher in Manchurian ash, regardless of substrate. Most notably, the rate of oleuropein oxidation due to peroxidases was approximately 6-7 times higher in Manchurian ash than black ash. To investigate potential mechanisms of action in emerald ash borer larvae, larval extract was reacted with oleuropein and proteins were separated via electrophoresis. Protein visualization revealed protein cross-linking and denaturation.

Our experiments are suggestive of a putative role of oleuropein in host resistance of Manchurian ash to emerald ash borer, though more experiments are needed to confirm this. It appears that oxidized oleuropein could cross-link with dietary or insect proteins, lowering the quality of digestive proteins or disrupting protein constituents of the peritrophic membrane.

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BIOLOGY, BEHAVIOR AND ECOLOGY

SILHOUETTE, COLOR, AND LIGHT SCATTERING: PARTITIONING THE COMPONENTS OF VISUAL ATTRACTION THAT ELICIT BEHAVIORS OF *AGRILUS* MALES

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ABSTRACT

Male buprestids like the emerald ash borer (EAB), *Agrilus planipennis,* perform visually mediated mating flights directly onto females poised on sunlit leaves (Lelito et al., 2007). Research into the nature of such responses has revealed factors that can be applied to trapping applications. This visual response has been observed across different *Agrilus* species and is not highly species-specific (Domingue et al., 2010). It also can be evoked with artificial decoys (Domingue et al., 2014 a,b). In high-density infestations, decoy-baited sticky leaf traps have proven as effective at detecting EAB as larger prism traps (Domingue et al., 2013). Furthermore, fully synthetic traps consisting of small green plastic sticky cards baited with 3D-printed beetles have proven effective at capturing EAB (Domingue et al., 2014). It was more recently determined that such traps were more effective if placed on the branches of healthy trees with no signs of EAB damage versus the epicormic sprouts of heavily damaged trees.

A nanofabricated decoy was also created that replicates the fine-scale surface structure of the dorsal surface of an emerald ashborer (Domingue et al., 2014b). These bioreplicated decoys scatter light with a similar pattern to that produced by real beetles when a white super-continuum laser is appled to them in a dark room. However, simple 3D-printed decoys without such structure do not scatter light. In a field experiment it was determined that real dead *Agrilus* beetles or the bioreplicated decoys could entice male *Agrilus biguttatus* to fly and land upon them if simply pinned to leaves. The simpler 3D-printed decoys triggered the initiation of such mating flights just as often, but the approaching males nearly always diverted away from the decoys and did not land on them. The more authentic bioreplicated decoys were used as part of an electrocution trap that caused approaching males to be stunned and collected when they approached and touched the decoys. Such traps were able to catch *A. biguttatus, A. angustulus,* and EAB. For EAB video evidence was obtained that showed a wild male flying onto the decoy of the electrocution trap and falling into the collection cup below. Further improvements to the traps were made to improve field usability, including the addition of a solar power source to allow continual operation.

Additional experiments indicate that in addition to light-scattering, the silhouette and color of decoys also influence the probability of successful mate attraction. For example, two parallel EAB elytra side by side will attract males to approach for mating as well as a pinned female, but elytra displayed in other configurations will not (Domingue et al., 2013). Color morphs of EAB that included violet, blue, copper, and red, in addition to the wild-type green were collected and presented to wild males. It was determined that wild *A. angustulus* males preferred the higher wavelength copper and red morphs, while *A. biguttatus males* more often flew toward the low wavelength violet and blue morphs.

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THE EFFECT OF EMERALD ASH BORER-CAUSED TREE MORTALITY ON FOREST REGENERATION

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis,* is an invasive pest that may cause extensive changes to forest structure and canopy composition in the forests of eastern North America. Mature ash, *Fraxinus* spp.*,* will succumb to larval feeding in 1-4 years, resulting in forest canopy gaps that could result in dramatic increases in light. Since, different species of woody plants are adapted to varying levels of understory light, this disturbance could greatly shift seedling and sapling communities. As ash trees die and create canopy gaps, changes in understory light conditions may favor some species over others, resulting in changes to understory composition which could influence long-term forest composition.

To investigate the response of understory woody plant communities to EAB-induced ash mortality, we tagged and measured tree seedlings in 24 sites (three plots per site) in 2012. Sites were established throughout western and central Ohio for long-term EAB monitoring by the United States Forest Service (USFS) and represent a range of time since EAB infestation. Nested within each plot ($400m^2$) were four $4m^2$ micro-plots, one in each cardinal direction, 6m from the center of the plot. At the plot ($400m^2$) level, live ash health was assessed using an ash condition rating on a scale of 1 (healthy) through 5 (dead) (Smith 2006). All trees <10cm diameter at breast height (DBH) located within the plot were measured and their canopy class recorded. Instead of using a simple average of ash condition for each plot, we calculated a weighted ash condition which gave greater consideration to larger trees which are more likely to influence forest understory conditions than smaller trees. Weighted ash condition for each plot was calculated by multiplying each ash tree's condition rating by its basal area, summing these, and dividing by the sum of ash basal area for the plot.

At the micro-plot ($4m^2$) level, all tree and shrub seedlings within the size range of 20 to 100cm were measured for height, and any new individuals which entered the size threshold in subsequent years were added. Canopy openness was also measured in each of the micro-plots ($4m^2$), using a concave spherical densiometer, taking four measurements within each micro-plot, one in each cardinal direction. Tree seedlings were ranked using a published shade tolerance scale, 0 (shade intolerant) to 5 (shade tolerant) (Niinemets and Valladares 2006). Seedling rankings were graphed and natural breaks in the data produced three distinct groupings. Species ranked 1-2.9 were classified as shade intolerant, those ranked 3-4 were classified as shade intermediate, and those ranked 4.1-5 were classified as shade tolerant.

Ash decline was most advanced near the initial infestation (northwest Ohio) and quickly advancing throughout western and central Ohio (Knight et al, this proceedings). Composition of tree seedling communities

was investigated using linear mixed models (LMM) and Akaike's Information Criterion (AIC) was used for model evaluation. Explanatory variables of interest included basal area of ash, weighted ash condition, total canopy basal area, and canopy openness. Response variables included total number of seedlings, number of tolerant seedlings, number of intermediate seedlings, number of intolerant seedlings, percent of shade intolerant seedlings, and mean seedling shade tolerance. Explanatory and response variables collected in 2012 and 2013 were evaluated; only 2013 results are reported. The best model for the total number of seedlings in a plot included only total tree canopy basal area; plots with greater basal area had fewer seedlings. The number of shade tolerant seedlings in a plot was best predicted by total canopy basal area and weighted ash condition; plots with greater basal area and less healthy ash trees had fewer shade tolerant seedlings. Weighted ash condition and canopy openness were the best predictors for the number of intermediate seedlings in a plot; plots with less healthy ash trees and less open canopies had fewer intermediate seedlings. The model that best predicted the number of intolerant seedlings in a plot was ash basal area; plots with greater ash basal area had less intolerant seedlings. The percent of intolerant seedlings in a plot was best predicted by weighted ash condition and canopy openness; plots with poorer ash condition and less open canopies had a greater percentage of intolerant seedlings. The best model for mean seedling tolerance in a plot included only total tree canopy basal area; plots with greater basal area had tree seedlings with lower mean shade tolerance. Similar results were obtained for both years of data and model support generally increased in 2013. Overall, results show that the abundance of tree seedlings is related to ash tree condition, canopy openness, and total canopy tree basal area. While all tree seedlings benefited from decreased competition from all species of overstory trees, differences in responses among different species of seedlings as well as competition among these species may lead to changes in understory composition.

Further analyses will be conducted using path analysis. Data will also be incorporated into a forest dynamics model to make long-term forest composition predictions that can assist in guiding resource management in forests impacted by emerald ash borer.

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IMPACTS OF EMERALD ASH BORER ON NATIVE AND INVASIVE PLANT COMMUNITIES

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ABSTRACT

As emerald ash borer (EAB; *Agrilus planipennis*) continues to spread throughout North America, land owners and managers are faced with the loss of untold millions of ash (*Fraxinus* spp.) trees. It is yet unclear how such widespread, simultaneous mortality will alter forest communities. Of particular concern is the potential for invasive plants to establish or spread in the disturbed areas, since canopy gaps, can be susceptible to plant invasions (Tilman 2004).

Our objective was to determine if gap formation due to ash mortality has altered the successional trajectory of the understory plant community. We tested the following hypotheses: 1) plots that contained more ash trees would have more and/or larger gaps; and 2) plots with higher gap fractions would have greater abundance and/or growth of invasive plant species. We focused on 10 woody invasive species that had been previously identified in the area: *Rosa multiflora*, *Lonicera* spp, *Berberis thunbergii*, *Elaeagnus umbellata*, *Celastrus orbiculatus*, *Rhamnus cathartica*, *Frangula alnus*, *Ligustrum vulgare* and *Euonymus alatus*. We also compared their growth to native plants that were growing in the vicinity of the invasive plants, with *Fraxinus* spp., *Lindera benzoin*, and *Carpinus caroliniana* being the most common.

We established 126 18-m radius circular plots in seven natural areas in southeast Michigan. Plots were classified according to soil moisture from dry xeric uplands previously containing white ash, to more organic mesic sites previously containing green ash, to wet hydric sites previously dominated by black ash (Smith 2006). Plots also spanned a gradient of ash density (prior to EAB-induced mortality) and time since EAB infestation. In this region, ash tree mortality exceeded 99% by 2009 (Smith et al., in press; Klooster et al. 2014), and the ash seed bank was depleted by 2007 (Klooster et al. 2014).

To estimate the understory light environment, hemispherical photographs were taken at four evenly spaced locations within each plot and analyzed using WinSCANOPY software. Individual pixels were labeled as

either "sky" or "canopy"; canopy gap fraction was calculated as a ratio of sky pixels to total pixels. Ash importance values (IVs) (relative basal area × relative density × relative dominance) were calculated using plot data collected prior to ash mortality; values were compared across moisture levels with the highest IVs in hydric plots and the lowest in xeric plots. Gap fraction was positively correlated with IV in xeric plots, but was not correlated in hydric or mesic plots.

To quantify plant growth and cover, we estimated percentage plant cover in four separate forest strata (0-1, 1-2, 2-5, and >5 m) for all plants combined, as well as for each individual invasive species. In the canopy layer (>5 m), hydric plots had less cover (63%) than mesic or xeric (83%) plots. Ash IV was negatively correlated with plant cover in the canopy layer (-0.38, $p < 0.05$).

Total number of native and invasive plants was assessed by counting individual stems in four 4-m^2 quadrats per plot. Simpson's index of diversity was calculated using the plot counts, and was higher in hydric (0.61 ± 0.05) than in mesic (0.24 ± 0.05) or xeric (0.25 ± 0.03) plots. Density of invasive plants was not correlated with plot mean gap fraction, ash importance, or ground layer percentage cover. Cover in the ground layer (0-1 m) was greatest in hydric plots (78%) compared to mesic (63%) or xeric (57%) plots.

Growth of selected invasive and native plants in the understory of EAB-impacted forests was measured annually as length × width × height. Invasive shrubs grew faster than native shrubs ($F = 4.61$; $p = 0.03$), but differences were not significant for trees or vines. Growth of native and invasive plants was positively correlated with gap fraction in hydric plots, but not in mesic or xeric plots. Growth was not related to ash IV for any moisture level.

In the understory (1-2 m layer), hydric plots had greater cover (63%) than mesic (42%) or xeric (36%) plots. Density of saplings did not vary by year or moisture level; however, shrub cover did vary by moisture level ($F = 6.96$; $p = 0.001$). Neither sapling density nor shrub cover was correlated with gap fraction or ash IV.

Gap fraction was negatively correlated with canopy cover in the subcanopy (2-5 m) layer, suggesting that any increase in light due to canopy gaps formed by ash mortality is likely being intercepted before reaching the ground or understory layers. This would explain the lack of correlation between plant metrics and ash IVs.

Ongoing research is focused on the response of non-ash overstory and understory trees to determine what effects, if any, ash mortality and canopy gap formation had on their growth. We are analyzing trunk cores collected from a variety of dominant, co-dominant, intermediate, and suppressed shade-tolerant and shade-intolerant trees to quantify their growth release in response to gap formation.

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EFFECTS OF CANOPY GAP FORMATION AND COARSE WOODY DEBRIS CAUSED BY EMERALD ASH BORER-INDUCED ASH MORTALITY ON GROUND BEETLE (COLEOPTERA: CARABIDAE) ASSEMBLAGES

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ABSTRACT

Ash mortality caused by emerald ash borer (EAB; *Agrilus planipennis*) has resulted in widespread, relatively simultaneous formation of canopy gaps in forests. Gap formation may alter environmental conditions on the forest floor by increasing light availability, soil temperature, and soil moisture, as well as increasing their variability. These changes on the forest floor have the potential to impact populations of ground-dwelling invertebrates, such as ground beetles, which are voracious predators that regulate many ecosystem processes and considered biological indicators of environmental change. However, as dead ash trees fall, accumulations of ash coarse woody debris (CWD; large branches and logs) may buffer environmental changes on the forest floor induced by increased light.

The objective of this study was to quantify the effects of canopy gap formation and accumulation of ash CWD caused by EAB-induced ash mortality on ground beetle assemblage diversity and composition. A two-year, manipulative experiment was conducted in forests at NASA Plum Brook Research Station in Ohio to determine the effects of canopy gaps, CWD, and their interaction on ground beetle assemblages. We predicted that 1) ash mortality would negatively impact ground beetle assemblages due to changes in the forest floor environment; 2) ground beetle species composition would shift as open habitat species became more dominant in canopy gaps than forest species; 3) in canopy gaps, ash CWD increased ground beetle abundance and diversity by providing a stable environment on the forest floor; and 4) ash CWD would buffer the forest floor environment from the effects of increased light.

The formation of canopy gaps altered ground beetle assemblages by decreasing activity-abundance and species richness. Canopy gaps changed ground beetle species composition by increasing open habitat species and decreasing forest species in gaps. CWD impacted ground beetle assemblages by increasing the activity-abundance of forest species under closed canopies. However, there was no evidence that CWD was providing a benefit to ground beetles in canopy gaps. Soil temperature and soil moisture increased in canopy gaps regardless of the presence of CWD. The impact of canopy gaps and CWD varied between years, and the response of ground beetle assemblages was dependent on the degree of environmental differences on the forest floor between canopy gaps and closed canopies. Emerald ash borer indirectly impacted ground beetle assemblages through changes in environmental conditions on the forest floor caused by ash mortality.

ASH DECLINE AND MORTALITY, TREE FALL, AND EMERALD ASH BORER POPULATION DYNAMICS AT FOREST STAND AND LANDSCAPE SCALES 2004-2014

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ABSTRACT

The accidental introduction of emerald ash borer (*Agrilus planipennis*, EAB) has resulted in millions of dead ash trees as the beetle has rapidly spread through much of the U.S. and adjacent Canada (Herms and McCullough 2014, www.emeraldashborer.info). Nearly 100% of infested trees of most species eventually die (Herms and McCullough 2014), although *F. quadrangulata* may show patterns of slower or decreased mortality (Tanis and McCullough 2014).

We began monitoring ash forest sites in southeast Michigan and northwest Ohio in 2004 and 2005, respectively. In 2006-2008, additional Ohio sites were added to encompass a range of ash density, ash species, and forest stand characteristics. Yearly data on ash tree canopy health, EAB symptoms, mortality, and tree fall were recorded (Knight et al. 2014). EAB traps were hung in a subset of the sites from 2008-2014, providing a seven year time course of EAB population dynamics in these sites. The dataset from these long-term ash monitoring plots, with yearly landscape-scale data spanning a decade, has provided many insights into effects of EAB on ash populations (Flower et al 2013, Klooster et al 2014, Knight et al 2013, Knight 2014, Smith 2006, Smith et al, in press).

The patterns of ash decline and mortality at the forest stand scale have shown that it generally takes 3 to 6 years for the ash trees to decline from healthy to nearly complete mortality (Figure 1), with some variation in the time course of mortality due to stand-level and tree-level factors (Knight et al 2013). We observed all five ash species native to the Midwest region, *Fraxinus americana, F. pennsylvanica, F. nigra, F. profunda*, and *F. quadrangulata*, with symptoms and mortality from EAB. Initially, most stands have mostly healthy trees as well as some trees declining or dead from factors other than EAB. EAB populations build gradually. Part-

way through the infestation, it is typical for the stand to have a mix of healthy, declining, and dead trees. As the infestation progresses, the EAB populations build to high levels, and mortality accelerates until nearly all the trees have died. Then, after nearly exhausting the host tree population, EAB populations rapidly decrease to low levels. Thus far in our sites, these EAB populations have persisted at low levels for at least 5 years after the ash population crashed, likely feeding on small ash saplings as the saplings become large enough to be attacked. After the ash trees die, they rapidly break up and fall down. Over 80% of the ash trees fell within five years of mortality in our sites.

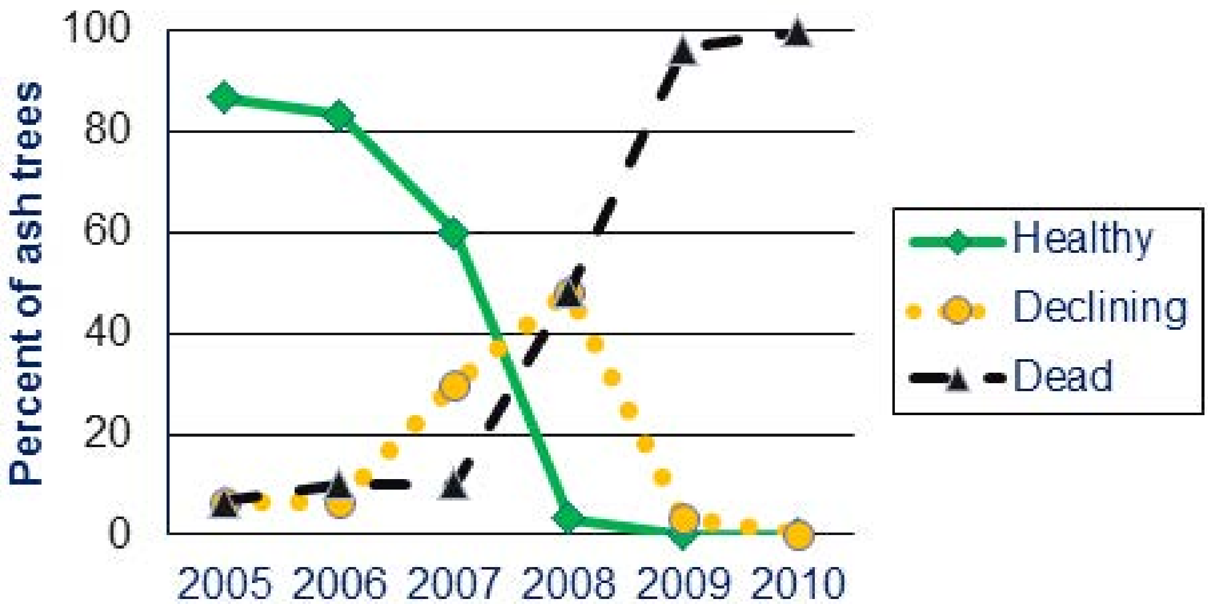

Figure 1. Ash mortality in long-term monitoring plots at Wildwood Metropark near Toledo, Ohio.

At the landscape scale, maps of stand-level metrics over time show how ash mortality spread from Michigan south throughout Ohio (Figure 2). Moderate levels of mortality were already occurring in the Michigan sites when monitoring began in 2004/2005, while mortality in northwest Ohio sites was very low at that time. Nearly complete mortality is apparent first in the Michigan sites in 2008, then in the Toledo area by 2009, all of northwest Ohio by 2011, and much of the Columbus area in 2014. Maps of EAB densities over time show the peak and crash of EAB populations in different regions of the state. EAB populations were high in Toledo in 2008 then crashed to lower densities, while EAB populations in Columbus varied among sites but generally peaked in 2012.

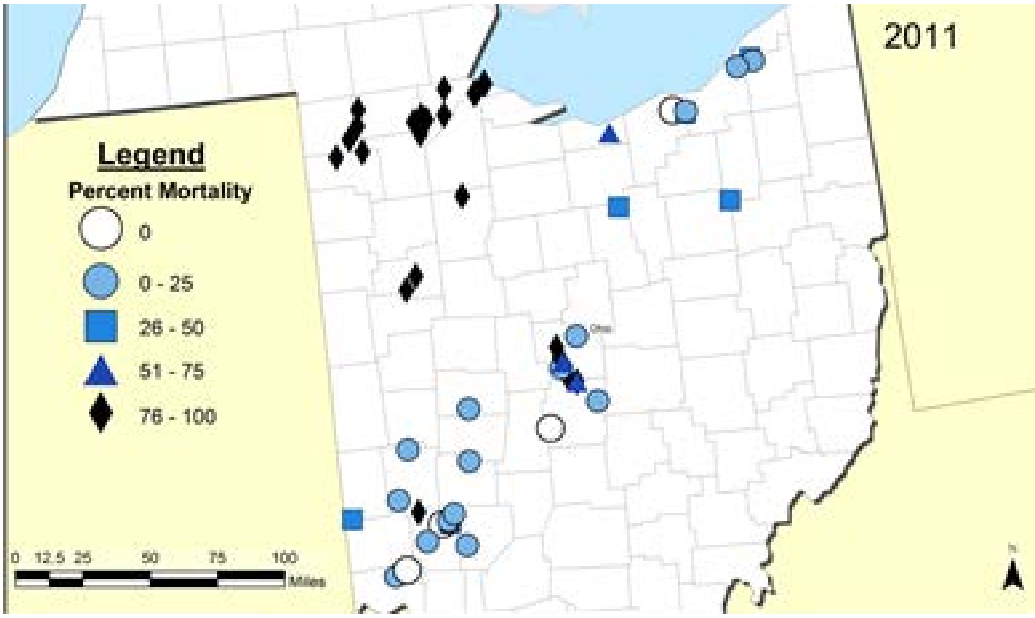

Figure 2. Mortality of ash trees >10cm DBH (Ohio) and 12.5cm DBH (Michigan) in long-term monitoring plots in 2011.

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INTERSPECIFIC PATTERNS OF ASH DECLINE AND MORTALITY IN A COMMON GARDEN

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ABSTRACT

We have characterized interspecific variation in ash resistance to emerald ash borer (EAB) in an on-going common garden study established at Michigan State University's Tollgate Education Center in Novi, Michigan in 2004. Patterns of ash decline and mortality have been largely consistent with the hypothesis that coevolved species indigenous to Asia are more resistant than evolutionary naïve hosts native to North America and Europe. The resident EAB population was low when the plot was established as most trees in the region had been killed. As EAB populations began to resurge and susceptible trees in the plot began to be killed, Manchurian ash has had the highest rate of survival and little canopy decline. Mortality of Manchurian ash that did occur was concentrated in the first few years after planting, perhaps due to transplant stress. The only tree killed after 2009 had its trunk badly injured by a deer rub. The high EAB resistance of this Manchurian ash population of seedling origin is consistent with that observed by Rebek et al. (2008) for the clonal Manchurian ash cultivar 'Mancana,' suggesting that EAB resistance is a species-level trait.

Fraxinus x 'Northern Treasure' ash, which is a Manchurian (Asian) x black ash (North American) hybrid, had similarly high survival and low canopy decline, suggesting introgression of Manchurian ash resistance genes into the hybrid. However, this pattern contrasts sharply with that observed by Rebek et al. (2008), who found 'Northern Treasure' ash to be highly susceptible to EAB. This suggests there may be taxonomic confusion in the nursery industry surrounding this cultivar that has yet to be resolved.

Most North American species and cultivars in the common gardens study experienced complete or nearly complete mortality, with green ash cultivars, black ash, and Oregon ash declining more rapidly than white ash cultivars. Blue ash has survived at a higher rate than other North American species, but by 2014 had lower survival and greater canopy decline than Manchurian ash. Decline and mortality of blue ash has increased over time, suggesting that surviving trees may ultimately succumb to EAB as other hosts are eliminated. The European species and cultivars evaluated in the common garden also experienced high decline and mortality, including *F. ornus*, *F. excelsior* 'Aureafolia', and *F. angustifolia* subsp. *oxycarpa* 'Raywood,', which suggests that EAB has the potential to cause widespread economic and ecological impacts in Europe as it continues to spread in Russia and beyond (Baranchikov et al. 2008, Orlova-Bienkowskaja 2014).

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IMPROVING DETECTION TOOLS FOR EAB: EFFECT OF HOST AND AGE OF TRAPS

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ABSTRACT

Green multifunnel traps, coated with fluon, a fluoropolymer, had been found to be comparable to or better than purple prism traps in terms of both trap catch (Francese et al. 2013) and detection, the ability of a trap to catch at least 1 beetle (Crook et al. 2014). As part of an ongoing project to improve survey tools for the emerald ash borer (EAB), several field assays were conducted in high EAB population density sites in south-eastern Michigan, as well as in low EAB population sites along the edge of the currently known infestation.

Multifunnel traps, while more expensive than prism traps are designed to be re-used from one year to the next. However, it is unknown whether the green pigment in the trap could fade over time, diminishing the trap's attractiveness to EAB. Also, since fluon is applied externally to the traps, the slick surface of the trap could also diminish. In a multifunnel trap durability / aging assay, we compared new traps (2014) with traps that had been placed in the field for one (2013), two (2012) or three field seasons (2011). Traps placed in the field since 2011 and 2012 caught significantly few EAB than traps placed in the field in 2013 and 2014. One reason for this may have been a switchover from fluoning traps by hand using a sponge (which can lead to an uneven coating) to having the manufacturer pre-fluon traps (using a dipping method) prior to shipment. We will continue this project in 2015.

In 2013 and 2014, as part of an ongoing detection tools comparison conducted along the edges of the currently known EAB infestation a trapping assay was conducted to compare trap catch and detection rates of EAB with four trap / lure combinations on ash as well as several non-hosts. Trapping on non-host was conducted to answer two questions that have been raised about surveying for EAB: 1) if an ash tree is not readily available to surveyors does placing the trap on another species affect trap catch and detection rates and 2) can these traps that have been developed for EAB be used for surveying other woodborers (ie. in a CAPS survey). The four traps were 1) an unbaited purple prism trap, 2) an unbaited purple multifunnel trap, 3) an unbaited green multifunnel trap, and 4) a green multifunnel trap baited with a 3Z-hexenol lure (50 mg/d release rate). In 2013 traps were placed on four tree genera: 1) *Fraxinus*, 2) *Acer*, 3) *Pinus* and 4) *Quercus* (red oak group only). In 2014, five tree groups were compared: 1) *Fraxinus*, 2) *Betula*, 3) *Populus*, 4) *Quercus* (red oaks) and 5) *Quercus* (white oaks).

Overall, baited green multifunnel traps had higher detection rates than unbaited purple multifunnel traps but were comparable to unbaited green multifunnel and purple prism traps. In 2013, of the sixteen replicates of traps placed in maple, red oak and pine, seven, eight and eight did not have a single detection recorded

among any of the traps, compared to 15 of 16 ash replicates (in the same areas) recording at least one positive catch. The 2014 EAB and all non-target catch data from 2013 and 2014 data is still being summarized and analysis will begin soon.

Besides being conducted in 5 states in 2013 and 8 states in 2014, this comparisons were also conducted in China, Russia and Poland (with adjustments made for locally available tree genera). In 2013, 3717 buprestids in 17 species (including 12 *Agrilus* species) and 60 cerambycids in 11 species were caught in Poland.

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REGULATORY, MANAGEMENT AND OUTREACH

CAN LOCAL PARASITOID HELP MITIGATE EAB DAMAGE AND PRESERVE OUR ASH?

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ABSTRACT

The emerald ash borer, *Agrilus planipennis* (EAB), is an exotic buprestid native to Asia. Since its introduction to North America near Detroit, MI EAB has spread to over 20 states, reaching Kentucky in 2009. Biological control has emerged as the most promising management strategy, and efforts are underway to release exotic hymenopteran parasitoids throughout the introduced range of EAB. Native parasitoids, several of which have been discovered in the northern portion of EAB's introduced range, may also play a key role in helping regulate EAB populations. Parasitoid communities in newly invaded areas farther from the initial infestation are unknown, and may vary considerably from areas that have been studied. This research aims to develop a sustainable management strategy for emerald ash borer by blending biocontrol releases with chemical applications. During spring of 2013 and 2014 ash in 60 forested plots across five sites were subjected to one of four treatments: 1) application of imidacloprid at recommended label rates, 2) releases of three species of classical biological control agents, 3) a combination of biocontrol releases with application of imidacloprid at reduced rates, and 4) untreated controls. In February 2014 ash from these plots were felled, cut into 60cm sections, and either debarked or placed in rearing enclosures to evaluate EAB infestation, establishment of introduced parasitoids, and recruitment of native parasitoids. Green multi-funnel traps were deployed May 1 - July 17, 2014 to evaluate adult EAB populations. EAB were first detected in mid-May and peaked during the first week in June, with reduced numbers in chemically treated plots (full and half strength). We see signs of successful establishment of the exotic *Tetrastichus planipennisi*; larvae and/or adults were recovered from three release sites. We also see recruitment of native parasitoids; seven morpho-types of hymenopteran parasitoids in three families were recovered from rearing enclosures, another two morpho-types in two families were collected from EAB galleries of debarked ash logs, and an additional two were collected from the trunks of dead or dying ash. These parasitoids, some of which have not yet been reported utilizing EAB as a host, add to our knowledge of native natural enemies and will aid in developing a localized management strategy for mitigating damage caused by emerald ash borer.

PREDICTING POTENTIAL TREE CANOPY LOSS IN CITIES DUE TO EMERALD ASH BORER

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ABSTRACT

It is estimated that the emerald ash borer (EAB) was introduced near Detroit, MI in the early to mid-1990's. In the last 15+ years, EAB has killed millions of ash trees within the Midwest region and has transformed the urban forest of many cities. As EAB continues to expand its range it is expected that many more cities will be impacted in the near future. i-Tree Canopy, was used to determine the change in land cover types (e.g., tree cover) using aerial images available in Google Maps in eight mid-west cities known to be infested with emerald ash borer (EAB) for 10+ years (Detroit, Flint, Lansing, Ann Arbor, Columbus, Toledo, Fort Wayne, and South Bend) and eight other cities where EAB has just arrived or expected in the near future (Boston, Rochester, St. Louis, Des Moines, Boulder, Denver, Fort Collins, Salt Lake City). i-Tree Canopy randomly generates sample points and zooms to each one so you can choose from your pre-defined list of cover types for that spot [tree canopy, pervious (shrub, grass, or soil), impervious (buildings, asphalt, or concrete), or water (lakes, rivers, pools)]. Up to 601 points were randomly selected within each city boundary. The average city size, population and interval evaluated were similar for each group. Past (2004-5) and current (2011-13) tree canopy cover was evaluated within the borders of 16 cities. Cities that have been infested by EAB for 10+ years have lost an average of 13% of their tree canopy (Table 1). In contrast, cities that have yet to experience EAB gained 22.7% more tree canopy. In sum, EAB-impacted cities lost their potential gain (nearly 23%) plus 13% of what they already had. Thus, the true loss approaches 36%. In addition, these cities lost benefits associated with trees, such as storm runoff mitigation, reduced shade, and pollution reduction (CO_2 sequestration). For example, the eight impacted cities lost nearly $10 million in CO_2 sequestration value. EAB has caused huge losses in tree canopy and associated benefits in several Midwest cities. Cities not yet impacted by EAB need to know that potential tree canopy losses can be extremely high (>35%). Losses in tree benefits can be in the millions of dollars. Losses can be reduced/eliminated through tree protection options, such as trunk injections of Arborjet's TREE-age® Insecticide.

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Table 1: Change in tree canopy cover and benefits over time in US cities with and without emerald ash borer infestation

City	Square miles within city limits	Population (2012)	Number of Observation Points	Percent Tree Canopy Cover (w foliage)		Gross CO^2 Sequestered Value (MM)		Difference	Year EAB first detected
Cities That Have Been Impacted by Emerald Ash Borer						2002-06	2010-13		
				2002	2010				
Detroit, MI	142.8	701,475	601	29.5 (1.9)	23.0 (1.7)	68.0	53.0	-15.0	2002
				-28.26%	**-6.5**				
				2005	2012				
Flint, MI	34.06	100,515	500	31.6 (2.1)	26.8 (2.0)	16.7	14.2	-2.6	2003
				-17.91%	**-4.8**				
				2005	2013				
Lansing, MI	36.7	113,996	601	32.8 (1.9)	31.8 (1.9)	18.8	18.2	-0.6	2003
				-3.14%	**-1.0**				
				2005	2010				
Ann Arbor, MI	28.7	116,121	601	44.3 (2.0)	39.3 (2.0)	19.9	17.7	-2.2	2002
				-12.72%	**-5.0**				
				2004	2010				
Columbus, OH	223.1	809,798	601	30.1 (1.9)	29.0 (1.9)	127.4	122.9	-4.5	2007
				-3.80%	**-1.1**				
				2003	2010				
Toledo, OH	84.1	284,012	601	30.4 (1.9)	27.2 (1.8)	47.5	42.4	-5.1	2004
				-10.50%	**-3.2**				
				2002	2011				
Fort Wayne, IN	79.1	254,555	601	25.1 (1.8)	21.8 (1.7)	51.0	44.3	-6.7	2006
				-13.50%	**-3.3**				
				2005	2011				
South Bend, IN	41.9	100,800	601	24.5 (1.8)	21.5 (1.7)	15.7	13.8	-1.9	2006
				-13.95%	**-3.0**				
				2004	2011				
Mean	83.8	310,159	588	**-13.0%**	**-3.5**	45.6	40.8	-4.8	
Cities Not Yet Impacted by Emerald Ash Borer						2002-06	2012-14		
				2002	2014				
Boston, MA	89.63	636,479	500	22.4 (1.9)	31.1 (2.1)	20.5	28.4	7.9	2014
				38.84%	**8.7**				
				2005	2013				
Rochester, NY	37.1	210,532	601	27.5 (1.8)	33.7 (1.9)	15.8	19.4	3.6	2013
				22.50%	**6.2**				
				2002	2013				
St. Louis. MO	66.2	318,178	601	19.6 (1.6)	20.3 (1.6)	19.1	19.7	0.6	NDY
				3.57%	**0.7**				
				2006	2013				
Des Moines, IA	82.6	206,688	601	25.5 (1.8)	30.3 (1.9)	38.9	46.2	7.3	2013
				18.80%	**4.8**				
				2006	2013				
Boulder, CO	25.7	101,808	601	15.7 (1.5)	24.5 (1.8)	7.6	11.9	4.3	2013
				56.10%	**8.8**				
				2006	2013				
Denver, CO	154.9	634,265	601	9.5 (1.2)	14.3 (1.4)	23.1	34.9	11.8	NDY
				22.50%	**4.9**				
				2005	2012				
Fort Collins, CO	47.1	146,812	601	12.8 (1.4)	15.0 (1.5)	11.1	12.9	1.8	NDY
				17.18%	**2.2**				
				2006	2013				
Salt Lake City, UT	109.1	189,314	601	16.3 (1.5)	16.6 (1.5)	28.1	28.7	0.6	NDY
				1.80%	**0.3**				
				2005	2013				
Mean	76.5	305,510	588	**22.7%**	**4.6**	20.5	25.3	4.7	

NDY = Not detected yet

DEVELOPMENT OF AN ARTIFICIAL DIET FOR ADULT EMERALD ASH BORER (*AGRILUS PLANIPENNIS*)

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ABSTRACT

Adult emerald ash borers (*Agrilus planipennis*) are reared on their preferred host in laboratory settings by harvesting ash leaves in the field during spring and summer months and in the greenhouse in fall and winter. Where greenhouse facilities are unavailable, leaves are imported from ash trees in warmer climates. Our objective was to develop an artificial diet for adult emerald ash borers (EAB), which will in turn support egg and larval parasitoid production in winter months. As a starting point we used cellulose acetate discs coated with sugars to establish sugar preference. Emerald ash borer preferred simple sugars such as fructose, galactose and sucrose, but not glucose. While some feeding did occur, the more complex sugars stachyose and raffinose did not elicit the strong feeding response of simple sugars. The sugar alcohol mannitol, commonly found in ash leaves during summer months, was not a feeding stimulant. Several common protein sources in artificial insect diets (i.e., brewer's yeast, soy flour, soy protein, cannellini bean powder, casein, wheat germ, lactoalbumin) were individually combined with a 2.5% sucrose solution (w/w) and presented to adult EAB on cellulose acetate discs, and feeding was monitored. There was no significant difference among the different proteins, suggesting all the tested protein sources were acceptable to EAB.

While feeding on sugar-coated discs, EAB releases a clear oral secretion onto the surface of the disc. After a few seconds the clear secretion turns brown. It was hypothesized the oral secretion aids in processing the host plant for consumption. Tests revealed the oral secretion contains polyphenol oxidase, likely used to neutralize phenolic defensive compounds produced by the host. In addition, the secretion contains a general protease and alpha-amylase, thought to aid in the digestion of host plant proteins and complex carbohydrates.

Though simple sugars appear to be strong feeding stimulants we also evaluated several extracts of ash leaves for feeding stimulant properties. Solvents ranging in polarity from polar to non-polar were used (i.e., water, methanol, hexane, pentane). Water extract of ash leaves was the only solvent that elicited a feeding response significantly higher than the control.

Our first attempts to create an adult EAB diet were based on the successful Colorado potato beetle diet. The diet, with and without added host material (ash leaf powder) was pressed into thin sheets with embedded card stock and suspended from the top of the cage. Adult beetles lived for only one week. The diet was then simplified and reformulated to incorporate known feeding stimulants such as ash-leaf water extract and simple sugars. Further observations suggested the thickness of the diet may be important for diet acceptance by adult EAB. This hypothesis was tested by presenting EAB adults with four forms of the diet:

diet with an embedded cellulose acetate disc pressed to <1 mm thick, plain diet pressed to <1 mm thick, diet in the form of a cone, and diet in block form. These were tested with leaves serving as the control. After 1 week all beetles on the cones and blocks died, while those on the sheet-like thinner diet and leaves survived, leading us to hypothesize adult EAB have the capacity to feed only on thin, leaf-like sheets. The gape of EAB mandibles was measured for 30 randomly selected individuals and found to rarely exceed 1 mm, suggesting anything thicker than 1 mm would be difficult for the insect to bite. Ash leaves are 0.2−0.3 mm thick, and diet approximately 0.5 mm thick was found to be ideal for feeding by most EAB except the smallest males.

To create thin sheets of adult EAB diet it was pressed between two 30.5 x 30.5 cm sheets of Plexiglas, but consistent thickness throughout the entire diet layer was difficult to obtain. Several alternate methods were attempted with limited success. This included use of a mandolin slicer, a tortilla press, and molds. A thin layer of diet with a reduced amount of gelling agent was also poured between two sheets of Plexiglas separated with a 0.5mm spacer, but none of these methods gave consistent diet thickness of less than 1 mm. EAB were also offered shredded diet, as has been done successfully for other edge feeding insects. Unfortunately, adult EAB would not feed on shredded diet.

To test the diet we pressed it between two panes of Plexiglas and excluded any discs over 1 mm thick. We also incorporated suspected phagostimulants (leaf water extract, simple sugars) into the artificial diet. Diet on cellulose acetate discs < 1 mm thick and 25 mm in diameter were obtained and used in feeding comparisons with leaf discs of a similar diameter. Adult EAB were kept in 118 ml cups with perforated lids at 25^{o}C and 65% RH at a light:dark cycle of 16:8. The diet or leaf section was suspended in the center of the cup on a needle inserted through the side of the cup and replaced daily. Adults on diet lived at a rate comparable to adults on leaves for > 3 weeks. This is the longest period adult EAB were sustained on a diet other than ash leaves. While improvements still remain to be made, the current progress toward an adult diet is encouraging. Future work is planned to evaluate adult survival and fecundity on the diet and to develop methods to obtain consistent diet thickness and simplify diet preparation for eventual use in a mass-production system.

EMERALD ASH BORER AND BIOLOGICAL CONTROL ACTIVITIES IN TENNESSEE: A SOUTHERN PERSPECTIVE

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ABSTRACT

The presence and impact of emerald ash borer, *Agrilis planipennis* (Coleoptera: Buprestidae), on forest and urban trees in the northern United States have been well documented since it was first discovered near Detroit, MI, in 2002. Emerald ash borer was first documented in the southern United States when it was found in Tennessee (Knox County in eastern Tennessee) in the summer of 2010 (27 July). At the time, this documentation represented the southernmost discovery of this invasive insect pest in the United States. Since emerald ash borer was first found in Tennessee, efforts have been underway to assess its distribution and spread, assess potential native natural enemies, and release and establish introduced parasitoids of emerald ash borer. The purpose of this paper is to provide a southern perspective on emerald ash borer and to:

1. Update efforts to assess incidence and distribution of emerald ash borer in Tennessee,
2. Discuss on-going activities to identify potential native natural enemies of emerald ash borer, and
3. Discuss releases and recoveries of introduced parasitoids of emerald ash borer.

Incidence and Distribution of Emerald Ash Borer in Tennessee

Since 2010, various state, federal, and private agencies and organizations (including USDA APHIS, Tennessee Department of Agriculture [TDA], Tennessee Division of Forestry, the University of Tennessee, and Delta-21) have worked together to assess the incidence and distribution of emerald ash borer in Tennessee. Since its initial detection in Tennessee, emerald ash borer has been documented in 28 counties (Fig. 1A), and tree decline and mortality have been observed at several locations. To reduce the further spread of emerald ash borer, the TDA has established quarantines in 39 counties in eastern and middle Tennessee (Fig. 1B).

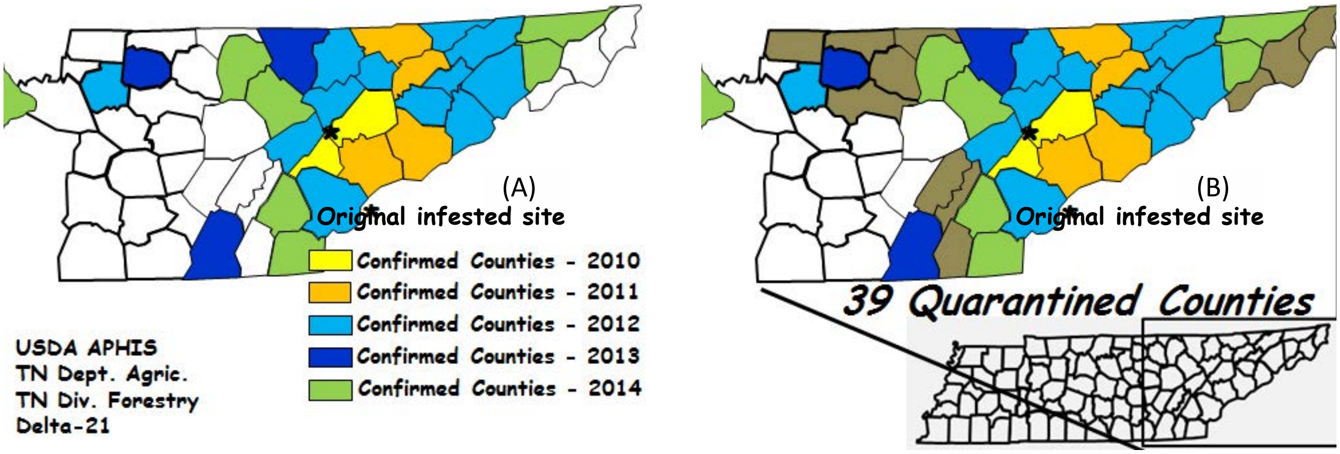

Figure 1. A) Distribution of emerald ash borer in Tennessee, 2010 to 2014, and B) current (October 2014) emerald ash borer quarantined area in Tennessee.

On-going Activities to Identify Potential Native Natural Enemies of Emerald Ash Borer

In northern states, parasitism of emerald ash borer by one native species (*Atanycolus cappaerti* [Braconidae]) averaged ca. 20%. Previous research indicates that some native parasitoids may have the potential to switch to emerald ash borer as a host. Studies were implemented in six counties in Tennessee to assess potential native natural enemies of emerald ash borer in the southern United States (Fig. 2).

During 2013, five species of potential parasitoids of emerald ash borer were recovered from two sites (Cactus Cove and Rowe Road) in two counties (Blount County in southern Tennessee and Claiborne County in northern Tennessee) (Fig. 2). The five parasitoid species are: *Atanycolus cappaerti* (mid to late May), *Spathius floridanus* (late May to mid June), *Spathius* nr. *elegans* (mid August), *Spathius* nr. *parvulus* (early August), and *Spathius* sp. (late July). All specimens of *Spathius* sp. are morphologically similar and are suspected to be the same species. One species, *S. floridanus*, was found at both locations. Specimens from samples collected in 2014 are still being processed and identified.

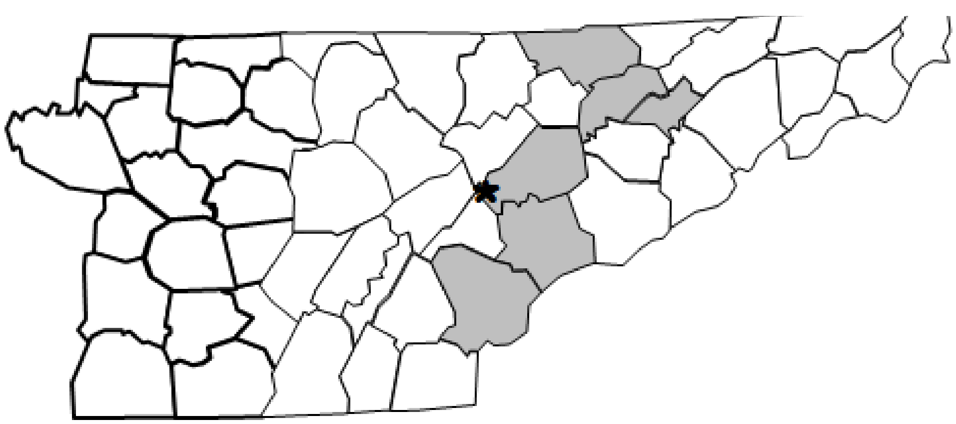

Figure 2. Counties where study sites were established to assess potential native natural enemies of emerald ash borer, as well as release and evaluate introduced parasitoid species.

Releases and Recoveries of Introduced Parasitoids of Emerald Ash Borer

Introduced parasitoid species (160,093 total) were released against emerald ash borer in six counties in eastern Tennessee (Fig. 2). Two larval parasitoid species (*Spathius agrili* [27,641 total] and *Tetrastichus planipennisi* [103,057 total]) were released in 2012, 2013, and 2014. One egg parasitoid species (*Oobiuis agrili* [29,395 total]) was released in 2013 and 2014. The two larval parasitoid species were released at four sites in three counties in 2013, and all three parasitoid species were released at six sites in 2013 and 2014.

Studies are underway to evaluate these three species of introduced parasitoids of emerald ash borer in Tennessee. These ongoing studies include:

1) open releases (trees are cut the year[s] following parasitoid releases and placed in barrels where they are regularly inspected and monitored for parasitoid emergence),
2) large cage releases with natural populations of emerald ash borer (caging trees naturally infested by emerald ash borer; then releasing parasitoids in the cages),
3) large cage releases with enhanced numbers of emerald ash borer (caging trees naturally infested by emerald ash borer and releasing emerald ash borer adults into the cages; then releasing parasitoids in the cages),
4) survival and overwintering of introduced parasitoids on stump sprouts in cages (releasing emerald ash borer adults in the cages, followed by parasitoid releases), and
5) survival and overwintering of introduced parasitoids on balled and burlapped ash trees in cages (releasing emerald ash borer adults in the cages, followed by parasitoid releases).

Thus far, four *Spathius* spp. have been recovered in pan traps from one of the open release sites (Study 1) at the Rowe Road site in Claiborne County. However, none of these species was identified as *S. agrili*.

In 2013, *S. agrili* was recovered from a caged tree in Study 2 (no additional emerald ash borer adults had been released into the cage) in Blount County. The infested tree was caged and parasitoid releases were made within the cages in 2012. *S. agrili* was recovered the following year on 22 May 2013, suggesting successful overwintering of this introduced species in Tennessee. In 2014, successful overwintering of *S. agrili* also was demonstrated in Study 3. In this study, 43 *S. agrili* were recovered from 4 to 19 August 2014 from trees caged the previous year with emerald ash borer adults released within the cages, followed by parasitoid releases in 2013. Recovered adult *S. agrili* represented a 1:1.5 male:female sex ratio.

To date, *T. planipennisi* has not been recovered from emerald ash borer larvae in any studies in Tennessee. The lack of a true diapause in this species may result in early and erratic emergence due to warmer temperatures in southern regions, such as Tennessee, interfering with its ability to overwinter. Studies are underway to further assess the survival, overwintering ability, and establishment of *T. planipennisi* in Tennessee.

Conclusions

Emerald ash borer is well distributed in eastern Tennessee and was recently documented in middle Tennessee (Nashville area), which should enhance outreach efforts to further distribute knowledge about this pest. Biological control is the most promising broad-scale management option for emerald ash borer, and studies on emerald ash borer and its parasitoids in the southern U.S. are at early stages. Continued monitoring of previous release sites, with the incorporation of new releases in 2015, will enable a more thorough evaluation of the success of these three parasitoid species against emerald ash borer in Tennessee. The documentation of five native parasitoid species, including *Atanycolus cappaerti*, *Spathius floridanus*, *Spathius* nr. *elegans*, *Spathius* nr. *parvulus*, and *Spathius* sp., is encouraging, and studies are underway to further assess their role in population dynamics of emerald ash borer. The recovery of one introduced parasitoid species, *S. agrili*, from an open release site and from one of the caged studies is encouraging and demonstrates its ability to survive and overwinter in Tennessee. However, continued long-term monitoring is necessary to determine if it is established and if it contributes to mortality of emerald ash borer populations, as well as recovery of tree health. The potential of *T. planipennisi* to overwinter and establish in Tennessee is uncertain, and concentrated efforts will be made in 2015 to further investigate its role against emerald ash borer in a southern climate. Collectively, these studies illustrate the potential use of large cages to enhance and assess the impact and establishment of *S. agrili* and possibly other introduced parasitoid species of emerald ash borer. These efforts, combined with the efforts of other researchers, will lead to a more thorough understanding of emerald ash borer in a southern climate to enhance integrated management of this invasive pest.

CHARACTERIZATION OF EMERALD ASH BORER (EAB) PUPAL CHAMBER LOCATION IN ASH LOG SECTIONS

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ABSTRACT

In order to develop appropriate phytosanitary measures for sawn ash wood, it is critical that we have a clear understanding of the location of EAB life stages in logs. If life stages are restricted to bark in larger diameter logs (>12") used in sawn wood production, then considerations regarding heat or fumigation required to kill life stages are largely irrelevant. White and green ash bolts ranging from 2" to 20" diameter were stood vertically on a portable bandsaw mill and processed into 1" thick discs. The EAB pupal chamber locations in each disc were measured for distance into the sapwood or bark, with reference to the cambium layer. Of the 9940 discs examined, total number of sapwood chambers was 6189, and the total number of bark chambers was 6879. There were 26 examples of pupal chambers in the sapwood for material > 12" diameter and having bark >1/2" thick, contrary to the finding of an earlier study that found no pupal chambers in the sapwood under those circumstances. Similarly, there were 95 instances where mean bark thickness combined with sapwood chamber depth exceeded 16.5mm, a finding not previously reported in the literature. These data will be used to support the discussion surrounding core regulation for sapwood removal in merchantable (>12") ash logs processed into lumber.

BIOLOGICAL CONTROL OF THE EMERALD ASH BORER: UPDATE 2014

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ABSTRACT

As of October 2014, emerald ash borer (EAB) had a known distribution of 24 states, infesting areas of the northeast and north central United States and Canada. New county records are being added at a consistent pace, and the possibility of eradicating EAB is a distant memory. Since 2003, researchers and program managers have been pursuing classical biological control as a potentially sustainable measure to control the spread and population increase of this invasive pest of ash (*Fraxinus*). Three parasitic wasps were discovered in China between 2003 and 2005; *Spathius agrili* (ectoparasitic gregarious larval parasitoid), *Tetrastichus planipennisi* (endoparasitic gregarious larval parasitoid), and *Oobius agrili* (solitary egg parasitoid). The wasps were imported into quarantine, where they underwent host specificity testing. After a thorough safety review (Federal Register 2007), permits for release were issued in 2007, and the first parasitoids were released in Michigan.

Since the USDA EAB Biocontrol Rearing Facility became operational in 2009, significant improvements in rearing efficiency have also enhanced rearing and release methods (USDA et al. 2013). Initially, trees containing EAB larvae were felled, cut into logs, and the logs were stored throughout the winter. Small ash branches were cut, flaps were created in the bark, and EAB larvae extracted from the logs were inserted in grooves in the branches. This was a cumbersome and time consuming process. The current method involves rearing adult EAB from cut ash logs and maintaining them in plastic containers with ash foliage, where they lay eggs on coffee filters. The EAB eggs are then secured onto small ash bolts with Parafilm, the eggs hatch, and neonates naturally infest the logs and develop to the proper stage for parasitization by the larval parasitoid adults. EAB eggs on coffee filters can also be presented to the egg parasitoid adults, who readily oviposit in these eggs. Ash bolts containing larval parasitoid pupae or coffee filters with egg-parasitoid pupae are then placed in the field where they emerge naturally. This greatly reduces the labor required to collect and ship parasitoid adults. The number of parasitoids reared and released has increased exponentially since 2009, with over half a million parasitoids shipped to state cooperators in 2014. Parasitoids have so far been released in 19 of the 24 infested states.

Through 2013, efforts to recover field-released parasitoids were made in 12 states. Over 640 recovery samples were collected at 88 release sites and 27 control locations (where releases were not conducted). Recovery samples taken soon after release can indicate that the parasitoids are reproducing in the field

(samples taken in the fall of a spring release), overwintering (samples taken in the spring following release), or "establishing" (parasitoids recovered two or more years after the last release). All three species reproduce in the field and overwinter quite well, but only *T. planipennisi* and *O. agrili* are persisting at release sites in the northern United States. *Tetrastichus planipennisi* was found to be established at 65% of the sites sampled two or more years after release, and *O. agrili* was found in 53% of the establishment samples. Both parasitoids were also recovered in control plots over 1 km from the release locations and *T. planipennisi* is frequently found parasitizing EAB at sites not associated with specific release locations. *Spathius agrili*, on the other hand, seemed to overwinter quite well (percentage parasitism was sometimes as high as 45%) but then it was rarely seen again. In fact, *S. agrili* was only found at one release and one control site after two years. Since *S. agrili* seems to overwinter, it does not seem limited by cold but rather it is possible that emergence of one or more of its generations is not synchronized with fourth-instar EAB larvae, its preferred host stage (Gould et al. 2011).

Beyond establishment, it is important to consider the effect that the three parasitoids, in combination with native parasitoids and woodpeckers, are having on EAB population growth (i.e. suppression) (Bauer et al. 2014). Long term life-table studies at six release and six control plots in Michigan are beginning to address this question (Duan et al. 2014). *T. planipennisi* was recovered in both release and control plots one year following release. After four years, over 90% of trees in the release plots and 80 % of trees in the control plots contained at least one brood of *T. planipennisi*. Percentage parasitism increased yearly to an average of 28% in the release plots and 25% in the control plots four years after release (Duan et al. 2013). Parasitism by *O. agrili* also increased yearly to an average of 22% in the release plots and 18% in the control plots by 2012, although this small parasitoid does not spread as quickly as *T. planipennisi* (Abell et al. 2014).

In summary, *T. planipennisi* and *O. agrili* are increasing in density and dispersing well, showing promise in helping suppress EAB densities. Spathius agrili does not persist in the northern United States, possibly due to asynchrony with its host. Climate matching models show that Tianjin China, the native range of S. agrili, is more similar to the central and southern U.S. Because of the lack of establishment, the EAB Biocontrol Program decided to release *S. agrili* only south of the 40th parallel. It is still too early to determine if populations can persist in those environments but sampling is ongoing. That leaves us with a gap in our biocontrol arsenal in the north; *T. planipennisi* has a short ovipositor and does not parasitize EAB larvae in trees more than ~11-cm DBH. Fortunately, *Spathius galinae* was discovered parasitizing EAB in ash trees in the Russian Far East near Vladivostok. This species has a long ovipositor, the climate of its native range is similar to northern regions of the United States, and it has a narrow host range. A petition for field release received unanimous support of the North American Plant Protection Organization in November 2013 and we are expecting a decision on the final petition in 2015.

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DIRECT AND INDIRECT IMPACTS OF EMERALD ASH BORER ON FOREST BIRD COMMUNITIES

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ABSTRACT

Since its discovery near Detroit in 2002, emerald ash borer (EAB, *Agrilus planipennis*) has killed millions of ash trees, billions more are at risk. Because ash trees represent a significant component of Midwestern forests the simultaneous decline and mortality of an entire genus is likely to initiate a cascade of direct and indirect ecological effects. The aim of this study, conducted from May 2011 to April 2013 in 51 forested sites was twofold. (1) To determine the direct impact of EAB infestation on the foraging behavior of bark-gleaning birds. Since EAB larvae feed under the bark, larvae become a novel food resource accessible to sub-cambial excavators (bark-gleaning birds). And (2) to quantify the indirect effects of EAB-induced ash mortality on forest habitat heterogeneity and bird community assemblages. By killing mature trees, invasive insects can disturb habitat structure by initiating growth in the understory (Bell and Whitmore 2000). Heterogeneity and increased complexity of vegetation structure following tree mortality may lead to an influx of bird species adapted to disturbed areas (Matsuoka et al. 2001, Tingley et al. 2002) within invaded forests.

Within sites comprising a gradient of EAB-induced ash decline from southeastern Michigan to southwestern Ohio, we monitored: (1) EAB-induced ash tree decline and bark-gleaning bird foraging activity. The stage of ash tree decline was assessed based on canopy vigor (Smith 2006) and other variables known to be associated with EAB infestation such as presence or absence of woodpecker holes. Foraging activity was monitored during winter months because EAB prepupae are readily accessible to foraging birds during a season when resources are limited. Sites were grouped categorically relating to the stage of EAB infestation. Sites deemed “healthy” were not known to be infested at the time of study, “infested” sites showed obvious symptoms of EAB induced decline and “aftermath” sites are those in which ash mortality was greater than 99 percent. Each year of the study between the months of December and March a meandering search at each of the 51 sites was conducted for bark foraging birds. When a bird was located, and for as long as sight could be maintained, the duration of foraging time was recorded for each tree type (ash, snags, others) the bird chose as a foraging substrate.

In the same sites we assessed (2) habitat variables associated with forest fragmentation and succession as well as abundance of all bird species present. Sites were grouped on a six point scale from “healthy” to “aftermath” based on numerical stage of ash decline modified from Smith 2006 (a ranking of one is assigned to sites where ash trees have a densely foliated canopy and no obvious signs of decline, an aftermath designation is assigned to sites where all ash trees have been dead for five or more years). Three plots separated by at least 150 meters were established at each of the 51 sites. Following forest inventory analysis (FIA) protocols (Schulz 2009), we quantified foliar density in each forest strata (groundcover, shrub, understory, and canopy) in all plots. In order to estimate bird community composition, point counts were conducted at

plot center in each of the three plots per site.

The forage preference of bark-gleaning birds was highly variable depending upon the stage of EAB infestation. Birds responded to forest infestation by increasing the proportion of time spent on ash trees from 15.1% ± 2.10 in healthy sites to 40.55% ± 2.16 in infested sites respectfully. Once the ash trees were dead and EAB were no longer abundant, they were less attractive to birds (19.3% ± 2.7) ($F_{8,1205}=27.59, p<0.001$).

Downy woodpeckers (*Picoides pubescens*) were about twice as abundant in EAB infested forests (1.03 ± 0.09) than in healthy (0.454 ± 0.09) or aftermath forests (0.578 ± 0.12) ($F_{2,50}$=8.45, P=0.001). Red-bellied woodpeckers (*Melanerpes carolinus*) were equally abundant in healthy (0.909 ± 0.07) and infested (0.960 ± 0.08) forests but were less abundant in aftermath forests (0.466 ± 0.08) ($F_{2,50}=8.43, p=0.001$). Hairy woodpecker (*Picoides villosus*) ($F_{2,50}=1.14, p=0.329$), Pileated woodpecker (*Dryocopus pileatus*) ($F_{2,50}=1.11, p=0.339$), and White-breasted nuthatch (*Sitta carolinensis*) ($F_{2,50}=0.33, p=0.718$) abundances were unaffected by EAB's presence in a forest.

Foliar density in the groundcover layer was greater in sites where complete ash mortality was recent (55.88% ± 8.97) than in healthy sites (37.94% ± 7.01). In aftermath sites average foliar density in the groundcover stratum was (49.90% ± 3.06) ($F_{5,45}=3.26,\ p=0.014$). Shrub density also peaked in sites suffering recent mortality (48.51% ± 5.37) ($F_{5,45}=8.07,\ p<0.001$). Though non-significant, there was a trend for understory foliar density to be greatest in aftermath sites (37.07 ± 3.75) and lowest in dead sites (25.04 ± 6.16) ($F_{5,45}=0.82, p=0.540$). As expected for sites where ash trees were dead, foliar density in the canopy was lowest (67.62% ± 4.81 ($F_{5,45}=8.38,\ p<0.001$).

A total of 84 bird species were recorded among 51 sites. Multi-Response Permutation Procedure of point count data showed that there were differences in bird community composition among stages of ash decline ($A=0.150, p<0.001$). Bird community richness ($F_{1,50}=7.00, p=0.011, R\text{-}Sq(adj)=10.71\%$) and Shannon's diversity ($F_{1,50}=6.80, p=0.012, R\text{-}Sq(adj)=10.40\%$) increased with the mean stage of ash decline such that aftermath sites were more diverse and speciose than healthy sites. Evenness did not differ ($F_{1,50}=0.89, p=0.348, R\text{-}Sq(adj)=0.21\%$).

In summary, by studying the inter-stand response by bark-gleaning birds to EAB invasion, we conclude that ash trees in infested sites are a more important forage substrate than ash trees in uninfested sites. We can also conclude that as ash trees decline, they become more attractive to the bark-gleaning guild. Our results suggest that not only does EAB-induced ash mortality create canopy gaps and promote regeneration in sub-canopy forest strata it may indirectly be driving forest bird assemblages because regeneration can provide additional habitat complexity for breeding birds. Single tree gaps may be beneficial to particular species and the perimeter of multi-tree gaps become edge type habitat which can be utilized by an additional cohort of breeding birds that would not otherwise be present in an undisturbed forest.

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POSTERS

BIOLOGY, BEHAVIOR, AND ECOLOGY

BIOLOGY, LIFE HISTORY, AND LABORATORY REARING OF *ATANYCOLUS CAPPAERTI* (HYMENOPTERA: BRACONIDAE), A LARVAL PARASITOID OF THE INVASIVE EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

Atanycolus cappaerti Marsh and Strazanac is a native North American parasitoid that has been found to parasitize the invasive emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, which has killed millions of ash trees since it was first detected in Michigan. A native parasitoid like *A. cappaerti* may be the preferred biocontrol agent as it has already developed a new association with the invasive pest and well adapted to North American habitats. In addition, this native parasitoid has a relatively longer (4 – 5 mm) ovipositor than the introduced larval parasitoids *Tetrastichus planipennisi* and *Spathius agrili*. More importantly, native parasitoids will minimize disruption of an ecosystem as they have already co-evolved with the ecosystem and have less adverse impacts on nontarget hosts. In this study, we investigated the biology, life cycle, rearing, and diapause behavior of *A. cappaerti* in the lab with normal rearing conditions (25 (±2) ºC, with 65 ± 10% RH and 16:8 (L:D) hr photoperiod). Our study shows that *A. cappaerti* can complete its life cycle in less than one month (26.5 days) but may frequently require more under our normal lab rearing conditions. The parasitoid larvae emerges from the egg and quickly molts through 6 instars in approximately six days and begins to spin a cocoon for pupation for which it may enter a diapause. Adult female wasps had a median survival time of 10.5 weeks which was found to be significantly different than males with a survival time of 9 weeks. The female oviposition peaked at 3 weeks old and had a mean of 11.38 (±1.9) progeny. The ratio of diapaused:emerging progeny started at 50:50 from week one and increased in percentage diapaused with each week until all progeny entered facultative diapauses starting at week 11.

The native range of *A. cappaerti* is widely distributed throughout the Midwest and northeast United States which receives temperatures around 25 ºC for many months (June-September) during the EAB growing season. The diapause patterns shown by *A. cappaerti* in this study show that it is likely for many populations to only have a single generation a year, which along with it being a solitary parasitoid may attribute it to being a less successful parasitoid for augmentative biocontrol.

FOREST RESPONSES TO EMERALD ASH BORER INDUCED MORTALITY

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ABSTRACT

Forests are routinely exposed to disturbances (e.g. pests, pathogens, fire, logging, etc.) and nearly all forests are in some stage of recovery. While our understanding of biogeochemical processes and forest successional dynamics to severe disturbances are relatively well known, uncertainty regarding the responses to biological forcing factors like pest outbreaks that cause diffuse mortality are less well known (Flower and Gonzalez-Meler 2015). The accidental importation of emerald ash borer (Agrilus planipennis, EAB) into North America and its selective targeting of ash (*Fraxinus* spp.) provides the opportunity to unravel ecosystem responses to diffuse disturbances. When EAB causes mortality of ash trees, the ash trees stop growing and storing carbon. Other tree species that co-exist with ash trees in forests may increase their growth as the ash trees die because they are no longer experiencing competition from the ash trees. These growth responses of individual trees combine to affect the productivity, biogeochemical cycling, and successional trajectories of forests. For example, under some circumstances the growth response of non-ash trees may offset the cessation of growth of the ash trees, maintaining forest productivity, while under other circumstances, forest productivity may drastically decline. We studied the effect of EAB on growth rates of ash and non-ash trees and used the results to model the effect on forest productivity (Flower et al 2013).

To assess differences in growth rates both between EAB and non-EAB impacted sites as well as among species in EAB impacted sites, we inventoried and measured >1100 ash and >1100 non-ash trees >10 cm DBH annually from long term forest plots across 7 impacted forests (45 plots; 2005-2011) and 5 non-impacted forests (25 plots; 2007-2011) in northern Ohio. Relative growth rates (RGR) of non-ash trees were greater in EAB-impacted forests compared to non-impacted forests (ANOVA; $P=0.014$). Additionally, sites with a greater initial basal area of ash experienced greater non-ash tree growth. This effect was more pronounced in EAB-impacted sites (ANOVA; $P<0.001$), with the slope of the regression ~15% higher in EAB impacted sites than in non-impacted sites. The elevated RGR's of non-ash trees may be due to increased availability of nutrients and light as these trees are released from competition with ash trees. A subsequent analysis of the RGR's from EAB impacted sites indicated that trees from the genera Acer and Ulmus exhibited the highest RGR's following EAB induced mortality. Acer spp. were the most abundant species in these EAB impacted forests and these results highlight the potential replacement of Fraxinus with Acer and its continued dominance.

In order to better understand forest productivity responses to EAB induced tree decline, we used the RGR's derived from the annual monitoring of the permanent plots in the impacted and non-impacted sites to

model annual aboveground biometric forest primary productivity (NPP_B) of the impacted forests. EAB-induced ash mortality reduced the productivity of the impacted forests by ~31% over the course of the study (ANOVA; $P<0.001$). Reduced productivity associated with ash tree mortality accounted for ~145 g C $m^{-2}y^{-1}$, and the compensatory growth of non-ash trees accounted for ~36 g C $m^{-2}y^{-1}$, and was insufficient to offset the loss of ash. The severity of this reduction in aboveground NPP was non-linear and related to the basal area of ash in the stand (Figure 1), highlighting how high diversity forests may maintain productivity during outbreaks of forest pests.

This study describes how forest pests can cause drastic changes to forest ecosystems by altering species composition, successional dynamics and forest biogeochemical cycling (Flower et al. 2013). While forests are somewhat resilient to disturbances when a small percentage of the canopy is impacted, as the proportion of the canopy impacted increases the losses become more severe. Furthermore, the non-linearity of this relationship indicates that there is a threshold beyond which forests exhibit considerable losses in productivity. When the remaining species are comprised of a dominant group, such as maple in the case of these riparian forests, their resilience to other forest pests or pathogens may be reduced. This knowledge can be used to plan restoration to help maintain and enhance future forest resilience.

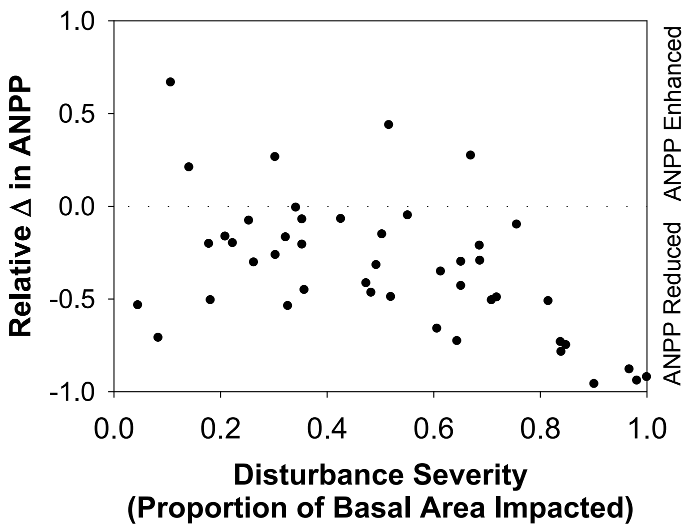

Figure 1: Relationship between disturbance severity and the relative changes in aboveground net primary production (ANPP) associated with EAB induced ash decline in forests of NW Ohio. Dashed line at y=0 differentiates enhanced ANPP from reduced ANPP.

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LIFE TABLE ANALYSIS OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) POPULATIONS IN MARYLAND

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis*, was first detected in Maryland in 2003, but presently still remains restricted to counties west of the Chesapeake Bay. In addition to treatment or removal of EAB infested ash trees, two non-native larval parasitoids (*Spathius agrili* and *Tetrastichus planipennisi*) and one egg parasitoid (*Oobius agrili*) have been introduced to the state since 2009 as part of a biological control program to slow the spread of this pest. In order to evaluate the effectiveness of the larval parasitoids at suppressing EAB populations, and examine the relative importance of other mortality factors (such as host tree resistance and predation) we used a large-scale field experiment and life table analyses to quantify the fates of EAB larvae for two years (2012 and 2013) at recently colonized sites in Maryland.

We selected a total of 12 study sites in western and southern Maryland (with *S. agrili* and *T. planipennisi* being released at eight of the sites), and within each site we identified five green ash trees (*Fraxinus pennsylvanica*) to host experimentally created cohorts of EAB (60 trees used in 2012, but only 50 in 2013 as two sites no longer had sufficient numbers of living ash). In late spring-early summer each year 30 EAB eggs were grafted onto the bark of host trees and covered in cotton balls and tree wrap for protection (see Jennings et al. 2013 for a detailed description of the methods used). Trees were debarked the following spring, and the fates of larvae were assigned to one of six categories: 1) developed to an adult, 2) alive, 3) diseased, 4) killed by tree resistance, 5) parasitized, and 6) depredated.

The overall egg hatching rate for both years was 52.7 ± 0.7%, which enabled us to assess the fates of 1,706 larvae. Most of the larvae we found were alive in both years (~60% of all larvae in this category), and tree resistance was the main source of mortality (~30% of all larvae in this category). Natural enemy predation combined to account for the fates of ~3-6% of all larvae, and we recovered both native and introduced parasitoids. *Spathius agrili* was only recovered one year after being released, but *T. planipennisi* was found in both years and also at two sites over 1.5 km away from where the nearest releases had occurred. In healthy trees and for early instars (L1 and L2), host tree resistance was the most important mortality factor. Conversely, in more stressed trees and for later instars (L3, L4, and overwintering stages), parasitism and predation were the major sources of mortality. However, life tables constructed from the EAB cohorts generated a mean net reproductive rate of 26.1 ± 3.4, showing that EAB populations were still able to grow rapidly at these sites. In conclusion, our results indicated that the fates of EAB larvae were dependent on life stage and host tree crown condition.

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MECHANISMS PROMOTING THE PERSISTENCE OF GREEN ASH IN THE PRESENCE OF THE EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB) has killed millions of ash trees near its introduction point in southeastern Michigan, and the potential for long-term ash persistence is unclear. Several studies have predicted the functional demise of ash where EAB is present because of the destruction of seed sources, but all studies to date have occurred in mixed stands where ash is a poor competitor with other canopy dominants. Ash persistence may be more likely, however, where ash relative density is high, and comprehensive studies that examine the ecological role of surviving trees, seed production and dispersal, and the degree of recruitment limitation are therefore fundamental. I therefore asked: 1) What is the current condition of surviving ash trees and how much viable seed do they produce? 2) Which factors of seed production influence its dispersion? and 3) How much seedling establishment has and will occur with current levels of seed production? Extensive sampling was completed at 17 sites in southeastern Michigan in 2010, 2011, and 2012; 2011 was a mast year for ash in southern Michigan. All stands were characterized by > 95% relative density of green ash that was 40-70 years old, were seasonally flooded between March and May, and were < 1 ha in area. Seed traps were arranged in a 15- m staggered array with 100 m^2 plots established around each plot; traps were checked monthly. All live and dead trees > 2.5 cm DBH were measured and all live trees > 4 cm were cored to determine age. Pre-EAB and post-EAB ash regeneration were tallied at the end of each growing season.

Mean ash density decreased from 1497 to 635 trees/ha due to EAB, and mean basal area decreased from 22.7 to 5.6 m^2/ha. Mean quadratic diameter (Qm) decreased from 11.6 to 8.2 cm. Almost 19% of remaining live trees (which included 55% of all seed trees) showed signs of EAB infestation. About 40- 60% of ash worsened in crown condition between 2010 and 2012. Sprouts are likely an important mechanism of green ash persistence, as they dominated total regeneration (mean = 45%) in every year of the study. Nearly 62% of EAB-killed trees had basal sprouts, and 31% of all current live trees originated from sprouts. Over 90% of sprouts were < 7 years old in 2012, and about 27% of sprouts > 4 cm DBH produced seed during the mast year (2011). Sprouts had a higher growth rate than saplings or trees, which likely provides an important competitive advantage during stand recovery. In addition to sprouts and pre-EAB regeneration, additional ash regeneration appears to be ongoing in these stands, as evidenced by a pulse of post-EAB seedlings occurred after the mast year. Seed production was nearly negligible in non-mast years but otherwise high (= 60 seeds/m^2 pre-EAB); germination trials of these seeds showed 83% viability. Seed dispersion across the stands was high, indicating universally available seed within each stand (= 40% pre-EAB). Seed dispersion was correlated to seed production and source dispersion in non-mast years, and to source dispersion in the mast year. Overall, seed dispersal is not restricted and seed availability is not limiting in these small stands.

Given the abundance of surviving trees and sprouts, their seed production during mast years, and ash advanced regeneration already in place, extirpation of green ash in these small, near-pure stands is not likely, even while EAB persists, although stand structure will differ greatly from pre-EAB stands.

HOST SUITABILITY OF EMERALD ASH BORER HOSTS IN GREEN AND BLUE ASH FOR *TETRASTICHUS PLANIPENNISI*: LABORATORY EXPERIMENT

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ABSTRACT

Within the current North American range of emerald ash borer distribution, blue ash (*Fraxinus quadrangulata*) is the native species of ash tree that is least susceptible and most likely to survive an emerald ash borer (EAB) attack. If natural enemies can parasitize EAB infested blue ash they may be better able to persist and protect regenerating ash trees. The suitability of EAB larvae reared on blue and green ash (*F. pennsylvanica*) bolts was determined for the gregarious endoparasitoid, *Tetrastichus planipennisi*. Ash bolts were infested with EAB eggs and caged in an incubator at 25 C and 14:10 LD. After larvae had developed to an estimated 3rd instar or larger, *T. planipennisi* were introduced to infested bolts. After two weeks these bolts were peeled to examine EAB larvae for the presence of parasitoids. Larvae were held to allow parasitoids to emerge. Parasitism rates, parasitoid abundance per brood, sex ratio, and adult parasitoid female tibia lengths were measured and calculated for each blue and green ash bolt.

The host ash species did not affect any of these performance parameters. These laboratory data suggest that when given the opportunity, *T. planipennisi* can attack EAB larvae on blue ash, and develop as well as on hosts feeding on green ash. Field tests need to be conducted in order to determine if *T. planipennisi* will attack blue ash in the field and establish in blue ash forests that have been infested with EAB.

BIOLOGICAL CONTROL

MOLECULAR APPROACHES FOR DEVELOPMENT OF EAB-RESISTANT BLACK ASH

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ABSTRACT

Black ash (*Fraxinus nigra*) is valued not only for commercial hardwood application such as cabinets, paneling, flooring, and veneer, but also for food and habitat for wildlife. The wood is preferred by Native Americans for making splints for basketry. However, the emerald ash borer (EAB), an exotic wood-boring beetle from Asia, has threatened all *Fraxinus* species growing in North America, and there are no means of complete eradication of the pest. As a long-term alternative, development of transgenic black ash with EAB-resistance is urgently needed. A naturally occurring toxin gene from *Bacillus thuringiensis* (*Bt*) was introduced into the black ash genome through *Agrobacterium*-mediated transformation using hypocotyl explants. Adventitious shoots were regenerated from transformed cells showing kanamycin-resistance, and the presence of the *Bt*-gene was confirmed. However, transgenic trees are not allowed to be routinely planted because of the potential environmental impacts of transgene flow. Reproductive sterility is one of the common strategies for gene containment, and it can be achieved by interfering with flowering control gene using current molecular biotechnologies of genome editing. An *AGAMOUS* homolog in black ash (*FnAG*), a C-class floral organ identity gene responsible for stamens and carpels, was isolated by conducting reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends. The 729 bp coding region of *FnAG* exhibited 93, 77, 75, and 74% identity at the amino acid level to green ash, poplar, black cherry, and Chinese chestnut AGAMOUS, respectively, with highly conserved MADS-domain. Expression of *FnAG* was detected in the reproductive tissues (female and male flowers) and in vitro seedlings, but rarely detected in the vegetative tissue (leaves). Expression was higher in reproductive tissues than in vitro seedlings. A functional analysis of *FnAG* is in the process through ectopic expression of *FnAG* in transgenic *Arabidopsis*.

AVAILABILITY OF SUGAR RESOURCES TO EMERALD ASH BORER PARASITOIDS AT NEW YORK STATE RELEASE SITES

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ABSTRACT

Since 2007, three hymenopteran parasitoids (*Tetrastichus planipennisi*, *Spathius agrili* and *Oobius agrili* have been released in emerald ash borer-infested stands in attempt to control emerald ash borer (EAB) population size and slow *Fraxinus* species mortality. Subsequent monitoring of parasitoid populations has shown variable establishment success and parasitism rates at the release sites. As adults, the parasitoids feed on sugar (Gould et al., 2011) and therefore their population growth and parasitism rates within EAB-infested stands may be limited by availability of mature fruits and floral nectar, extrafloral nectaries, and honeydew produced by leaf- and twig-dwelling homopterans. Our study is part of a larger ongoing study in New York to quantify ash mortality and attempt to retain it as a canopy species. Our research objectives are to (1) quantify potential sugar resources available to adult parasitoids at ten release sites and (2) correlate abundance of sugar resources with observed parasitoid population growth and parasitism rates.

Mature tree (>10cm diameter at breast height, dbh) density and dbh were measured in twelve systematic 200-m^2 plots. Sapling (1.4cm to10cm dbh) density and dbh and shrub species density and diameter at ground level (dgl) were measured in 24 15-m^2 sub-plots. Within our 15-m^2 sub-plots, shrub flower and fruit counts were taken bi-weekly for 18 wks. Herbaceous species flowers and fruits were counted bi-weekly from 48 1-m^2 subplots at each site. We collected canopy flowers with five litter traps per site.

To measure honeydew, we tacked 64-cm^2 filter papers to horizontal wooden boards bi-weekly 0.5 m high on 30 systematic 1-m wooden stakes. We later treated filter papers with methanolic aniline phthalate at 130°C for 10 minutes to reveal sugar drops.

Nearly all species with extrafloral nectaries had leaf extrafloral nectaries. We will use allometric equations to estimate total abundance of extrafloral nectaries using field measurements of number of nectaries per leaf and number of leaves per plant of specific diameters.

The total number of herbaceous flowers differed among sites ($p<0.001$) and weeks ($p = 0.038$). The total number of shrub flowers also differed among sites and weeks ($p<0.001$). Honeydew abundance differed across weeks ($p<0.001$) and was greatest from late June through July. Among the highest three weeks of honeydew production, honeydew differed among sites ($p=0.047$). Although the total number flowers or flowers and fruits differ, certain flowers or sugar resources may be more accessible to parasitoids or may be available at a critical time. Parasitoid yellow pan trap data are still in the process of being collected and analyzed. Once parasitoid data is available, correlations will be constructed.

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INVESTIGATING INDIVIDUAL DISPERSAL CAPABILITIES OF THE EAB PARASITOIDS *Oobius agrili* AND *Tetrastichus planipennisi*

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ABSTRACT

Emerald ash borer (*Agrilus planipennis* Fairmaire, EAB) is a highly-destructive invasive forest pest of ash trees (*Fraxinus* spp.) and is now the target of an extensive national biological control campaign intended to mitigate EAB driven ash mortality. Three species of exotic hymenopteran parasitoids from the native range of EAB in northeast Asia and are currently being released as biological control agents throughout the United States: *Oobius agrili* Zhang & Huang (Hymenoptera: Encyrtidae), an egg parasitoid; *Spathius agrili* Yang (Hymenoptera: Braconidae), a larval ectoparasitoid; and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), a larval endoparasitoid. Following release, monitoring is needed to determine population establishment and dispersal; however, local dispersal remains difficult to investigate and has received relatively little attention due to challenges associated with observing these small parasitoids in the field.

To construct a basic understanding of dispersal distance, direction, and elucidate other potential patterns, new releases of the biological control parasitoids *O. agrili* and *T. planipennisi* were performed during summer 2014 in a forested wetland in Chili, NY. Eight parasitoid release points were selected in areas dominated by green (*F. pennsylvanica*) and black ash (*F. nigra*). Data for ash stems >5.0 cm DBH within a 200 m^2 circular fixed-area plot surrounding each release point were recorded. Releases were performed by placing "oobinators" containing EAB eggs parasitized by *O. agrili* and small ash bolts containing EAB larvae parasitized by *T. planipennisi* in the field and allowing adults to emerge naturally. Dispersal of newly emerged parasitoids was monitored in eight areas separated by at least 50 m using yellow pan traps (YPTs) placed on ash trees in rings with a radius of 10 m (n = 4) or 20 m (n = 4) with parasitoid release points as the center of each ring. Eight YPTs were distributed around the circumference of each 10 m ring and 16 YPTs were distributed around the circumference of each 20 m ring. Samples were collected from YPTs weekly for a period of five weeks for both species of parasitoid.

Once all YPT monitoring had ended, parasitized EAB eggs and ash bolts with parasitized larvae used for releases were dissected to determine parasitoid emergence. For all release points combined, 3,008 *O. agrili* and 1,056 *T. planipennisi* are estimated to have successfully emerged over the five week period. Of these, no *O. agrili* were recovered from YPTs in 10 m replicates and four *O. agrili* individuals were recovered from YPTs in 20 m replicates. Four *T. planipennisi* individuals were recovered from YPTs in 10 m replicates and seven *T. planipennisi* were recovered from 20 m replicates. Multiple individuals of the same species were never recov-

ered from the same YPT, nor did the same YPT recover individuals of both species.

Results demonstrate both biological control parasitoids were able to disperse the distances investigated during this study and are likely able to exert parasitism pressure over a large geographic area within a relatively short period of time. This is an important finding for *O. agrili*, which due to its minute size (~0.95 mm) is often assumed to have limited dispersal capabilities and so was thought to remain on or near the tree of origin after release/emergence.

Combined recoveries of both species demonstrated a tendency for parasitoids to disperse in a northern or northeastern trajectory away from release points. Given the sheltered nature of the release points, it is considered unlikely that prevailing wind was the most important factor determining dispersal direction. YPTs placed on trees to the north and northeast of release points were positioned on the southern and southwestern faces of ash trees that receive greater sunlight and which may have had greater numbers of EAB eggs and larvae. Therefore, it is suggested parasitoids might have spent more time foraging in these locations and increased exposure time to YPTs.

Ash basal area within each fixed-area plots was positively correlated with total parasitoid recoveries from YPTs in the associated replicate, although ash stem density was not. Further attention should be given to stand characteristics at release sites, as the relationship between ash basal area and total parasitoid recovery shows that ash tree component likely plays an important role in determining the degree of dispersal/retention of parasitoids and likelihood of recovery. It is also suggested that checking YPTs over a shorter time frame than weekly may provide more insights into dispersal distances. Relationships such as this might have further implications for overall establishment success and subsequent levels of EAB parasitism achieved at release sites.

AN IMPROVED METHOD TO ESTIMATE EMERALD ASH BORER EGG PARASITISM BY THE INTRODUCED PARASITOID *OOBIUS AGRILI*

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ABSTRACT

Classical biological control of emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) in North America began with releases of three EAB parasitoids from China into stands of EAB-infested ash in Michigan. The EAB-biocontrol agents are an egg parasitoid *Oobius agrili* (Encrytidae) and two larval parasitoids: *Tetrastichus planipennisi* (Eulophidae) and *Spathius agrili* (Braconidae) (Bauer et al. 2014). In 2008, we began monitoring the outcome of these releases annually where the sustained establishment and spread of *O. agrili* and *T. planipennisi* have been confirmed. Each release site in our study is comprised of a parasitoid-release plot and a paired non-release control plot separated by one km or more. Over the years, these sites have been used: 1) to develop and optimize parasitoid-recovery methods and 2) to evaluate stage-specific factors regulating EAB population dynamics (Duan et al. 2013; Abell et al. 2014).

Estimating EAB-egg parasitism requires collecting EAB eggs from the bark of ash trees. Not only are these eggs small (~1 mm in diameter) and cryptically colored, but they are laid between layers and in crevices of ash bark, making their recovery difficult. Initially, we estimated EAB-egg parasitism by sampling eggs from ash trees in the field using a timed, visual-search method. In 2011, we developed another sampling method -- bark-sifting, and compared it to the visual-search method for two field seasons. The bark-sifting method involves the removal of an area of bark from ash trees in the field using a draw knife. The bark is then returned to the laboratory, dried, sifted, and the eggs sorted from debris in a white ceramic tray under a dissecting microscope.

Oobius agrili adults were released from 2007 to 2009 at three study sites, and egg parasitism was estimated from 2008 to 2010 using the visual methodology, exclusively. Three additional *O. agrili* release sites were added from 2009 to 2010, and in 2011, we began using both sampling methods to estimate egg parasitism at all six paired study sites. Ten EAB-infested ash trees were sampled per plot each year.

Using the visual-search method at release plots from 2008-2012, we found average egg parasitism increased from 0.7% to 10.6% and percentage of ash trees with *O. agrili* increased from 3.3% to 18.3% (Table 1). In 2011 at the release plots, the bark-sifting method resulted in 21.8% egg parasitism on 34.6% of ash trees compared to the visual-search method that showed only 2.3% egg parasitism on 7.6% of ash trees. Similar

results were observed in 2012. Results for the control plots were lower and more variable as *O. agrili* spread from release plots, but were generally higher for the bark-sifting than for the visual-search method (Abell et al. 2014). Overall, bark sifting provides a more sensitive and consistent method of sampling EAB eggs, independent of sampler capabilities and experience, bark texture and color, and weather and lighting conditions in the field.

Table 1. Comparison of the visual-search vs. the bark-sifting methods for egg-parasitism estimates at six Michigan study sites for the 2011 and 2012 field seasons (Abell et al. 2014).

Year	Plot type	% *O. agrili* parasitized eggs[1]		% ash trees with *O. agrili*	
		Visual-search	Bark-sifting	Visual-search	Bark-sifting
2008	release	0.7	n/a	3.3	n/a
	control	0	n/a	0	n/a
2009	release	4.2	n/a	10.0	n/a
	control	0	n/a	0	n/a
2010	release	2.4	n/a	10.0	n/a
	control	0	n/a	0	n/a
2011[2]	release	2.3[A]	21.8[B]	7.6[A]	34.6[B]
	control	0.4[A]	3.3[A]	3.2[A]	12.5[A]
2012[2]	release	10.6[a]	18.9[a]	18.3[a]	25.0[a]
	control	8.6[a]	4.3[a]	7.1[a]	10.7[a]

[1]Likelihood chi-square tests based on a logistical regression model were used to compare the visual-search vs. bark-sifting methods for each treatment and year separately.

[2]Letters indicate significant differences between methods and treatments analyzed separately in 2011 (upper case letters) and 2012 (lower case letters).

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PARASITOID RELEASES AGAINST EMERALD ASH BORER IN NEW YORK STATE

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ABSTRACT

Parasitoid releases in New York have been ongoing at multiple sites since 2011, two years after the discovery of emerald ash borer in the state. All three parasitoid species were released in southwestern NY (in and around Randolph NY) and in the Hudson River Valley. We are evaluating multiple forest stands yearly for parasitoid establishment and ash health . We are also monitoring seedling and sapling status to determine if ash will remain as a component in aftermath forests. Parasitoids have also been released along the Genessee Greenway (south of Rochester) where we will document parasitoid dispersal and establishment along a linear forest corridor. In related research, we are testing methods for more efficient quantification and assessment of parasitoid populations. We work closely with multiple state agencies on these projects and assist with coordination of systematic releases.

MORTALITY FACTORS AFFECTING EMERALD ASH BORER POPULATIONS IN CHINA, WITH NEW RECORDS OF PARASITISM BY TWO SPECIES OF BEETLES

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ABSTRACT

In order to further explore potential natural enemies of the emerald ash borer and their role in regulating the pest population dynamics, we conducted field surveys at multiple forest sites with variable host densities in the pest's native ranges (north and northeast China) during 2007-2013. Our field surveys revealed a complex of natural enemies (eight species of hymenoptera parasitoids and two species of coleopteran beetles) attacking immature stages of EAB. Besides hymenopteran and coleopteran parasitoids, immature stages of EAB egg and larvae were also attacked by predators (e.g., woodpeckers on larvae), and some undetermined biotic factors such as diseases, competition and/or putative plant resistance. Findings from our surveys also indicated that the abundance of the natural enemy (parasitoid) complex as well as its impact on EAB mortality (parasitism) varied with different sampling times and geographic areas in north and northeast China, the EAB's native range. For example, the egg parasitoid *Oobius agrili* and the larval parasitoid *Tetrastichus planipennisi* were not observed in Tianjin, but these two species were frequently observed attacking EAB eggs or larvae in Beijing, Jilin, Liaoning and Heilongjiang. On the other hand, *Spathius agrili* is a major parasitoid of EAB in north China, especially in Tianjin, but not in the northeast areas (Jilin, Liaoning and Heilongjiang). In addition, we discovered two parasitic beetles *Tenerus lewisi* and *Xenoglena quadrisignata* attacking EAB larvae or pupae in one of the northeast provinces (Liaoning), one of them (*T. lewisi*) being a dominant parasitoid of overwintering EAB larvae or pupae there. Together, our findings suggest that classical biocontrol introduction of EAB natural enemies (parasitoids) from the pest's native range to the newly invaded region (e.g. U.S. and Canada) needs to consider the spatial and temporal variations as well as dominance in natural enemy complex and the EAB's native range. Further studies are needed to determine whether the two species of parasitic beetles can be potentially used for classical biocontrol introduction against EAB in North America.

MATING STATUS DIFFERENTIALLY AFFECTS ATTRACTION OF *SPATHIUS AGRILI* MALES AND FEMALES TO MALE-PRODUCED "AGGREGATION-SEX PHEROMONE"

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ABSTRACT

Male and female *Spathius agrili* Yang were tested for attraction to the synthetic male pheromone. Lures consisting of a 3-component pheromone blend were placed in the center of a white filter paper target used to activate upwind flight in the wind tunnel. When virgin males and females were tested for attraction, both sexes were attracted to the lure prior to mating. However, only males were attracted to the pheromone lures after mating. In another experiment, of females that flew to the lure as virgins, half were subsequently mated and the other half were not, and mated females were no longer attracted. Then both mated and virgin females were provided with host material (emerald ash borer larvae in sticks of ash) to determine if oviposition affected attraction. They were supplied with fresh hosts, *ad libidum*, and subsequently tested for attraction for 50 d, and results showed that oviposition did not affect attraction to the pheromone. Additionally, it was found that mating status did not affect oviposition rate. The key factor in attraction to the pheromone by females was mating status. Because this pheromone is released by one sex and is attractive to both sexes for the purpose of mating, it qualifies as an "aggregation-sex pheromone".

CHEMICAL AND MICROBIAL CONTROL

WHEN IS IT TOO LATE? RECOVERY OF ASH TREES TREATED WITH EMAMECTIN BENZOATE

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ABSTRACT

Many private landowners and municipal foresters are using systemic insecticides to protect high value ash trees from emerald ash borer (EAB). To be effective, trees must be healthy enough to transport insecticide from the base of the trunk to leaves and branches in the canopy. Feeding by EAB larvae, however, disrupts the vascular system of trees. As larval densities build and damage accumulates, the ability of trees to translocate insecticide to the canopy is diminished. Canopies typically become thin, i.e., transparent, as trees are increasingly stressed by larval feeding. Large branches may also be girdled and die.

Often, arborists must determine whether treatment is likely to be successful in trees that are already infested. Numerous field studies have shown the insecticide product sold as TREE-äge™, with the active ingredient emamectin benzoate, provides nearly 100% control of EAB for 2 to 3 years. Given this level of efficacy, it seemed likely that trees with low or perhaps moderate canopy decline might recover if treated with TREE-äge. At some level of damage, however, trees unable to successfully transport the insecticide to the canopy will succumb.

To assess the ability of trees treated with TREE-äge to recover from EAB injury, we selected pairs of ash trees with varying levels of EAB damage in 2009. One tree of each pair matched for damage-level was randomly assigned to be treated with the TREE-äge™ insecticide, while the other tree was left untreated. Trees treated with TREE-äge™ in 2009 were subsequently retreated in 2011 and 2013. Treated and untreated trees were evaluated annually from 2009 to 2014 to monitor survival and assess canopy condition of live trees.

Methods: In May 2009, we selected 11 pairs of green ash (*Fraxinus pennsylvanica*) trees in two sites in lower Michigan (22 trees total). Paired trees were similar in size and canopy condition, and were growing within 10 m of each other. Size of trees ranged from 15.2 to 35.1 cm and average (± SE) DBH was 23.9 ± 1.0 cm. Five pairs of trees were in an unmanaged portion of a recreation area in Genesee Co. and six pairs were in a wooded area of a state park in Clinton Co. Both sites were dominated by green ash trees and EAB populations in 2009 were very high.

In June 2009, experienced evaluators visually estimated percent canopy transparency and canopy dieback (10% classes) of each tree, along with other symptoms of EAB infestation. Trees were categorized as relatively healthy, moderately injured, or severely injured, with 3 to 5 pairs of trees per category. Relatively healthy trees had less than 30% canopy decline, trees with 30-60% canopy decline were considered to be moderately injured, and trees with >60% canopy decline were considered to be severely injured. For each

pair, the tree randomly assigned to the insecticide treatment was trunk-injected with TREE-äge™ at the lowest label rate (4%; 0.1 g. AI per DBH inch) using the ArborJet QUIK-Jet™ system. Trees were treated on 16 June 2009, then retreated on 20 May 2011 and 18 June 2013 at the same rate.

We evaluated canopy condition of treated and control trees on 2 Sept 2009, 29 June 2010, 22 July 2011, 9 August 2012, 18 July 2013, and 2 July 2013. Variables recorded included canopy transparency (10% = nearly full canopy; 90% = few leaves) and canopy dieback (10% = little damage; 90% = nearly dead). Epicormic sprouts and holes left by woodpeckers preying on late instar EAB larvae were recorded separately on each tree as absent, few (1-5) or many (>5).

Results: By August 2012, all untreated control trees were dead, including trees that appeared healthy in June 2009. All trees treated with TREE-äge™ in alternate years survived if canopy transparency estimated in 2009 was less than 60%. A severe summer drought in 2012 affected the surviving trees, but all recovered by 2013. Branches that were killed by EAB remain dead; dieback estimates in 2014 ranged from 0 to 40%. However, there was little evidence of transparency in 2014; live branches had ample foliage.

Trees that had already sustained substantial damage from EAB larvae and had >60% decline in 2009 were not protected by the TREE-äge™ treatment. One tree died by late August 2009 and the others were dead by 2012. However, while the main leader and branches of these heavily injured trees died despite the TREE-äge™ injection, at least one epicormic sprout remained alive. These sprouts have continued to grow; some are now 10 cm in diameter and producing new shoots on the lower portion of their trunk. In a genetic and a functional sense, these trees remain alive and, assuming they can be protected over time, should continue to grow and recover. In contrast, none of the control trees had any shoots or epicormic sprouts that remained alive; these trees are truly dead.

Overall, results indicate the importance of treating valuable landscape ash trees before serious canopy decline (>60% transparency) is apparent. Trees with low or moderate levels of canopy transparency in 2009 (<60%) were protected by the TREE-äge™ and are recovering from previous EAB damage. As of 2014, surviving trees are laying new wood over old EAB galleries and appear to be recovering from previous injuries.

HOST INTERACTIONS

HOST SPECIES MATTERS: INTERACTIONS BETWEEN EMERALD ASH BORER AND FIVE *FRAXINUS* SPECIES

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ABSTRACT

All North American *Fraxinus* (ash) species assessed to date can be colonized by *Agrilus planipennis* Fairmaire, but interspecific differences in *A. planipennis* host preference and host resistance have been documented. Among North American ash species, black (*F. nigra* Marsh.) and green ash (*F. pennsylvanica* Marsh.) are highly preferred by *A. planipennis* while white ash (*F. americana* L.) is considered intermediate. Blue ash (*F. quadrangulata* Michx.) is relatively resistant to *A. planipennis* compared to other North American ash species. Although interspecific differences in *A. planipennis* host preference are apparent, it is not clear whether these differences reflect adult selection of feeding and oviposition sites, the ability of larvae to feed and develop, or both.

In April 2006, a common garden consisting of 21 trees of each of four North American (black, blue, green and white ash) and one Asian species (Manchurian ash) was established at the Michigan State University Tree Research Center. In July 2010 and 2011, we assessed adult host suitability (leaf consumption) and adult beetle mortality using excised (beetles were caged with leaves in a Petri dish) or intact (beetles were caged on leaves still attached to trees) leaf bioassays.

Despite our efforts to protect trees with physical barriers, wild populations of *A. planipennis* were so high by August 2010, that 14 of the 21 black ash trees and 15 of the 21 green ash trees were dead. In September 2011, all trees were harvested and carefully peeled with a draw knife to assess larval density. We recorded: number of exit holes, larval condition (dead or alive), larval stage (early instars = first, second and third instars; late instars = fourth instars + prepupae) and number of woodpecker and parasitoid attacks. Larval density was standardized per m^2 of exposed phloem. Larvae were progeny of wild *A. planipennis* adults.

In 2010, survival was highest for beetles caged with excised black ash leaves (53%), intermediate for beetles on excised green (30%), white (32%), or Manchurian ash (30%) leaves and lowest for beetles caged with excised blue ash leaves (15%). Leaf area consumed per beetle day was higher for beetles caged on excised white and Manchurian ash foliage than the other three species and lowest for beetles caged on blue ash foliage.

In 2011, survival was higher for beetles caged on white (72%) or Manchurian ash (80%) than for beetles caged on blue ash (33%). Leaf area consumed per beetle day was lowest for beetles caged with excised blue ash leaves and higher for beetles caged with intact blue or white ash leaves than for beetles caged with excised leaves of the same species. Leaf area consumed was not different between beetles caged with excised or intact Manchurian ash foliage.

Larval gallery density was highest on black (mean ± SE = 235.9 ± 36.4 per m2) and green ash trees (220.1 ± 39.8), intermediate on white ash trees (40.7 ± 11.6) and lowest on blue (2.0 ± 0.9) and Manchurian (1.5 ± 0.7) ash trees. Among the 21 trees of each species, all black and green ash trees had larval galleries while 15 blue ash, five white ash and 13 Manchurian ash trees had zero larval galleries. Among the 4,135 *A. planipennis* larval galleries assessed, 13% produced an adult *A. planipennis*, 24% contained live larvae (8% of these were early instars, 92% were late instars), 24 and 29% were killed by woodpeckers or parasitoids, respectively and 10% contained dead larvae (61% of these were early instars, 39% were late instars).

SUSCEPTIBILITY OF SELECTED ASIAN, EUROPEAN, AND NORTH AMERICAN ASH SPECIES TO EMERALD ASH BORER: PRELIMINARY RESULTS OF NO-CHOICE BIOASSAYS

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ABSTRACT

Many species of *Fraxinus* have not been adequately evaluated for their susceptibility to emerald ash borer (EAB). We assessed the susceptibility of several North American, European and Asian ash species to EAB under controlled laboratory conditions in no-choice bioassays using bolts collected from the limbs of non-infested ash trees growing at the Arnold Arboretum of Harvard University in eastern Massachusetts. Arnold Arboretum holds a collection of ash species numbering 175 individual trees across 100 accessions, comprising approximately 20 species. Asian ash species, which co-evolved with EAB, are presumably relatively resistant to this pest. Several species from North America and Europe, however, have yet not been encountered by EAB. In this study, we focused on the relative suitability of various *Fraxinus* species for EAB larval feeding and development.

In 2013-2014, two laboratory trials were conducted at Michigan State University (MSU) using small bolts collected from 19 different ash species. Branches of 2-3 individual ash trees from selected North American, European and Asian species at the Arnold Arboretum were collected in 2013, first in June (Trial 1) and next in July (Trial 2). For Trial 1, samples included North American green ash (*F. pennsylvanica*), white ash (*F. americana*), pumpkin ash (*F. tomentosa* [syn. *F. profunda*]), and Texas ash (*F. texensis*); European species included Manna ash (*F. ornus*), narrow-leafed ash (*F. angustifolia ssp. oxycarpa*), Pallis' ash (*F. pallisae*), and European ash (*F. excelsior*); while Asian species included Chinese ash (*F. chinensis*), Manchurian ash (*F. mandshurica*), and Japanese ash (*F. longicuspis*). For Trial 2, North American species included green ash, white ash, black ash (*F. nigra*), blue ash (*F. quadrangulata*), Oregon ash (*F. latifolia*), and velvet ash (*F. velutina*); Asian species included Korean ash (*F. chinensis var. rhynchophylla*), Chinese flowering ash (*F. sieboldiana*), Japanese flowering ash (*F. lanuginosa*), and Chinese red ash (*F. platypoda*). Cut ends of the branches were sealed after cutting to reduce desiccation and delivered to MSU within 48 hours for further processing.

At MSU, 12 sections from the branches of each ash species (n = 132 sections in Trial 1; n = 120 sections in Trial 2) were cut to 14 cm lengths. One cut end of each section was waxed to prevent drying. Approximately 100 eggs collected from laboratory-reared EAB were placed on each section and wrapped with parafilm. Sections were placed in trays of water and held in growth chambers to allow eggs to hatch and EAB larvae to feed, following rearing methods developed by Jonathan Lelito, formerly of USDA APHIS. After 3 to 4 months, sections were moved to cold storage for an additional 4 months, then returned to growth chambers

to allow larvae to complete development, pupate and emerge as adults. Sections from Trial 1 and Trial 2 were debarked and survivorship of EAB larvae was assessed in late February and mid-April 2014, respectively.

Results of our no-choice bioassays indicate that EAB larvae could feed and develop on all North American, European and Asian ash species included in our trials. Overall, average (± SEM) survivorship of EAB tended to be greater on North American ash species (63.3 ± 7.2%) compared to ash species native to Europe (51.4 ± 17.0%) or Asia (26.6 ± 10.5%). The percentage of EAB larvae that developed to prepupal or adult stages, however, was highly variable among ash species, including species of similar continental origin. For instance, survivorship of EAB reared on European species ranged from 10.5 to 79.5% (European ash and narrow-leafed ash, respectively) and 0 to 80.0% on Asian species (Japanese ash and Chinese ash, respectively). North American species exhibited similar variability, ranging from a high of 93.2% survivorship on green ash in Trial 1 to a low of 25.7% survivorship on velvet ash in Trial 2. Whether this variability represents actual differences in susceptibility among ash species or merely an artifact of using cut sections is unknown.

While all ash species included in our trials appear to be potential hosts for EAB, survivorship values should be interpreted with caution. Some differences, for example, could reflect varying rates of desiccation among ash species that subsequently affected larval feeding and development. Furthermore, larvae feeding on cut sections would not encounter any inducible defenses that could affect development rates or survival. Further research is needed to evaluate relative susceptibility of live trees of these ash species to EAB colonization and reproductive success.

LINGERING ASH: POPULATION DYNAMICS AND THE SEARCH FOR RESISTANCE TO EMERALD ASH BORER

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ABSTRACT

Rare natural resistance or tolerance to exotic pests and pathogens has been found in many tree species, including American elm (*Ulmus americana*) tolerant to Dutch elm disease and American beech (*Fagus grandifolia*) with resistance to beech scale insects. After emerald ash borer (*Agrilus planipennis*) (EAB) has swept through natural populations of ash (*Fraxinus spp.*), it is possible that some lingering (surviving healthy individuals after the initial mortality wave) ash trees may exhibit rare natural resistance or tolerance to this exotic beetle. Herein we summarize progress on the four goals of the field component of our lingering ash research:

1. Understand ash and EAB population dynamics to provide context for understanding ash survival in natural populations
2. Monitor lingering ash populations to determine factors that predict long-term survival
3. Locate additional lingering ash trees of each ash species
4. Identify the most promising trees for propagation for the EAB Resistance Breeding Program

The rapid time course of ash tree mortality and the peak and crash of EAB populations has been documented in monitoring plots throughout Ohio for nine years. Less than 1% of the original population of mature ash trees remained after the initial wave of ash mortality. In 2010, two years after EAB had caused >95% mortality of ash trees at Oak Openings Metropark in northwest Ohio, the ash trees along the seven mile stretch of Swan Creek floodplain within the metropark was surveyed. The complete survey of surviving ash >10cm DBH revealed 284 surviving ash trees of which 108 were healthy from an original population of >11,000 ash trees. These lingering ash trees were scattered among dead ash trees and were therefore unlikely to have simply been missed by EAB (figure 1). The surviving trees were tagged and re-surveyed in 2011, 2012, 2013, and 2014. Initial canopy health

and absence of evidence of woodpecker feeding activity were the best predictors of subsequent survival. From 2010 to 2013, 40% of the initially healthy trees remained healthy while nearly all of the severely declining trees died. All lingering ash trees exhibited some evidence of past EAB infestation; EAB is persisting at low densities and may be continuing to attack these trees.

Figure 1. A healthy lingering green ash tree (left) beside a dead ash tree (right) that was killed by EAB at Oak Openings Metropark in northwest Ohio.

Thus far, nearly all of the lingering ash trees identified have been green ash (*F. pennsylvanica*) or white ash (*F. americana*). There is a need to identify and test lingering black ash (*F. nigra*) for tolerance or resistance to EAB because it is both an ecologically and culturally important species. To this end, we are using models of black ash habitat in long-infested areas of southeast Michigan for targeted helicopter surveys to detect lingering black ash. For 13 counties in southeast Michigan, we used multiple regression trees to model black ash occurrence (from previous monitoring plots and FIA data in the focal area) with habitat, climate, and soil variables as predictors. The model identified 5 km^2 as likely habitat for black ash in these areas, and those areas will be targeted in helicopter surveys.

We launched a new website http://www.nrs.fs.fed.us/SurvivorAsh for reporting lingering ash trees in October 2014. The target area is restricted to counties in southeast Michigan and northwest Ohio and will be expanded over time. Submissions of healthy, naturally-occurring (not planted cultivars) ash trees >10 in DBH that have not been treated with insecticides are encouraged.

The healthiest, largest lingering ash trees identified are being propagated and tested for resistance or tolerance to EAB as part of the EAB Resistance Breeding Program (Mason et al., this proceedings). As of October 2014, twelve of the lingering ash trees from Oak Openings have been successfully propagated and replicated, and nine have been tested in EAB resistance assays. As more genotypes are tested, the information on the performance of the lingering ash trees in the field context of ash and EAB dynamics will be compared with results from resistance assays to understand how resistance traits may lead to long-term survival of ash trees in infested sites.

REGULATORY, MANAGEMENT AND OUTREACH

PRESERVING ASH REGENERATION AND STABLE AGE STRUCTURE BY PROTECTING MATURE ASH DURING THE EMERALD ASH BORER INVASION

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ABSTRACT

Wide-scale ash mortality by emerald ash borer (EAB) (*Agrilus planipennis*) has stopped ash reproduction and new regeneration in southeast Michigan (invasion epicenter), creating an unstable population structure that threatens the persistence of ash in this area. If the same trend occurs as EAB continues to spread, it could threaten to functionally extirpate susceptible species range-wide, creating a need to preserve the ash gene pool. Systemic insecticides are highly effective at protecting individual ash trees from EAB (McCullough et al., 2011; Smitley et al., 2010). Furthermore, a simulation model concluded that annual insecticide treatments of clusters of ash with emamectin benzoate in an urban forest may provide nearly complete protection of ash populations in an urban forest over a 10 year period (McCullough and Mercader, 2012). This suggests that treating a subset of trees could also provide a strategy for conserving ash in a natural forest.

In an attempt to maintain ash regeneration and conserve the ash gene pool, the Five River MetroParks (FRMP) in Dayton, OH, initiated an ongoing program in 2011 to protect 600 mature (reproductive) ash trees with emamectin benzoate every 2 years. This offered a unique opportunity to quantify the effects of treating clusters of ash trees of various sizes across a large-scale, natural forest experiencing a gradient of EAB-induced decline and mortality. The objectives of our study are to determine treatment effects on survival of mature trees, saplings, and seedlings; seed production and seedling germination; and genetic variation in each size cohort. We hypothesize that treating larger proportions of ash trees will maintain a larger mating population, thus preserving reproduction, new regeneration, and stable population structures (i.e., Type III survivorship curve with > 50% of the ash stand composed of new seedlings) than treating smaller clusters of trees. We also hypothesize that the insecticide treatment will provide associational protection to untreated trees, and that this effect will diminish with distance from the cluster of treated trees.

In 2014, we established 24-one hectare quadrats (100 m x 100 m) across gradients of treatment intensity and EAB-induced ash decline. Treatment intensity was identified as high (average of 12% ash trees treated), medium (average of 8% ash treated), low (average of 3% ash treated), or untreated (0% ash treated). EAB impact was categorized as severe (>65% ash mortality), moderate (15-65% ash mortality), or low (<15% ash mortality). Within each quadrat, we assessed canopy decline on a scale of 1-5 (1 = full canopy, 2-4 = progressive degrees of canopy thinning, 5 = dead, open canopy; Smith, 2006) of treated and untreated ash trees. We also quantified densities of new (first year seedlings with cotyledons) and established (<1.5 m tall, without cotyledons) seedlings, and ratio of new to established seedlings (population structure) to determine the effects of both EAB impact (EAB-induced ash decline) and treatment intensity on new ash regeneration and population structure.

Overall, treated ash trees were healthier (lower canopy rating) than untreated ash, suggesting insecticides are directly protecting treated ash trees. Across the EAB-induced ash decline gradient (low, moderate, and severe), there was an increase in ash canopy decline of untreated ash trees as the severity of EAB increased (2.6 ± 0.1, 3.2 ± 0.1, and 4.5 ± 0.1; respectively, $p<0.0001$), but no difference in the treated ash (1.6 ± 0.2, 1.7 ± 0.1, and 1.4 ± 0.1; respectively). However, ash canopy decline was not affected by treatment intensity (high, medium, low, and untreated) for either untreated ash (3.1 ± 0.1, 4.1 ± 0.1, 3.3 ± 0.1, and 3.1 ± 0.1; respectively) or treated ash (1.5 ± 0.1, 1.5 ± 0.1, 1.9 ± 0.3, and N/A; respectively).

As the severity of the EAB-induced ash decline increased, new seedling density decreased ($p<0.003$), but established seedling density did not change. Furthermore, severely impacted quadrats had mostly established seedlings, causing the ratio of new to established seedlings to fall below one, which reflects an unstable population structure. However, we found no effect on seedling density (both new and established seedlings) or population structure as treatment intensity increased.

We will continue to monitor ash demography over time. We anticipate that as mortality of untreated trees increase and treated trees become an increasingly larger proportion of the surviving ash population, the treatment effects will intensify and the spatial relationship between treated trees and density of ash seedlings will strengthen.

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THE INVASIVE SPECIES CANNONBALL RUN: A CASE STUDY OF FIREWOOD MOVEMENT TO THE NEW HAMPSHIRE MOTOR SPEEDWAY

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ABSTRACT

Firewood transportation has been identified as a significant vector for dispersing invasive forest insects, such as emerald ash borer (EAB) and Asian longhorned beetle (ALB), much greater distances than they could disperse through natural spread. Several factors exacerbate the risk of moving pests in firewood compared to other wood products. For instance, minimal processing and incremental burning increase the likelihood of firewood harboring live insects which may emerge over time to initiate new infestations. Seasoning firewood may reduce the risk in some cases, but is not effective against certain life stages of wood-boring insects. The firewood movement pathway includes wood harvested for both commercial and recreational purposes, which makes it a challenge to effectively regulate. While commercial firewood enterprises can be identified and targeted for education on how to safely move their product, people moving firewood for camping and recreation are poorly reached through regulatory and outreach efforts.

In July 2011, New Hampshire implemented an out-of-state firewood quarantine, generally prohibiting the importation of any tree material intended for use as fuel for fires, to reduce the risks to its forest resources through firewood movement. The New Hampshire Motor Speedway (NHMS) is a popular camping and recreational destination for people from across North America, making it a prime candidate for firewood outreach activities. Moreover, NHMS hosts the two largest camping activities in the state – the days leading up to the July and September NASCAR races – with approximately 75% of its 5,000 campsites occupied by out-of-state race fans. In cooperation with other state and federal partners and NHMS, New Hampshire's Forest Rangers coordinated outreach to NASCAR fans regarding the risks of moving out-of-state firewood. Outreach has included messaging on the NHMS website, displays at NHMS fan days, brochures in mailings and grab bags, and surveys of fans on their firewood transportation habits.

Firewood quarantine enforcement activities at NHMS were developed and implemented prior to the two NASCAR races in 2013 and before the July race in 2014. Enforcement activities were conducted from 9 a.m. to 10 p.m. Wed.-Fri. preceding each race, when most campers arrive. The NHMS provided space at the entrance so NH Forest Rangers could identify violators and issue notices of violation and summonses upon entry. Cooperating state and federal partners assisted the Rangers in confiscation of firewood, documentation of violations and, most importantly, providing outreach to violators about invasive pests of concern and the risks associated with moving firewood long distances. Confiscated firewood was examined and burned at a nearby transfer station to mitigate risk. Vouchers for local heat-treated firewood were provided as available.

The firewood quarantine enforcement at NHMS resulted in 225 confiscations of out-of-state firewood over 9 days. Confiscated firewood included both green and seasoned wood, with volumes ranging from only a few sticks to full truckloads per violation. Most intercepted firewood (80%) originated in neighboring northeastern states and was transported 49 to 200 miles to NHMS (primarily from Maine, Massachusetts and Vermont). As expected, the number of firewood confiscations from out-of-state decreased with increasing distance from NHMS, with 12% being transported 201-300 mi, 6% transported 301-400 mi, and 2% transported 401-700 mi. This reduction in confiscations with distance may be due to the geographic draw of NHMS rather than to a change in the willingness to transport firewood based on distance. For instance, transcontinental firewood movement from as far as Florida, California and Washington were also observed. Additionally, confiscated firewood originating from the Canadian provinces of Quebec and Prince Edward Island was also found during this enforcement activity in spite of Customs and Border Protection inspections. No firewood originating from known ALB-infested areas was intercepted, although 2% of confiscated firewood originated from towns adjacent to ALB quarantine areas in Massachusetts and New York. Fifteen percent of confiscated firewood originated in areas with known EAB infestations.

The targeted firewood quarantine enforcement activities conducted at NHMS are a model of an effective outreach partnership between private industry and state and federal government. These enforcement activities would not have been possible without the support and cooperation of NHMS and results have provided resource managers and policymakers considerable information on the prevalence of transportation of recreational firewood into New Hampshire. Ongoing and continued outreach efforts about the risks to forests posed by the transportation of firewood are necessary to effect long-term change in the habits of attendees at large recreational camping events. Although expensive, providing firewood vouchers lessens the inconvenience associated with confiscation. Future firewood quarantine enforcement and outreach activities at large recreational events should consider including parallel enforcement at private campgrounds in proximity to the event, conducting additional outreach throughout the event grounds rather than solely on the entry road, and collaborating with local firewood vendors so out-of-state attendees may be readily aware of locally-sourced and affordable firewood at or near the event.

IMPROVED UNDERSTANDING OF *FRAXINUS NIGRA* BIOLOGY AND ECOLOGY USED TO PREDICT HIGH-QUALITY LOCATIONS: AN APPROACH TO PRIORITIZING PREPAREDNESS AND MANAGEMENT OF EMERALD ASH BORER IN NORTHERN NEW ENGLAND

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ABSTRACT

All ash tree species in North America are currently threatened by the invasion of the emerald ash borer (*Agrilus planipennis*; EAB). One threatened species, black ash (*Fraxinus nigra*), is of particular importance both ecologically and socio-economically (Benedict L. 2010; Benedict, M. 2001; Benedict and Frelich 2008; Palik et al. 2011) and requires further attention and protection if it is to subsist in the Northeastern United States.

Black ash is commonly found in moist lowland sites and is typically thought of as a wetland species. While there is not a large population of black ash throughout the eastern United States, Native American tribes have used the species for centuries to weave baskets, which are a key component in the tribes' histories, cultures, and economies. Black ash also fills a particular ecological niche; it can colonize wetland sites and is one of the most drought-tolerant species of *Fraxinus* (Percival et al. 2006). To maintain black ash on the landscape, it is essential to identify and map high-quality black ash sites so the species may be better monitored and protected from environmental stressors, such as the impending arrival of EAB in Maine and northern New York.

This study investigated the characteristics associated with high-quality sites, which are defined as sites where black ash (1) can regenerate successfully; (2) have wide increments of annual growth at DBH (greater than 2mm for 10 consecutive years); and (3) show little to no decline in annual growth levels over time. Characteristics significantly correlated with high-quality sites were then used to develop a comprehensive spatial model predicting high-quality sites. The model was applied to 24 sites in Maine where quality was already known; the model accurately predicted site-quality on 83.33% of sites.

To strengthen model validation, the model will be applied to an additional 15 sites in northern New York and 10 additional sites in Maine. Once model validation is complete, the model can serve as a tool for resource managers to help monitor and protect black ash, as well as local basket-tree harvesters adapting to ash mortality and scarcity in the face of the emerald ash borer.

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FACTORS INFLUENCING THE SPREAD OF EMERALD ASH BORER IN AN URBAN FOREST: A CASE STUDY IN SYRACUSE, NEW YORK

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ABSTRACT

In 2009, the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) was detected in Randolph, New York and has since been detected throughout the state. During delimitation of the Randolph infestation, hyper-infested trees (*Fraxinus* spp.) were identified from extensive woodpecker foraging and within tree larval densities. These trees were termed "mother-trees" and could act as source or superspreader trees, infesting a disproportionately high number of surrounding trees. The phenomenon of superspreader trees has not been extensively explored in invasive ecology and may be a factor influencing the spread of EAB. Understanding emerging infestations of EAB in NY and the role of superspreader trees could provide valuable data for developing effective strategies for long-term management of EAB infestations.

In 2013, EAB was detected in Syracuse, NY, presenting an opportunity to study the distribution and dispersal of an emerging EAB infestation and to determine the presence and possible influence of superspreader trees in an urban forest. We used two assumptions to detect superspreader trees: 1) trees had a significantly higher number of exit holes, presence of woodpecker foraging, and within tree larval densities when compared to surrounding infested trees and 2) trees likely had been infested for a short period of time. Dendrochronological data and within tree larval densities were collected from felled ash throughout the Syracuse area infestation to test our definition of superspreader trees. Stand surveys were conducted in observably infested areas (species, DBH, health rankings, and land use type were recorded for each 100 x 4 m transect plot) and a delimitation study was conducted to determine the extent and distribution of the infested area. A metric (score 1-17; 17 indicating tree killed by EAB) was developed using visible signs of an infestation to determine the health ranking of infested trees. Data was imported into ArcMap 10.1 (ESRI Redlands, CA) to determine the spatial relationship of data points.

Surveys and trapping in the Syracuse infestation revealed the infested area to be ~175 km^2 in size and included portions of the city of Syracuse and surrounding towns and villages. Spatial analysis of data collected from stand surveys and sampled trees suggested the infestation likely originated near the 90 Thruway. Surveys (n = 65) of infested sites across different urban land use areas (e.g. greenspaces and right-of-ways) suggested 60% of ash surveyed were infested and 40% of woodlot ash surveyed were infested. The delimitation study suggested EAB was spreading away from urban areas and into old fields stocked with ash. Fifteen street trees were sampled from across the infested area and showed moderate signs of infestation based on the metric we developed (mean score of 9). Dendrochronological data suggested the Syracuse area had been infested for five to seven years. Sampled infested trees indicated a mean larval density of 38 (±24)

larvae/m^2 of phloem. Two trees with moderate signs of infestation were heavily infested with a mean larval density of 75 and 74 larvae/m^2 phloem and were infested for three and two years, respectively. These two trees represented the best examples of superspreader trees detected to date. One of the sampled tree was the only ash tree located on a street and the other tree was surrounded by several other ash trees, all exhibiting minor to moderate symptoms of infestation.

No trees sampled to date have met our definition of a true superspreader tree. Recently detected heavily infested clusters of trees near the edge of the infestation may provide the best data for identifying superspreader trees and the role they may play in the dispersal of an urban infestation.

SURVEY AND TRAPPING

EVALUATION OF TWO TRAP DESIGNS IN STANDS WITH VARYING LEVELS OF WHITE ASH MORTALITY

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ABSTRACT

Emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) has become the most destructive forest insect to ever invade North America, killing hundreds of millions of ash (*Fraxinus* spp.) trees and threatening more than 8 billion trees in U.S. forests (Poland and McCullough 2006). Catastrophic levels of white ash (*Fraxinus americana* L.), green ash (*F. pennsylvanica* Marsh.) and black ash (*F. nigra* Marsh.) mortality caused by EAB have been recorded in plots established in southeast Michigan and Ohio (Burr and McCullough 2014, Flower et al. 2013, Klooster et al. 2014, Knight et al. 2014). We have observed, however, that an unexpectedly high proportion of overstory white ash trees remain alive in some sites in southeast Michigan, despite the presence of EAB for more than six years. Documenting site or stand level conditions associated with relatively high or low rates of white ash survival would be valuable, particularly for forest managers.

One aspect of our project involved monitoring the relative abundance of EAB in sites with low, high and intermediate white ash survival rates. In 2014, we set up two double-decker (DD) traps in each of 30 sites located in southeast and central lower Michigan. Each DD trap consists of two coroplast prisms (36 cm × 60 cm panels) zip-tied to a 3 m tall PVC pipe (10 cm diam) that slides over a t-post. One of the DD trap designs consisted of two "standard" dark purple prisms baited with two *cis*-3-hexenol bubble cap lures (release rate of 3.7 mg/d for a combined release rate of 7.4 mg/d; Contech Enterprises Inc.) on the upper prism (3.0 m high) and a Manuka oil pouch lure (release rate of 50 mg/d; Synergy Semiochemicals, Corp.) on the lower prism (1.8 m high). The other DD trap design consisted of a dark green prism on top, while the lower prism was a light shade of purple (Francese et al. 2013). Two *cis*-3-hexenol lures were attached to the top prism and two more were suspended from the lower prism. Surfaces of each prism were coated with clear Pestick™ to capture beetles. Lures were replaced mid-season to ensure sufficient volatile release throughout the entire season. Beetles were collected at 2-3 week intervals and identified to species and sex in the laboratory.

A fixed radius plot (18 m radius) was established around each DD trap at each site. Species, DBH and canopy condition of overstory trees (>6 cm) were recorded. Proportion of white ash trees that were alive and dead were determined overall and by DBH class for each site. Total area of white ash phloem and proportion of the phloem that remained alive were calculated following methods of McCullough and Siegert (2007).

A total of 580 adult EAB were captured in the 30 sites between June and September 2014. More beetles

were captured on the dark green-light purple DD traps baited with *cis*-3-hexenol on both panels than on the standard purple-purple DD traps baited with Manuka oil and *cis*-3-hexenol. Captures were heavily male-biased throughout the summer, regardless of trap type; overall, 79% of the captured beetles were male. Adult EAB were captured in all sites, including areas where less than 10% of the white ash trees remained alive. The linear relationship between the proportion of white ash phloem alive and the total number of EAB captured in each site was significant ($P < 0.05$), but explained relatively little variation ($R^2 = 0.011$). In 2015, we plan to continue to evaluate EAB abundance, more intensively assess condition of overstory white ash and regeneration, and evaluate soil, land use, or vegetation data that may be associated with varying levels of white ash survival in these sites.

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EFFECTS OF TRAP DESIGN, COLOR, AND LURE ON EMERALD ASH BORER ATTRACTION

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ABSTRACT

A number of promising trap designs incorporating attractive olfactory and visual cues have been developed to detect and monitor populations of emerald ash borer (EAB, *Agrilus planipennis* Fairmaire). EAB is attracted to volatiles emitted by stressed ash trees including the green leaf volatile, *cis*-3-hexenol (de Groot et al. 2009), and bark sesquiterpenes found in Manuka oil (Crook et al. 2008). They are also attracted to particular shades of green and purple (Francese et al. 2010, Crook et al. 2012). We conducted three trap-comparison experiments from June to August 2014. The first experiment compared different colored double-decker traps consisting of two three-sided prisms (60 cm tall × 40 cm wide on each side), made of corrugated plastic, mounted to the top and 120 cm from the top of a 2.4 m tall, 10 cm diameter PVC pipe slid over a T-post that was driven into the ground. Traps were baited with two *cis*-3-hexenol bubble caps (hex) (7.4 mg/day, Contech Enterprises, Inc., Delta, B.C.) on both the top and bottom prisms (hex-hex) or two *cis*-3-hexenol bubble caps on the top prism and one Manuka oil pouch (man) (50 mg/day, Synergy Semiochemical, Burnaby, B.C.) on the bottom prism (hex-man). Colors tested included dark purple, and a lighter shade of purple and dark green that are more attractive to EAB (Francese et al. 2010, Crook et al. 2102). There were 10 replicates of 5 treatments at a site with very low EAB population density: 1) dark purple top and bottom prisms baited with hex-hex; 2) dark purple top and bottom prisms baited with hex-man; 3) dark green top and light purple bottom prisms baited with hex-hex; 4) dark green top and light purple bottom prisms baited with hex-man; and 5) light purple top and bottom prisms baited with hex-hex. Preliminary results indicated that traps with dark green top prisms and light purple bottom prisms captured significantly more EAB than traps with dark purple prisms on the top and bottom, regardless of lure. Traps with light purple top and bottom prisms captured an intermediate number of EAB. For traps of the same color, there was no significant difference in attraction of EAB to the hex-hex lures versus the hex-man lures. The detection rate (i.e., percentage of traps that captured at least one EAB) for traps with dark green tops and light purple bottoms was 90% for traps baited with hex-hex, and 100% for traps baited with hex-man. Traps with dark purple top and bottom prisms had the lowest detection rates (60% and 70% for traps baited with hex-hex or hex-man, respectively). The detection rate of traps with light purple top and bottom prisms that were baited with hex-hex was 80%.

The second experiment compared several different trap designs. Five replicates were set up at each of two sites, one with moderate and one with very low EAB population density. Treatments included: 1) double-decker trap with dark purple top and bottom prisms baited with hex-man; 2) double-decker traps with dark green top prism and light purple bottom prism baited with hex-hex; 3) dark green prism trap baited with hex and hung in the ash canopy; 4) dark green funnel trap coated with Fluon, baited with -hex and hung in the ash canopy; and 5) dark green modified boll weevil traps baited with hex and hung in the ash canopy. Standard boll weevil traps were modified by replacing the bottom portion with a 40-cm long green

cylinder. At the site with very low EAB population density, significantly more EAB were captured in the double decker traps of either color than in the boll weevil traps which did not capture any EAB. The green prism traps and green funnel traps captured an intermediate number of EAB. All of the dark green and light purple double-decker traps captured at least one EAB, 80% of the dark purple double-decker traps, 60% of green canopy prism traps and 40% of green funnel traps captured at least one EAB. At the site with moderate EAB population density, 20% of the boll weevil traps captured a few EAB. For all of the other trap types, 100% of the traps captured EAB. Significantly more EAB were captured in the green funnel traps than on the dark purple double-decker traps or boll weevil traps and captures on green prism or dark green and light purple double-decker traps were intermediate.

The third experiment was designed to determine if pre-glued prism traps were as effective at entrapping and retaining EAB as prisms with a thicker application of insect-trapping glue. It also compared *cis*-3-hexenol released from two bubble caps at 7.4 mg/day (ConTech Enterprises, Delta, B.C., Canada) on each prism versus one pouch releasing 50 mg/day on each prism (Scentry, Billings, Montana, USA). All traps were double-decker traps with dark green prism on the top and light purple prism on the bottom. The experiment was set up as a 2 x 2 factorial design with 10 replicates of 4 treatments: 1) pre-glued trap with bubble caps; 2) pre-glued trap with pouches; 3) trap with glue added with bubble caps; and 4) trap with glue added with pouches. Captured EAB were tallied and marked each week with different colored pins but were not removed from the traps. EAB that had fallen off, as indicated by colored pins with no EAB beside them, were also tallied each week. Traps with glue added tended to capture slightly more EAB than pre-glued traps. Furthermore, significantly more EAB fell off traps that were pre-glued compared to traps with glue added. Overall, approximately half of the captured beetles fell off the pre-glued traps within 1 to 2 weeks. There was no difference in number of EAB captured or the number that fell off traps baited with bubble caps compared to traps baited with pouches.

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