Fall 2014

Suitability of blue ash (fraxinus quadrangulata) and green ash (f. pennsylvanica) to emerald ash borer (agrilus planipennis) and its larval parasitoid tetrastichus planipennisi.

Donnie "L. " Peterson
Purdue University

Recommended Citation

Peterson, Donnie "L. ", "Suitability of blue ash (fraxinus quadrangulata) and green ash (f. pennsylvanica) to emerald ash borer (agrilus planipennis) and its larval parasitoid tetrastichus planipennisi." (2014). *Open Access Theses*. 459.
https://docs.lib.purdue.edu/open_access_theses/459

Graduate School Form 30
(Revised 08/14)

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By Donnie L Peterson

Entitled
Suitability of Blue Ash (Fraxinus quadrangulata) and Green Ash (F. pennsylvanica) to Emerald Ash Borer (Agrilus planipennis) and its Larval Parasitoid Tetrastichus planipennisi.

For the degree of Master of Science

Is approved by the final examining committee:

Clifford Sadof	Sadof
Steve	Yaninek
Matthew	Ginzel

To the best of my knowledge and as understood by the student in the Thesis/Dissertation Agreement, Publication Delay, and Certification/Disclaimer (Graduate School Form 32), this thesis/dissertation adheres to the provisions of Purdue University's "Policy on Integrity in Research" and the use of copyrighted material.

Approved by Major Professor(s): Clifford Sadof
Steve Yaninek

Approved by: Steve Yaninek 11/14/2014
Head of the Department Graduate Program Date

SUITABILITY OF BLUE ASH (*FRAXINUS QUADRANGULATA*) AND GREEN ASH (*F. PENNSYLVANICA*) TO EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) AND ITS LARVAL PARASITOID *TETRASTICHUS PLANIPENNISI*.

A Thesis
Submitted to the Faculty
of
Purdue University
by
Donnie L. Peterson

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

December 2014
Purdue University
West Lafayette, Indiana

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

Peterson, Donnie L. M.S., Purdue University, December 2014. Suitability of Blue Ash (*Fraxinus quadrangulata*) and Green Ash (*F. pennsylvanica*) to Emerald Ash Borer (*Agrilus planipennis*) and its Larval Parasitoid *Tetrastichus planipennisi*. Major Professors: Clifford Sadof and Steve Yaninek.

Emerald ash borer (EAB) (*Agrilus planipennis*) is a primary pest that has killed tens of millions of North American ash (*Fraxinus* spp.) trees. The larval parasitoid *Tetrastichus planipennisi* was introduced from China as part of a classical biological control program for long-term EAB management. The high mortality rates of ash trees greatly reduce the number of EAB hosts and may make it difficult for parasitoids to persist. However, blue ash (*F. quadrangulata*) is relatively resistant and appears to be able to survive EAB infestation. If natural enemies can attack EAB in infested blue ash they may be better able to persist and protect regenerating ash trees. I compared the capacity of EAB larvae and its larval parasitoid to survive and develop in blue ash and the more susceptible green ash (*F. pennsylvanica*). Blue and green ash trees were infested with EAB eggs in the field and laboratory and their bark was peeled to determine larval survivorship and developmental stages. A subset of blue and green ash was exposed to *T. planipennisi* so that parasitism rates, brood sizes, sex ratios, and adult sizes could be determined. EAB larvae survivorship was high (>96%) and mortality due to wound periderm formation was low (<4%) on green and blue ash in the field. However, EAB larvae developed slower in blue ash than in green ash in the field and laboratory. In contrast, *T. planipennisi* brood sizes, sex ratios, and adult female sizes had no significant difference between ash species suggesting that hosts have no apparent affects. In the field, *T. planipennisi* was able to attack and develop on EAB larvae in caged blue and green ash trees. As such, if *T. planipennisi* can find blue ash trees, then they should readily attack and survive on living EAB. When joined with higher blue ash survival, it is possible that this ash species could serve as refuge for EAB and its parasitoids after susceptible ash are killed by this pest. Thus, blue ash presence may improve the capacity of parasitoids to parasitize, persist, and protect more susceptible ash species in forests.

CHAPTER 1. BIOLOGY, BIOLOGICAL CONTROL, AND HOST PLANT RESISTANCE OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*)

1.1 Emerald ash borer, a tree killer:

The emerald ash borer (EAB), *Agrilus planipennis* is a primary pest of North American ash trees with the potential to change North American forests. It was first discovered in 2002 as the cause of a massive number of tree deaths in Michigan, US and Ontario, Canada (Haack et al. 2002). Since its arrival in North America from China on infested wood packing material during the early- to mid-1990s (Siegert et al. 2008, 2014; Cappaert et al. 2005), tens of millions of ash trees have died (Poland and McCullough 2006) and potentially eight billion ash trees are threatened (Mercader et al. 2009). EAB has the potential to cost tens of billions of dollars for treating, removing, and replacing ash trees in urban areas over a 10 year period (Kovacs et al. 2010). In addition, declining and dead trees pose a hazard and have the potential to injure or kill people (Beering and Scott 2010).

Besides the economic costs, there are also ecological costs from the loss of ash trees. Many vertebrates use ash trees. White-tailed deer and moose use ash trees for protection, browse, and thermal cover. The bark of seedlings and saplings can provide food for beaver, rabbits and porcupines (Heyd 2005). Ash loss could endanger 43 monophagous native arthropods and harm an additional 30 arthropods which use ash trees as one of their hosts (Gandhi and Herms 2010a). The loss of ash can cause the formation of canopy gaps, increase coarse woody debris, alter biogeochemical cycling, and many ecological interactions among organisms (Gandhi and Herms 2010b). Recent reports of EAB attacking the white fringetree (*Chionanthus virginicus*) in Ohio suggests that this pests host range may expand as its geographic range encompasses a wider range of potential host species.

1.2 Life history of EAB:

In the spring, “J-shaped larvae” become prepupae and shed their cuticles. The pupae develop into adults and emerge from the ash tree at 230-260 C° DD_{10} (Brown-Rytlewski and Wilson 2004) by chewing through wood and leaving their diagnostic D-shaped exit holes. Adults feed on ash tree leaves for five to seven days before mating. Females feed for five to seven more days after mating and lay 50-90 eggs (Poland and McCullough 2006). Females lay eggs in the cracks and crevices of the bark of ash trees. Eggs develop into larvae that emerge in two weeks. Larvae chew their way through the outer bark into the phloem where they feed and create S-shaped galleries. They feed and develop until October or November at which time EAB larvae burrow into the sapwood of the ash tree and overwinter (Cappaert et al. 2005). EAB develops through four instars, when 4th instars mature they bore into the sapwood, create a pupal chamber, and overwinter as “J-shaped” larvae. Individuals that do not reach the J-shape stage overwinter in the phloem layer and need an additional year to feed and develop to complete a generation. EAB development can range between one to two years. In regions with shorter summers, it may take EAB two years to develop to adults, while in warmer climates they need only one year (Wei et al. 2007). Development of EAB from eggs to adults in resistant species of ash can also take longer due to slowed development (Chakraborty et al. 2014).

1.3 Management of EAB:

Soon after its detection in the US, federal and state agencies tried to contain the incipient Michigan and Ontario EAB infestations and eradicate early satellite populations (Cappaert et al. 2005, Herms et al. 2004). Eradication was stopped after this effort was judged to be a failure because of the discovery of new satellite populations in many new states. Since its accidental introduction in the early- to mid-1990s (Siegert et al. 2014) near Detroit, MI, EAB has spread into 24 U.S. states (Cooperative Emerald Ash Borer Project 2014) and two provinces in Canada (Canadian Food Inspection Agency 2013). Nationally, management efforts have focused on reducing the spread of EAB by restricting the movement of infested ash wood. EAB can fly up to 7 kilometers per day (Taylor et al. 2005, 2010), but can be moved hundreds or thousands of kilometers per year by humans. To manage for anthropomorphic spread, laws have been implemented to regulate the transportation and sale of ash trees and logs from quarantined counties (Cappaert et al. 2005). Other management efforts have focused on reducing EAB populations and slowing ash mortality.

The SLow Ash Mortality (SLAM) program, a collaboration of state and federal agencies and universities, is designed to slow down the rate of ash decline by reducing EAB population growth. In the first step, sanitation, infested ash trees are removed before adults emerge to reduce the abundance of *A. planipennis*. This effort is followed by girdling selected ash trees that attract ovipositing females who prefer them over healthy ash trees (McCullough et al. 2009a). Harvesting and destroying these trap trees before adult emergence, kills all overwintering EAB. Another version of trap trees, lethal trap trees, can also be used to kill EAB adults and larvae by treating ash trees with an insecticide (McCullough et al. 2009), such as emamectin benzoate, and then girdling after a few weeks. This method can be used best in areas that are difficult to access. The lethal trap tree can be left standing as long as it doesn't threaten harm to humans or property (SLAM 2014). In simulations, insecticide and girdling treatments reduced radial spread and larval consumption of phloem (Mercader et al. 2011).

Systemic insecticides can effectively protect ash trees from EAB in urban settings (Herms et al. 2014). These chemicals can be applied via soil or injections, and lower trunk sprays in the mid- to late spring. EAB can be when adult beetles feed on the leaves of treated ash trees and ingest a lethal toxin (insecticide). Alternatively, newly hatched larvae that emerge from eggs can be killed when they feed on the phloem of treated trees. The capacity of insecticides to kill EAB in the urban landscape provides opportunities to develop area wide strategies that can reduce the rate of EAB spread (Mercader et al. 2011).

Classical biological control is being implemented to suppress EAB populations by re-establishing natural enemy/prey relationships found in this pest's native range. In 2003, an exploratory survey was conducted in China for natural enemies where EAB populations were low (Liu et al. 2003). Three parasitoids were found, imported, tested for host range, and released in the US (Liu et al. 2003, Bauer and Liu 2007, Gould et al. 2007, 2013). A fourth larval parasitoid, *Spathius galinae* has recently been found (Belokobylskij et al. 2012) and is under study for release (Yang et al. 2012).

1.4 *Fraxinus* resistance to EAB:

In China, native Asian ash appear to be less likely to be infested by EAB compared to North American ash trees because recent outbreaks of EAB have been on North American ash (Liu et al. 2003). For example, Manchurian ash (*Fraxinus mandshurica*) and Oriental ash (*F. rhynchophylla*), both Asian ash trees, have lower levels of infestations of beetles in Eastern Russia in comparison to North America's green ash (*F. pennsylvanica*) (Duan et al. 2012b). Specifically in the Khabarovsk area of Russia, green ash had higher mean densities of EAB than artificially stressed Manchurian ash, 11.3-76.7 and 2.2 /m^2 respectively. In the Valdivostok area of Russia, green ash also had higher densities of EAB than Oriental ash, 77-245 EAB/m^2 and 10-59 EAB/m^2 respectively. In a second study, Korean ash (*F. chinensis var. rhynchophylla*), a native to Asia, appears to be more resistant to EAB because it was not infested, but 95% of green ash trees found nearby were infested (Liu et al. 2007).

Since EABs introduction to the US, numerous studies provide evidence that North American ash trees are highly susceptible to EAB (Anulewicz et al. 2006, 2007, Pureswaran and Poland 2009, Chen and Poland 2010, Rebek et al. 2008). For example, both green ash (92%-100%) and white ash (*F. americana*) (75%) had high rates of mortality from EAB compared to Manchurian ash (*F. mandschurica*) (<20%) in a common garden study (Rebek et al. 2008). There appears to be a hierarchy of EAB oviposition and feeding preference of native ash trees with green ash>white ash>black ash (*F. nigra*)>blue ash (*F. quadrangulata*) (Anulewicz et al. 2006, 2007, Pureswaran and Poland 2009, Chen and Poland 2010, Tanis and McCullough 2012, Carson 2013, Rigsby et al. 2014). Mechanisms of resistance are not well understood and are the subject of ongoing research.

Even among susceptible North American ash species, e.g. *F. pennsylvanica*, about 10-17% of EAB die during larval development (Duan et al 2010). Most research has focused on defenses within the phloem, particularly on phenolics and defensive proteins (Eyles et al. 2007, Cipollini et al. 2011, Whitehill et al. 2011, 2012, Chakraborty et al. 2014). Eyles et al. (2007) and Cipollini et al. (2011) have compared Manchurian ash phloem chemistry to green and white ash trees and found several unique or higher concentrations of phenolic components that may contribute to its higher level of resistance. Interestingly, blue ash has been demonstrated to have a unique secondary metabolite profile (Whitehill et al. 2012). These studies investigated constitutive defenses. When induced defenses were analyzed in trees artificially infested with EAB, Manchurian and black ash had an increase in defensive compounds (Chakraborty et al. 2014). Specifically, Manchurian ash had much higher levels of the phenolics, pinoresinol A and B compared to black ash. Chakraborty et al. (2014) hypothesized that these phenolics may contribute to the higher levels of resistance in Manchurian ash. In general, it has been demonstrated that Asian ash are more resistant than North American ash; however, one North American ash tree appears to have some resistance to EAB - blue ash.

Blue ash appears to be the most resistant and least preferred North American ash as a host for EAB (Anulewicz et al. 2007, 2008, Pureswaran and Poland 2009, Tanis and McCullough 2012). Blue ash is an uncommon species with square twigs that typically grows in limestone outcrops (Sibley 2009). Phylogenetically, blue ash is distantly related to other North American ash species that EAB has encountered in the US (Wallander

2008). Specifically, blue ash is in the Dipetalae clade with its closest relatives which also have square stems, but these ash trees occur in the SW US. In comparison, most US ash trees are within the Melioides clade and appear quite susceptible to EAB. In a mixed ash forest in Michigan, up to 71% of the blue ash survived EAB infestations (Tanis and McCullough 2012). In contrast, all white ash trees were dead except for 29 saplings that were too small to be infested at the time of the tree census. Eighty percent of the remaining blue ash had signs of infestation which suggests that these trees survived the invasion of EAB (Tanis and McCullough 2012). Similar observations have been made in Canada (Carson 2013), where 82% of blue ash were alive with crowns that had >90% of their foliage. In contrast, all but 6.5% of the green ash in the survey died (n=31). This resistance of blue ash to EAB may be due to its unique secondary metabolite profile and being distantly related to other native ash species.

1.5 Biological control of EAB:

Biological control is a management tactic that can contribute to pest suppression using ecological principles and co-evolved relationships. Classical, augmentative, and conservation controls are three types of biological control (Eilenberg et al. 2001). Classical biological control is the intentional introduction of an exotic biological control agent for establishment, persistence, and long-term pest management. In comparison, inoculation or augmentative biological control is the release of a biological control agent with the expectation that it will multiply and control the pest for a limited period. Generally, augmentative releases are conducted in greenhouses, orchards, or other highly managed ecosystems. A third biological control, conservation control, focuses on modification of the environment to protect and enhance the capacity of native natural enemies to reduce pests. Often, this control can involve providing alternate hosts, floral resources, or harborage from other natural enemies or pesticides (Nomikou et al. 2002, Rebek et al. 2006, Schmidt 2014). By improving habitats, refuges can provide natural enemies sites for reproduction and harborage.

For EAB management, classical biological control was adopted as a long-term strategy after eradication attempts failed (Herms and McCullough 2014, GAO 2006). The search for natural enemies of EAB began in 2002 in the US, and 2003 in China (Liu et al. 2003). Initially, two species of larval parasitoid wasps were found in China, *Spathius agrili* (Hymenoptera: Braconidae) Yang and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) Yang (Liu et al. 2003, Yang et al. 2005, 2006). Later, *Oobius agrili* (Hymenoptera: Encyrtidae) Zhang and Huang, an egg parasitoid, was discovered in 2004 and described by Zhang et al. (2005).

Oobius agrili is parthenogenic and has a female to male sex ratio of 14.5:1 (Bauer and Liu 2007). In China, *O. agrili* was found to complete two generations per year in the field and parasitize >60% of EAB eggs (Liu et al. 2007).

Spathius agrili is a gregarious ectoparasitoid with up to four generations a year (Yang et al. 2005). As an idiobiont, this parasitoid does not permit its host (EAB larvae) to develop beyond the stage that was attacked (Alphen and Jervis 1996). *Spathius agrili* appears to be effective at reducing beetles with up to 90% parasitism in the field (Yang et al. 2005). Typically, an average of 8.4 larvae emerges per host (range of 1-18) with a reported sex ratio of 3:1 (females to male) (Yang et al. 2005).

Tetrastichus planipennisi is a gregarious larval endoparasitoid that has up to four generations a year (Liu et al. 2007). *Tetrastichus planipennisi* is a koinobiont, a parasitoid that allows its host to feed and develop after being parasitized (Alphen and Jervis 1996). *Tetrastichus planipennisi* find hosts from EAB larval feeding vibrations (Ulyshen et al. 2010). On average, this wasp parasitized 22.4% of EAB larvae in the field with rates as high as 40.4% (Liu et al. 2007). *Tetrastichus planipennisi* was reported to reduce EAB densities by 73.6% in the 2005 field season in China (Liu et al. 2007). This wasp has a high reproductive capacity with an average fecundity of 35 *T. planipennisi* progeny per EAB larvae (Liu et al. 2007) and a sex ratio of 3:1 (females to male) (Duan et al. 2012c).

These three parasitoids have been imported, quarantined, screened, and released to suppress EAB in the United States (Gould et al. 2007, 2013, Liu and Bauer 2007, Bauer and Liu 2007). *Oobius agrili* has successfully established in Michigan and Indiana (Duan et al. 2011, 2012a, Mapbiocontrol 2014). In Michigan, *O. agrili* was able to parasitize up to 48% of the EAB eggs in the field (Duan et al. 2012a). *Spathius agrili* has been less successful and to date, has not established in any of the US release sites north of the 40th parallel. Consequently, *S. agrili* currently is only being released south of the 40th parallel (Gould et al. 2013). *Tetrastichus planipennisi* has successfully established in Michigan, Maryland, Minnesota, and Indiana (Mapbiocontrol 2014, Duan et al. 2013, Jennings et al. 2013). EAB parasitism rates have been reported as high as 28.6% by *T. planipennisi* in the field (Duan et al. 2013). *Tetrastichus planipennisi* has been observed to have parasitized EAB larvae in small regenerating ash trees years after the death of the original ash forest (Duan et al. 2013, per. comm.). Although this observation occurred in

Michigan, follow-up surveys at Huntington, IN in 2013 and 2014 failed to recover *T. planipennisi* from green ash saplings (Mapbiocontrol 2014), even though it had been recovered previously in 2011 (Mapbiocontrol 2014). *Tetrastichus planipennisi* released in 2009 and 2010 persisted in green ash saplings at several Indiana sites (Huntington, Orleans, and Ritchey Woods; Mapbiocontrol 2014); however, these saplings may be insufficient to support EAB and its parasitoids.

1.6 Blue ash, America's Manchurian ash:

In Asia, EAB remains a secondary pest through a coevolved relationship with host plants and natural enemy suppression. Biological control by woodpeckers and parasitoids contribute to decreasing EAB populations (Wang et al. 2010, Duan et al. 2012b, Liu et al. 2007, Yang et al. 2005). The addition of host plant resistance can slow larval development (Chakraborty et al. 2014) and lead to EAB being more prone to natural enemy attack. This relationship demonstrates the combined effects of host plant resistance and biological control to decreasing pest populations.

In North American, EAB is a primary pest of ash trees because most native trees are highly susceptible and there are no specialist natural enemies to suppress populations. *Tetrastichus planipennisi* has successfully established in the US and blue ash is able to survive EAB infestation (Tanis and McCullough 2012, Carson 2013). Therefore, blue ash may be able to serve as a refuge for EAB and its larval parasitoid, *T. planipennisi*. If introduced parasitoids, such as *T. planipennisi,* are able to persist in EAB larvae on blue ash, then they have the potential to protect more susceptible regenerating ash trees. A study on the suitability of blue ash to serve as host for EAB and its natural enemies would contribute to determining whether this tree species could be used as a refuge.

1.7 Research Objectives:

The main goal of this study was to determine the extent to which blue ash has the potential to serve as a refuge for *T. planipennisi.* This goal was broken down into two main objectives with the following sub-objectives:

1) Determine the extent to which green and blue ash are able to support EAB larvae (Chapter 2).
    a. Do EAB larvae survive at the same rate on blue and green ash?
    b. Do EAB differ in size during or after larval development on blue and green ash?
2) Determine the extent to which EAB larvae in green and blue ash can support *T. planipennisi* (Chapter 3).
    a. Can *T. planipennisi* attack EAB larvae on green and blue ash?
    b. Do parasitism rates by *T. planipennisi* differ between blue and green ash?
    c. Do the sex ratios, brood size, or adult female size of *T. planipennisi* differ between green and blue ash?

This work will quantify the relative suitability of blue and green ash trees to support larval stages of EAB, and serve as hosts for *T. planipennisi.* This work will also contribute to determining the extent to which blue ash could serve as a refuge for EAB and parasitoids that can protect more susceptible North American ash. Additionally, if this blue ash refuge concept can work for *T. planipennisi*, it has the potential to work for other larval parasitoids which could further contribute to the suppression of EAB populations.

1.8 Literature cited

Alphen, J. J. and M. A. Jervis. 1996. Foraging behavior, pp. 26. *In* M. A. Jervis and N. Kidd. (Eds.). Insect Natural Enemies (1st ed.). New York, New York: Chapman and Hall.

Anulewicz, A., D. McCullough, and D. Miller 2006. Oviposition and development of emerald ash borer (*Agrilus planipennis*) (Coleoptera: Buprestidae) on hosts and potential hosts in no-choice bioassays. The Great Lakes Entomologist 39:99-112.

Anulewicz, A., D. McCullough, and D. Cappaert. 2007. Emerald Ash Borer (*Agrilus planipennis*) Density and Canopy Dieback in Three North American Ash Species. Arboriculture & Urban Forestry 33: 338-349.

Anulewicz, A., D. McCullough, D. Cappaert, and T. Poland. 2008. Host range of the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in North America: Results of multiple-choice field experiments. Environmental Entomology 37: 230-241.

Bauer, L., and H. Liu. 2007. *Oobius agrili* (Hymenoptera: Encyrtidae), a solitary egg parasitoid of emerald ash borer from China, pp. 63-64. *In* V. Mastro, D. Lance, R. Reardon, and G. Parra (Comps.), Proceedings, Emerald ash borer and Asian longhorned beetle research and development review meeting 29 October- 2 Novemeber 2006, Cincinnati, OH. USDA publication.

Belokobylskij, S. A., G. I. Yurchenko, J. S. Strazanac, A. Zaldivar-Riveron, and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius Nees* (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Annuals of the Entomological Society of America 105: 165-178.

Beering, P. and J. Scott. 2010. Premises Liability and Your Trees. Arboricultural Consultant: 6-10, 23.

Brown-Rytlewski, D.E., and M.A. Wilson. 2005. Tracking the emergence of emerald ash borer adults, pp. 13-14. *In* V. Mastro and R. Reardon (Comps.), Proceedings, Emerald ash borer research and technology development meeting, 5-6 October 2004, Romulus, MI. USDA publication.

Canadian Food Inspection Agency. 2013. Areas regulated for the emerald ash borer. http://www.inspection.gc.ca/plants/plant-protection/insects/emerald-ash-borer/areas-regulated/eng/1347625322705/1367860339942

Cappaert, D., D. G. McCullough, T. Poland, and N. Siegert. 2005. Emerald ash borer in North America: a reserach and regulatory challenge. American Entomologist 51: 152-165.

Carson, S. L. 2013. Spatiotemporal dynamics and host selection of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae, at Point Pelee National Park, Ontario, Canada. PhD dissertation.

Chakraborty, S., J. Whitehill, A. Hill, S. Opiyo, D. Cipollini, D. Herms, and P. Bonello. 2014. Effects of water availability on emerald ash borer larval performance and phloem phenolics of Manchurian and black ash. Plant, cell, & environment DOI: 10.1111/pce.12215.

Chen, Y., and T. Poland. 2010. Nutritional and defensive chemistry of three North American ash species: possible roles in host performance and preference by emerald ash borer adults. The Great Lakes Entomologist 43: 20-32.

Cipollini, D., Q. Wang, J. Whitehill, J. Powell, P. Bonello, and D. Herms. 2011. Distinguishing defensive characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. Journal of Chemical Ecology 37: 450-459.

Cooperative Emerald Ash Borer Project. 2014. Multistate EAB poster. http://emeraldashborer.info/files/MultiState_EABpos.pdf

Duan, J., M. Ulyshen, L. Bauer, J. Gould, and R. Van Driesche. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). *Environmental Entomology* 39: 1513-1522.

Duan, J., L. Bauer, M. Ulyshen, J. Gould, and R. Van Driesche. 2011. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biological Control 56: 170-174.

Duan, J., L. Bauer, J. Hansen, K. Abell, and R. Van Driesche. 2012a. An improved method for monitoring parasitism and establishment of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid introduced for biological control of the emerald ash borer (Coleopetera: Buprestiade) in North America. Biological Control 60: 255-261.

Duan, J., G. Yurchenko, and R. Fuester. 2012b. Occurrence of Emerald Ash Borer (Coleoptera: Buprestidae) and Biotic Factors Affecting Its Immature Stages in the Russian Far East. Environmental Entomology 41: 245-254.

Duan, J., L. Bauer, K. Abell, and R. Van Driesche. 2012c. Population responses of hymenoptera parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in the north central United States. BioControl 57:199-209.

Duan, J., L. Bauer, K. Abell, J. Lelito, and R. V. Driesche. 2013. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 106: 1145-1154.

Eilenberg, J., A. Hajek, and C. Lomer. 2001. Suggestions for unifying the terminology in biological control. BioControl 46: 387:400.

Eyles, A., W. Jones, K Riedl, D. Cipollini, S. Schwartz, K. Chan, D. Herms, and P. Bonello. 2007. Tree phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). Journal of Chemical Ecology 33: 1430-1448.

Gandhi, K. and D. Herms. 2010a. North American arthropods at risk due to widespread *Fraxinus* mortality caused by the alien emerald ash borer. Biological Invasions 12: 1839-1846.

Gandhi, K. and D. Herms. 2010b. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biological Invasions 12: 389-405.

Government Accountability Office (GAO). 2006. Invasive forest pests: Lessons learned from three recent infestations may aid in managing future efforts. Rep. US GAO, GAO-06–353. http://www.gao.gov/assets/250/249776.pdf

Gould, J., J. Ayer, Y. Zhong-qi, and W. Xiao-yi. 2007. Host specificity of *Spathius agrili*, a parasitoid of the emerald ash borer, pp. 65-66. *In* V. Mastro, D. Lance, R. Reardon, and G. Parra (Compliers), Proceedings, Emerald Ash Borer Research and Asian Longhorned Beetle Technology Development Meeting, Cincinnati, OH. USDA publication.

Gould, J. S., L. S. Bauer, J. Lelito, and J. Duan. 2013. Emerald ash borer biological control release and recovery guidelines. USDA, APHIS, FS Northern Research Station, and Agricultural Research Service. pp. 1-76.

Haack, R., J. Jendek, H. Liu, K. Marchant, T. Petrice, T Poland, and H. Ye. 2002. The emerald ash borer; a new exotic pest in North America. Newsletter of the Michigan Entomological Society. 39: 1-5.

Herms, D., and D. McCullough. 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. Annual Review of Entomology 59: 13-30.

Herms, D., A. Stone, and J. Chatfield. 2004. Emerald ash borer: the beginning of the end of ash in North America?, pp. 62-71. *In*, J. Chatfield, E. Draper, H. Mathers, De. Dyke, P. Bennett, and J. Boggs (eds.), Ornamental plants: annual reports and research reviews 2003, Columbus, Ohio. Ohio State University Extension.

Herms, D. A., D. G. McCullough, D. R. Smitley, C. S. Sadof, and W. Cranshaw. 2014. Insecticide options for protecting ash trees from emerald ash borer. North Central IPM Center Bulletin. 2nd Edition. 16 pp.

Heyd, R. 2005. Ash (*Fraxinus* sp.) management guidelines. Emerald ash borer response strategy. Draft report. Michigan Department of Natural Resources. March 2005.

Jennings, D., J. Gould, J. Vandenberg, J. Duan, and P. Shrewsbury. 2013. Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (*Agrilus planipennis*). PLOS ONE 8: 1-9.

Kovacs, K., R. Haight, D. G. McCullough, R. Mercader, N. Siegert, and A. Liebhold. 2010. Cost of potential emeralds ash borer damage in U.S. communities, 2009-2019. Ecological Economics 69: 569-578.

Liu, H., L. Bauer, R. Gao, T. Zhao, T. Petrice, and R. Haack. 2003. Exploratory Survey for the Emerald Ash Borer *Agrilus planipennis* (Coleopetera: Buprestidae), and its Natural Enemies in China. The Great Lakes Entomologist 36: 191-204.

Liu, H., L. Bauer, D. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42: 61-71.

Mapbiocontrol. 2014. Parasitoid Recoveries.EAB Biocontrol. http://www.mapbiocontrol.org/eab/recovery/

McCullough, D. G., T. Poland, A. Anulewicz, and D. Cappaert. 2009a. Emerald ash borer (Coleoptera: Buprestidae) attraction to stressed or baited ash trees. Environmental Entomology 38: 1668-1679.

McCullough, D. G., T. M. Poland, and D. Cappaert. 2009b. Attraction of the emerald ash borer to ash trees by girdling, herbicide treatment, or wounding. Canadian Journal of Forest Research 39: 1131-1345.

Mercader, R., N. Siegert, A. Liebhold, and D. McCullough. 2009. Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. Agricultural and Forest Entomology 11: 421-424.

Mercader, R., N. Siegert, A. Liebhold, and D. McCullough. 2011. Simulating the effectiveness of three potential management options to slow the spread of emerald ash borer (*Agrilus planipennis*) populations in localized outlier sites. Canadian Journal of Forest Research 41: 254-264.

Nomikou, M., A. Janssen, R. Schraag, and M. Sabelis. 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Experimental and Applied Acarology 27: 57-68.

Poland, T.M., and D.G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and threat to North America's ash resource. Journal of Forestry 104: 118-124.

Pureswaran, D., and T. Poland. 2009. Host Selection and Feed Preference of *Agrilus planipennis* (Coletoptera: Burprestidae) on Ash (*Fraxinus spp.*). Environmental Entomology 38: 757-765.

Rebek, E., C. Sadof, and L. Hanks. 2006. Influence of floral resource plants on control of an armored scale pest by the parasitoid *Encarsia citrina* (Craw.) (Hymenoptera: Aphelinidae). Biological Control 37: 320-328.

Rebek, E., D. Herms and D. Smitley. 2008. Interspecific Variation in Resistance to Emerald Ash Borer (Coleoptera: Buprestidae) Among North American and Asian Ash (*Fraxinus spp.*). Environmental Entomology 37: 242-246.

Rigsby, C. M., V. Muilenburg, T. Tarpey, D. Herms, and D. Cipollini. 2014. Oviposition preferences of *Agrilus planipennis* (Coleoptera: Buprestidae) for different ash species support the mother knows best hypothesis. Annals of the Entomological Society of America 107: 773-781.

Schmidt, R. 2014. Leaf structures affect predatory mites (Acari: Phytoseiidae) and biological control a review. Experimental and Applied Acarology 62: 1-17.

Sibley, D. 2009. Olive family: ashes, pp. 379. *In* D. Sibley, The Sibley guid to trees. Alfred Knopf, Inc., New York, USA.

Siegert, N., D. McCullough, A. Liebhold, and F. Telewski. 2008. Reconstruction of the establishment of emerald ash borer through dendronological analysis. *In* K. McManus and K. Gottschalk (eds.), Proceedings, 19th U.S. Department of Argriculture interagency research forum on invasive species 2008; 8-11 January 2008, Annapolis, MD: U.S. Department of Agriculture, Forest Service, Northern Research Station: 70.

Siegert, N., D. McCullough, A. Liebhold, and F. Telewski. 2014. Dendrochronological reconstruction of the epicenter and early spread of emerald ash borer in North America. Diversity and Distributions 1-12.

SLAM (SL.ow A.sh M.ortality). 2014. Implementation: SLAM steps in implementing a strategy to SL.ow A.sh M.ortality. http://www.slameab.info/implementation/

Tanis, S., and D. G. McCullough. 2012. Differential persistence of blue ash and white ash following emerald ash borer invasion. Canadian Journal for Forest Research 42: 1542-1550.

Taylor, R., L. Bauer, D. Miller, and R. Haack. 2005. Emerald ash borer flight potential, pp. 15-16. *In* V. Mastro and R. Reardon (Comps.), Proceedings of the emerald ash borer research and technology development meeting, 15-16 October 2004,Romulus, MI. USDA publication.

Taylor, R., L. Bauer, T. Poland, and K. Windell. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on flight mill and in free flight. Journal of Insect Behavior 23: 128-148.

Ulyshen, M D., J. J. Duan, L. S. Bauer, and I. Fraser. 2010. Suitability and accessibility of immature *Agrilus planipennis* (Coleoptera: Buprestidae) stages to *Tetrastichus planipennisi* (Hymenoptera: Eulophidae). Journal of Economic Entomology 103: 1080-1085.

Wang, X.-Y., Z.-Q. Yang, J.R. Gould, Y.-N. Zhang, G.-J. Liu, and E. Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10: 1–23.

Wei, X., Y Wu, R. Reardon, and T.-H. Sun. 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* Fairmaire) in China. Insect Science 14: 367-373.

Whitehill, J., A. Popova-Butler, K. Green-Church, J. Koch, D. Herms, and P. Bonello. 2011. Interspecific proteomic comparisons reveal ash phloem genes potentially involved in constitutive resistance to the emerald ash borer. PLOS One DOI: 10.1371/journal.pone.0024863.

Whitehill, J., S. Opiyo, J. Koch, D. Herms, D. Cipollini, and P. Bonello. 2012. Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to emerald ash borer. Journal of Chemical Ecology 38: 499-511.

Yang, Z.-Q., J. Strazanac, P. Marsh, C. Van Acterber, and W.-Y Choi. 2005. First recorded parasitoid from China of *Agrilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 95: 636-642.

Yang, Z.-Q., J. Strazanac, Y.-X. Yao, and X.-Y. Wang. 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). Proceedings of the Entomological Society of Washington 108: 550-558.

Yang, S., J. J. Duan, T. Watt, K. Abell, and R. G. van Driesche. 2012. Responses of an idiobiont ectoparasitoid, *Spathius galinae* (Hymenoptera: Braconidae), to host larvae parasitized by the koinobiont endoparasitoid *Tetrastichus planipennisi* (Hymenoptera: Eulophidae): implications for biological control of emerald ash borer (Coleoptera: Buprestidae). Environmental Entomology 41: 925-932.

Zhang, Y., D. Huang, T. Zaho, H. Liu, and L. Bauer. 2005. Two New Species of Egg Parastioids (Hymenoptera: Encyrtidae) of Wood-Boring Beetle Pests from China. Phytoparasitica 33: 253-260.

CHAPTER 2. EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) DEVELOPMENT IN BLUE ASH (*FRAXINUS QUADRANGULATA*) AND GREEN ASH (*F. PENNSYLVANICA*): FIELD AND LAB EXPERIMENTS

2.1 Abstract

Emerald ash borer (EAB) (*Agrilus planipennis*) is a primary pest that has killed tens of millions of North American ash (*Fraxinus* spp.) trees. Although all native ash species are susceptible to EAB, blue ash (*F. quadrangulata*) is relatively resistant and appears to be able to survive EAB infestation. I compared the capacity of EAB larvae to survive and develop in blue ash and the more susceptible green ash (*F. pennsylvanica*). Blue and green ash trees were infested with EAB eggs in the field and laboratory, bark was peeled to extract larvae, and larval developmental stages were determined. EAB larvae reared in field and laboratory studies in both ash species had similar survivorship (>96%), while larval mortality in both ash species due to encapsulation by wound periderm was low (<4%). Larvae reared in field grown blue ash were significantly smaller with fewer overwintering (15%) as J-shape (prepupae) than those in green ash. Similarly, larvae reared in blue ash in the laboratory were significantly smaller and developed slower than in green ash. The consistent finding in both field and laboratory data suggests that blue ash slows the larval development rate. Therefore, more EAB larvae have a two year life cycle in blue ash than green ash. Longer development time also increases EAB exposure to larval mortality factors and could help explain why blue ash survive infestation at a higher rate than green and white ash in forests.

2.2 Introduction

The emerald ash borer (EAB), *Agrilus planipennis* is a primary pest of North American ash trees with the potential to dynamically impact North American forests. It was first discovered in 2002 as the cause of extensive ash tree mortality in Michigan, US and Ontario, Canada (Haack et al. 2002). Since its arrival in North America in infested wood packing material from China during the early to mid-1990s (Siegert et al. 2008, 2014; Cappaert et al. 2005a), it has killed tens of millions of ash trees (Poland and McCullough 2006). This pest has the potential to kill eight billion North American ash trees (Mercader et al. 2009) and cost tens of billions for treating, removing, and replacing 37.9 million ash trees on developed land over 10 years (Kovacs et al. 2010). In addition, declining and dead trees pose a hazard and have the potential to injure or kill people (Beering and Scott 2010).

In the spring, EAB pupae develop into adults and emerge from the ash tree at 230-260 C° DD_{10} (Brown-Rytlewski and Wilson 2004) by chewing through wood and forming diagnostic D-shaped exit holes. Adults feed on ash tree leaves and look for mates. After mating, females feed to mature their eggs and lay 50-90 eggs individually in bark cracks or crevices (Poland and McCullough 2006). Eggs develop and larvae emerge within two weeks. Larvae feed and develop in the phloem layer until October or November when they overwinter (Cappaert et al. 2005a). During their fourth instar, larvae will bore into the host’s sapwood to overwinter as a pre-pupae called “J-shaped larvae”. EAB can take one to two years (univoltine and semivoltine, respectively) to develop depending on temperature and host plant health (Wei et al. 2007, Cappaert et al. 2005a, Tluczek et al. 2011).

In China, EAB occurs at low densities (Liu et al. 2003), which likely results from a combination of host tree resistance and suppression by natural enemies. EAB outbreaks in China were rare until after the North American white ash and velvet ash were introduced (Liu et al. 2003). North American green ash (*F. pennsylvanica*) is attacked more frequently and with higher infestation densities of EAB than Asian congeners in China (Duan et al. 2012a, Liu et al. 2007).

Most North American ash species are far more susceptible to EAB than Asian species like Manchurian ash (*F. mandshurica*) (Anulewicz et al. 2007, Rebek et al. 2008, Pureswaran and Poland 2009, Tanis and McCullough 2012). In a common garden study, Manchurian ash suffered 20% mortality from EAB compared to 92-100% for green ash, and 75% for white ash (Rebek et al. 2008). There is a hierarchy of EAB oviposition and feeding preference among North American ash species with green ash>white ash>black ash (*F. nigra*)>blue ash (*F. quadrangulata*) (Anulewicz et al. 2006, 2007, Pureswaran and Poland 2009, Chen and Poland 2010, Tanis and McCullough 2012, Carson 2013).

Mechanisms of resistance to EAB are not well understood. Current research has focused on antixenosis (Groot et al. 2008, Pureswaran and Poland 2009, Rigsby et al. 2014) and antibiosis resistance (Eyles et al. 2007, Cipollini et al. 2011, Whitehill et al. 2011, 2012, Chakraborty et al. 2014), and their respective potential roles in host plant preference and secondary metabolites that may affect the EAB biology. Electroantennograms (EAGs) of emerald ash borer adults show strong responses to green leaf volatiles (GLV) (Groot et al. 2008). Pureswaran and Poland (2009) found EAB preference for green ash over Manchurian ash may be from differences in volatile concentration and composition. And more recently, Rigsby et al. (2014) demonstrated that ovipositing female EAB prefer North American ash trees (e.g. black, green, and white ash).

Whitehill et al. (2012) examined the phenolic and lignin levels of the phloem across one resistant and several susceptible *Fraxinus* species. They found Manchurian ash has unique lignans and lignan derivatives that may contribute to its higher resistance, while blue ash also had a unique secondary metabolite profile that included coumarins, esculetins, lignans, and secoiridoids. Additionally, the phloem of blue ash also contains several coumarins that were present in significantly higher levels than other ash species. Whitehill et al. (2012) postulated that these secondary metabolites may contribute to the allopatric resistance in blue ash.

Blue ash is an uncommon species of ash that tends to grow in limestone outcroppings and has square twigs (Sibley 2009). Phylogenetically, it is distantly related to other North American ash species. Blue ash appears to be the most resistant and the least preferred North American ash host for EAB (Anulewicz et al. 2007, 2008, Pureswaran and Poland 2009, Tanis and McCullough 2012). Recent work conducted by Tanis and McCullough (2012) in Michigan demonstrated that blue ash is able to survive EAB infestation. In a mixed ash forest, up to 71% of the blue ash survived EAB infestations (Tanis and McCullough 2012). In contrast, 100% of the white ash were dead except for 29 saplings, which were too small to be infested at the time of the tree census. Of the surviving blue ash, 80% had signs of infestation suggesting that these trees were able to survive an EAB attack (Tanis and McCullough 2012). Similar results have been demonstrated in mixed forests in Canada (Carson 2013), where 82% of blue ash crowns had >90% of their foliage after the initial wave of EAB mortality. In contrast, all but 6.5% of the green ash in the survey had died.

There have been no studies to explain how blue ash survives after successfully being attacked by EAB. So, I examined the potential for antibiosis by evaluating the relative suitability of blue and green ash logs for the development and survivorship of EAB larvae.

2.3 Materials and Methods

2.3.1 Description of field sites and selection of experimental ash trees

In the April 2013, field sites with existing stands of green ash in Celery Bog, West Lafayette, Indiana, and blue ash in the Mississinewa reservoir, Peru, Indiana were selected as a source of host material for EAB larval development studies. All ash trees used in this study had a diameter at breast height (DBH) between 8-12 cm. Selected trees had no obvious symptoms of EAB, such as vertical bark splits, epicormic shoots, D-shaped exit holes, canopy dieback, and woodpecker holes.

2.3.2 Field: infestation of standing ash trees with EAB eggs

Experimental trees in each field site were infested with EAB eggs acquired from the USDA Forest Service at Michigan State University, East Lansing, Michigan. Eggs laid onto coffee filter paper by gravid EAB females, as described in Abell et al. (2012), were incubated at 24°C, 75% humidity, and 16:8 h (L:D) photoperiod until they were 13 days old, three days before expected eclosion. Eggs were attached to trees in the field using a modified version of Abell et al. (2012) (Appendix 1) at 54 eggs/m^2 between 28 May-6 June 2013 along a 1.5 m main stem length 0.3 m above the soil surface. Leftover eggs were maintained in incubated conditions for two weeks and used to determine hatch rates by monitoring for exit holes by neonates. The hatch rate was used to estimate the number of viable eggs transferred to ash trees.

2.3.3 Field: harvest and dissection of ash tree logs

On 4 December 2013 and 11 April 2014, five and then six green and blue ash trees were felled and cut into 2 m sections, and then transported to our lab at Purdue University. The logs were stored in a walk-in cooler set at 5.5°C until they were removed for dissection. At which time, the diameter and bark thickness were recorded for both the top and bottom end of each log. The bark and phloem were moved from each log using a draw knife until EAB galleries were revealed, at which point a chisel was used to carefully remove any remaining bark covering individual larva. EAB larvae were removed with soft forceps and placed into 24 cell well plates. EAB gallery width and prothoracic width were recorded. Collected EAB larvae were categorized as healthy (H) or dead (D). Healthy individuals were white in color, while dead individuals were discolored (e.g. black), covered with fungal fruiting bodies, or engulfed in callus tissue formed by the tree. Additionally, I noted whether individuals were larvae or J-shaped larvae (prepupae) defined as being folded in a "J-shape" in a pupal chamber in the xylem. Weight was also recorded for EAB larvae from trees that were peeled in April 2014.

2.3.4 Field: statistical analysis

Chi-squared tests were used to compare the proportion of overwintering J-shaped larvae in green and blue ash, proportion of EAB larvae recovered from viable transferred eggs, and survival of larvae recovered from harvests. A mixed model PROC GLIMMIX (SAS® 9.4 Institute Inc., Cary, NC) was used to determine if ash species affected weights of J-shaped larvae collected during the April harvest of EAB, and prothoracic width and gallery width of larvae from December and April EAB harvests.

2.3.5 Lab: infesting ash bolts with emerald ash borer

I conducted a laboratory experiment to determine the relative suitability of blue and green ash as hosts for EAB. On 19 February 2014, EAB eggs between 8 and 12 days old were obtained from the USDA laboratory mentioned previously. Eggs younger than 12 days old were allowed to develop at 24°C until they reached 12 days of development at which point all eggs were stored at 10°C. On 24 February, 4 blue and 4 green ash trees between 3.0 - 6.0 cm in diameter were felled at Mississinewa and Celery Bog, respectively, and the main stems were transported back to Purdue University. These trees were cut into 84 ash bolts 25 cm in length. Following the standard operating procedure for laboratory rearing of EAB (Duan et al. 2011), I soaked green ash and blue ash bolts in a 10% sodium hypochlorite (bleach) solution for 30 minutes to kill pathogens on 25 and 26 February. Bolts were then rinsed with water to remove bleach. Eggs were individually cut out from clutches laid on coffee filters. I attached 13 eggs individually or in pairs using strips of parafilm wrapped around each bolt to secure the eggs. Each ash bolt contained eggs from at least 3 different cohorts of eggs. Leftover eggs were incubated in the laboratory and used to determine hatch rate. Eggs were monitored for exit holes by neonates for two weeks. The hatch rate was used to determine the number of viable eggs transferred to ash trees.

Eighty-four blue and green ash bolts were maintained in growth chambers for up to six weeks to determine EAB development. To keep bolts moist, they were inserted 3.8 cm into water soaked floral foam (Oasis floral foam deluxe, Smithers-Oasis Company, Kent, OH) after eggs were transferred. Three bolts were inserted into each floral block and kept in 5.7 liter clear plastic bins. Floral blocks were kept moist by watering to the top of each block on Monday, Wednesday, and Friday of each week. Ash bolts were kept inside growth chambers at 27°C 16:8 (light: dark) cycle with 70% humidity.

Ten green and blue ash bolts were removed at days 23, 30, and 37 (24, 31 March and 7 April 2014, respectively) to harvest EAB larvae using the peeling method previously described. Twelve green and blue ash bolts were peeled on day 33 and 34 (3-4 April), referenced as day 33.5. EAB length, prothoracic width, weight (g), and gallery width were recorded. EAB were recorded as alive or dead, and as larvae or J-shaped larvae as previously described. Larvae were then stored in 24 cell well plates.

2.3.6 Lab: statistical analysis

A Chi-squared test was used to compare the proportion of EAB larvae recovered from viable transferred eggs. PROC GLIMMIX for Generalized Linear Mixed Models was used in order to determine the extent to which ash species affected the prothoracic width, gallery width, and weight of developing EAB. EAB weights were square root transformed to correct for non-normality based on the shape of residual plots.

2.4 Results

2.4.1 Field study

Out of 550 eggs transferred to trees, 86.7% were estimated to be viable. In total, 272 EAB larvae were recovered from the 477 viable eggs placed on blue and green ash trees. There was no significant difference between the percentage of viable EAB eggs recovered as larvae in green ash (52.99%) and blue ash (61.5%) (χ^2= 3.52; df = 1; p = 0.061; Table 2.1) in December and April. Similarly, there was no difference between the percentage of healthy EAB larvae that were recovered from green and blue ash in December (χ^2= 0.05; df = 1; p = 0.830; Table 2.1) and April harvests, (χ^2= 0.2333; df = 1; p = 0.629; Table 2.1). In December, 3.17% and 3.85% of the EAB larvae recovered from green ash and blue ash, respectively, died encased in callus tissue. In April only 1.37% and 1.54% of the recovered EAB larvae died in this manner in green and blue ash, respectively. Significantly more J-shaped larvae overwintered in green ash (82.93%) compared to blue ash (67.63%) (χ^2= 8.11; df = 1; p = 0.004; Table 2.1). EAB gallery width was significantly larger in green ash (F = 24.52; df = 1, 18; p = <0.001; Table 2.2; Figure 2.1); however, harvest time (Dec. and April) (F = 1.34; df = 1, 18; p = 0.263) and the interaction of time and species (F = 0.15; df = 1, 18; p = 0.705) did not affect this measure of larval development. Prothoracic width was significantly larger in green ash larvae (F = 23.55; df = 1, 18; p = <0.001; Table 2.2; Figure 2.2) and different between sampling times (F = 18.30; df = 1, 18; p = 0.001) with no significant interaction between time and ash species (F = 0.23; df = 1, 18; p = 0.638). Weights of overwintering J-shaped larvae reared were larger greater in green ash (67.9 mg) than blue ash (61.9 mg), but the differences were not significant (F = 4.07; df = 1, 10; p = 0.071; Table 2.2).

2.4.2 Laboratory study

Out of 1,097 eggs placed on ash bolts, 445 EAB larvae were recovered over the four sampling periods that covered 1st instars up to J-shaped larval development. There were no significant differences in the proportion of larvae recovered from the estimated 91.9% viable eggs in green ash (45.62%) and blue ash (43.03%) (χ^2 = 0.68; df = 1; p =

0.409; Table 2.3). EAB larvae had significantly larger prothoracic widths in green ash than blue ash (*F* = 12.12; df = 1, 76; *p* = 0.001; Figure 2.3). Prothoracic widths increased significantly across sample days (*F* = 57.55; df = 3, 76; *p* = <0.001). There was no significant interaction between ash species and time (*F* = 0.28; df = 3, 76; *p* = 0.842). EAB larvae had consistently larger gallery widths in green ash (*F* = 19.43; df = 1, 76; *p* = <0.001; Figure 2.4). Larval galleries increased in both species over time (*F* = 42.69; df = 3, 76; *p* = <0.001), but there was no interaction between species and time (*F* = 42.69; df = 3, 76; *p* = <0.001). After a square root transformation to normalize data, green ash were found to have significantly heavier larvae (*F* = 10.99; df = 1, 76; *p* = 0.001; Figure 2.5). All larvae increased their weights over time (*F* = 60.66; df = 3, 76; *p* = <0.001), and there was no significant interaction between species and time (*F* = 2.13; df = 3, 76; *p* = 0.103).

2.5 Discussion

In my study, EAB larvae survive at the same rate in blue ash when compared to green ash, suggesting that these plant hosts do not affect survivorship. In both the field and laboratory experiments, the percentage of EAB larvae recovered from viable eggs was similar in blue and green ash hosts. Over 96% of larvae recovered from both species of ash in the field and laboratory studies were healthy. This survival rate is similar to that reported by Hietala (2013) who examined remnant green and white ash trees at the tail end of an EAB invasion in several parks. A subgroup of these ash trees were also artificially infested with EAB. Most larvae extracted from apparently tolerant and intolerant ash trees were healthy and were able to develop up to the J-shape stage. In the 2013-14 winter (December through February), Northern Indiana experienced the sixth coldest winter with an average daily temperature -6.5°C compared to the normal average of -2.8°C (NOAA 2014). EABs appear unaffected by these extreme cold temperatures because I observed the same percentage of live larvae (Table 2.1) before (December harvest) and after (April harvest) the winter season.

Few larvae (< 4%) were killed by encapsulation in either ash species. In contrast, this phenomenon is a common response of North American birch species (*Betula* spp.) to the native *Agrilus anxius*, the bronze birch borer, (Muilenberg and Herms 2012). Paper birch (*B. papyrifera*) is relatively resistant to the bronze birch borer, which is hypothesized to come from defenses that slow bronze birch borer larval feeding in order for wound periderm to encapsulate larvae (Muilenburg and Herms 2012). Although I found wound periderm to form callus tissue in green and blue ash, this response was unable to kill significant numbers of larvae suggesting that wound periderm may not contribute to the observed capacity of blue ash to tolerate EAB attack in the forest (Anulewicz et al. 2007, 2008, Pureswaran and Poland 2009, Tanis and McCullough 2012).

EAB larvae developed more slowly in blue ash compared to green ash in both the laboratory and field studies. In the laboratory, three measurements of EAB larval size; prothoracic width, gallery width, and larval weight suggested that larvae grew larger and more quickly in green ash. Similarly in another study, EAB larvae appear to grow more slowly in the relatively resistant Manchurian ash than those in the susceptible black ash under normal conditions (Chakraborty et al. 2014). However, it is interesting to note that toward the end of EAB larval development, the magnitude of this size difference is reduced (Figures 2.3, 2.4, and 2.5). Nevertheless, differences between larval development rates were clear in green and blue ash growing in the field. EAB larvae entering the winter (December harvest) and the spring (April harvest) in green ash had both significantly wider galleries and prothoracic segments compared to blue ash. In addition, significantly more J-shape larvae overwintered in green ash than in blue ash. The overwintering J-shape larvae collected from green and blue ash weighed the same. Although it is not clear whether the slow growth of EAB larvae has any effect on the reproductive fitness or survival of adults, it may be some indication of antibiosis in blue ash.

Callus tissue is not a major source of mortality in green and blue ash. However, the capacity of blue ash defenses to slow development in larvae may be a critical part of host plant resistance as suggested by the slowed EAB development observed in Manchurian ash (Chakraborty et al. 2014). Blue ash is known to have a unique secondary metabolite profile (Whitehill et al. 2012). One chemical group in particular, the coumarins, are known to be antifeedants in a wide variety of taxa where they can completely inhibit the development of an organism, slow the growth of larvae, or affect adult fertility (Meisner and Skatulla 1975, Mansour 1982, Fung and Herrebout 1987, Silva et al. 2006). Effects of these and other secondary metabolites should be investigated to determine if they are causing the slowed development of larval EAB in resistant ash species. Although EAB are able to successfully complete development in blue ash (Anulewicz et al. 2008), ash host effects on adult survival and fitness are unknown. Female EAB preferentially feed on leaves of green, black, and white ash over blue ash (Pureswaran and Poland 2009) and may hint at some effect of blue ash on maturation feeding by females. Studies to determine the extent to which host leaves affect female fitness should be conducted to demonstrate these effects.

The slower development and lower occurrence of J-shaped larvae in blue ash might indicate that a higher proportion of EAB have a semivoltine life history. Longer EAB development time in blue ash may increase attack rates from natural enemies that have the capacity to find concealed larvae beneath bark. For example, woodpeckers account for a significant source of EAB larval mortality (Lindell et al. 2008, Jennings et al. 2013, Cappaert et al. 2005b). Additionally, larval parasitoids both native and introduced can also contribute to EAB mortality (Duan et al. 2012b, Duan et al. 2013). Slowed growth and increased mortality from natural enemies has been demonstrated in a number of plant herbivore systems including both specialist and generalist defoliators (Haggstrom and Larsson 1995, Benrey and Denno 1997, Williams 1999, Havill and Raffa 2000).

In conclusion, blue ash does not appear to kill EAB larva. However, blue ash does slow larval development and demonstrates that blue ash is more tolerant to EAB feeding than green ash. The slowed development in blue ash may make larvae more susceptible to natural enemies that attack immature stages of EAB. Future studies are needed to determine the effect of slowed larval development in blue ash on adult fitness. Additionally, the extent to which blue and green ash leaves affect host plant choice and female fecundity needs to be explored.

2.6 Literature cited

Abell, K., J. Duan, L. Bauer, J. Lelito, and R. Van Driesche. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). Biological Control 63: 320-325.

Anulewicz, A., D. McCullough, and D. Miller 2006. Oviposition and development of emerald ash borer (*Agrilus planipennis*) (Coleoptera: Buprestidae) on hosts and potential hosts in no-choice bioassays. The Great Lakes Entomologist 39:99-112.

Anulewicz, A., D. McCullough, and D. Cappaert. 2007. Emerald ash borer (*Agrilus planipennis*) density and canopy dieback in three North American ash species. Arboriculture & Urban Forestry 33: 338-349.

Anulewicz, A., D. McCullough, D. Cappaert, and T. Poland. 2008. Host range of the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in North America: Results of multiple-choice field experiments. Environmental Entomology 37: 230-241.

Beering, P., and J. Scott. 2010. Premises liability and your trees. Arboricultural Consultant: 6-10, 23.

Benrey, B., and R. Denno. 1997. The slow-growth-high-mortality hypothesis: a testing using the cabbage butterfly. Ecology 78: 987-999.

Brown-Rytlewski, D.E., and M.A. Wilson. 2005. Tracking the emergence of emerald ash borer adults, pp. 13-14. *In* V. Mastro and R. Reardon (Comps.), Proceedings, Emerald ash borer research and technology development meeting, 5-6 October 2004, Romulus, MI. USDA publication.

Cappaert, D., D. G. McCullough, T. Poland, and N. Siegert. 2005a. Emerald Ash Borer in North America: a reserach and regulatory challenge. American Entomologist 51: 152-165.

Cappaert, D., D. McCullough, and T. Poland. 2005b. The upside of the emerald ash borer catastrophe: a feast for woodpeckers, pp. 69–70. *In* Mastro and Reardon (2005).

Carson, S. L. 2013. Spatiotemporal dynamics and host selection of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), at Point Pelee National Park, Ontario, Canada. PhD dissertation.

Chakraborty, S., J. G. Whitehill, A. L. Hill, S. O. Opiyo, D. Cipollini, D. A. Herms, and P. Bonello. 2014. Effects of water availability on emerald ash borer larval performance and phloem phenolics of Manchurian and black ash. Plant, Cell, and Environment 37: 1009-1021.

Chen, Y., and T. Poland. 2010. Nutritional and defensive chemistry of three North American ash species: possible roles in host performance and preference by emerald ash borer adults. The Great Lakes Entomologist 43: 20-32.

Cipollini, D., Q. Wang, J. Whitehill, J. Powell, P. Bonello, and D. Herms. 2011. Distinguishing defensive characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. Journal of Chemical Ecology 37: 450-459.

Duan, J. J., T. Watt, and C. Oppel. 2011. An alternative host plant-based method for laboratory rearing of emerald ash borer to produce larval parasitoids for biological control, p. 3. *In* G. Parra, D. Lance, V. Mastro, R. Reardon, and C. Benedict (eds.), Proceedings of Emerald Ash Borer National Research and Technology Development Meeting, October 12 & 13, 2011 Wooster, Ohio. USDA Technology Transfer FHTET-2011-06, Raleigh, North Carolina.

Duan, J., G. Yurchenko, and R. Fuester. 2012a. Occurrence of emerald ash borer (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far East. Environmental Entomology 41: 245-254.

Duan, J., L. Bauer, K. Abell, and R. Van Driesche. 2012b. Population responses of hymenoptera parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in the north central United States. BioControl 57:199-209.

Duan, J., L. Bauer, K. Abell, J. Lelito, and R. V. Driesche. 2013. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 106: 1145-1154.

Eyles, A., W. Jones, K Riedl, D. Cipollini, S. Schwartz, K. Chan, D. Herms, and P. Bonello. 2007. Tree phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). Journal of Chemical Ecology 33: 1430-1448.

Fung, S.-Y., and W. M. Herrebout. 1987. Coumarins in *Prunus maahaleb* and its herbivore, the small Ermine moth *Yponomeuta mahalebellus*. Journal of Chemical Ecology 13: 2041-2047.

Groot, P., G. G. Grant, T. M. Poland, R. Scharbach, L. Buchan, R. W. Nott, L. Macdonald, and D. Pitt. 2008. Electrophysiological response and attraction of emerald ash borer to green leaf volatiles (GLVs) emitted by host foliage. Journal of Chemical Ecology 34: 1170-1179.

Haack, R., J. Jendek, H. Liu, K. Marchant, T. Petrice, T Poland, and H. Ye. 2002. The emerald ash borer; a new exotic pest in North America. Newsletter of the Michigan Entomological Society. 39: 1-5.

Haggstrom, H., and S. Larsson. 1995. Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. Oecologia 104:308-315.

Havill, N. P., and K. F. Raffa. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. Ecological Entomology 25: 171-179.

Hietala, K. G. 2013. Evaluation and monitoring of ash (*Fraxinus* spp.) to long-term emerald ash borer (*Agrilus planipennis* [Coleoptera: Buprestidae]) exposure. Master's Thesis, Michigan Technological University. http://digitalcommons.mtu.edu/etds/664

Jennings, D. E., J. R. Gould, J. D. Vandenberg, J. J. Duan, and P. M. Shrewsbury. 2013. Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (*Agrilus planipennis*). PLOS One 8: e83491.

Klooster, W. S., D. A. Herms, K. S. Knight, C. P. Herms, D. G. McCullough, A. Smith, K. J. K. Gandhi, and J. Cardina. 2014. Ash (*Fraxinus* spp.) mortality, regeneration, and seed bank dynamics in mixed hardwood forests following invasion by emerald ash borer (*Agrilus planipennis*). Biological Invasions 16: 859-873.

Kovacs, K., R. Haight, D. G. McCullough, R. Mercader, N. Siegert, and A. Liebhold. 2010. Cost of potential emeralds ash borer damage in U.S. communities, 2009-2019. Ecological Economics 69: 569-578.

Lindell, C. A., D. G. McCullough, D. Cappaert, N. M. Aposolu, and M. B. Roth. 2008. Factors influencing woodpecker predation on emerald ash borer. The American Midland Naturalist 159:434-444**.**

Liu, H., L. Bauer, R. Gao, T. Zhao, T. Petrice, and R. Haack. 2003. Exploratory Survey for the Emerald Ash Borer *Agrilus planipennis* (Coleopetera: Buprestidae), and its Natural Enemies in China. The Great Lakes Entomologist 36: 191-204.

Liu, H., L. Bauer, D. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42: 61-71.

Mansour, M. H. 1982. The chronic effect of some allelochemics on the larval development and adult reporductivity of the cotton leafworm *Spodoptera littoralis* Boisd. Chem. Abstr. 97:51188c.

Meisner, J., and U. Skatulla. 1975. Phagostimulation and phagodeterrency in the larva of the gypsy moth *Portheiria dispar* L. Phytoparasitica 3:19-26.

Mercader, R., N. Siegert, A. Liebhold, and D. McCullough. 2009. Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. Agricultural and Forest Entomology 11: 421-424.

Meyhofer R. and J. Casas. 1999. Vibratory stimuli in host location by parasitic wasps. Journal of Insect Physiology 45: 967-971.

Poland, T.M., and D.G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and threat to North America's ash resource. Journal of Forestry 104: 118-124.

Pureswaran, D., and T. Poland. 2009. Host Selection and Feed Preference of *Agrilus planipennis* (Coletoptera: Burprestidae) on Ash (*Fraxinus spp.*). Environmental Entomology 38: 757-765.

Rebek, E., D. Herms and D. Smitley. 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus spp.*). Environmental Entomology 37: 242-246.

Sibley, D. 2009. Olive family: ashes, pp. 379. *In* D. Sibley, The Sibley guid to trees. Alfred Knopf, Inc., New York, USA.

Siegert, N., D. McCullough, A. Liebhold, and F. Telewski. 2008. Reconstruction of the establishment of emerald ash borer through dendronological analysis. *In* K. McManus and K. Gottschalk (eds.), Proceedings, 19th U.S. Department of Argriculture interagency research forum on invasive species 2008; 8-11 January 2008, Annapolis, MD: U.S. Department of Agriculture, Forest Service, Northern Research Station: 70.

Siegert, N., D. McCullough, A. Liebhold, and F. Telewski. 2014. Dendrochronological reconstruction of the epicenter and early spread of emerald ash borer in North America. Diversity and Distributions 1-12.

Silva, M. C. P., T. R. Walter, and C. Ferreira. 2006. Absorption of toxic β-glucosides produced by plants and their effect on tissue trehalases from insects. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 143: 367-373.

Tanis, S., and D. G. McCullough. 2012. Differential persistence of blue ash and white ash following emerald ash borer invasion. Canadian Journal for Forest Research 42: 1542-1550.

Tluckzek, A., D. McCullough, and T. Poland. 2011. Influence of host stress on emerald ash borer (Coleoptera: Buprestidae) adult density, development, and distribution in *Fraxinus pennsylvanica* trees. Environmental Entomology 40:357-366.

Rigsby, C. M., V. Muilenburg, T. Tarpey, D. Herms, and D. Cipollini. 2014. Oviposition preferences of *Agrilus planipennis* (Coleoptera: Buprestidae) for different ash species support the mother knows best hypothesis. Annals of the Entomological Society of America 107: 773-781.

Wallander, E. 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. Plant Systematics and Evolution 273: 25-49.

NOAA. 2014. A look back at the 2013-2014 winter season. http://www.crh.noaa.gov/iwx/?n=2013_2014winter

Wei, X., Y Wu, R. Reardon, and T.-H. Sun. 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* Fairmaire) in China. Insect Science 14: 367-373.

Whitehill, J., A. Popova-Butler, K. Green-Church, J. Koch, D. Herms, and P. Bonello. 2011. Interspecific proteomic comparisons reveal ash phloem genes potentially involved in constitutive resistance to the emerald ash borer. PLOS One DOI: 10.1371/journal.pone.0024863.

Whitehill, J., S. Opiyo, J. Koch, D. Herms, D. Cipollini, and P. Bonello. 2012. Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to emerald ash borer. Journal of Chemical Ecology 38: 499-511.

Williams, I. S. 1999. Slow-growth, high-mortality – general hypothesis or is it? Ecological Entomology 24:490-495.

Table 2.1 Survival and development of emerald ash borer eggs and larvae in caged green and blue ash trees in West Lafayette and Peru Indiana.

	Combined 2013 and 2014 harvests				2013 harvest	2014 harvest
Hosts	Eggs placed	Viable eggs	Total larvae (% of viable eggs)	J-shaped larvae[1] (% of total larvae)	Live larvae (% survival)	Live larvae (% survival)
Green ash	289	251	133 (52.99)[2]	102 (82.93)	61 (96.83)	71 (97.26)
Blue ash	261	226	139 (61.50)	94 (67.63)	75 (96.15)	64 (98.46)
Effect of host	$\chi^2{}_1$=		3.52	8.11	0.05	0.23
p-value			0.061	0.004	0.830	0.629

---

[1] EAB larvae that were killed by callus tissue or lost during peeling were excluded because they could not be determined if they reached the J-shape stage.

[2] Values followed by the same letter are not significantly different according to Chi-squared test $p < 0.05$.

Table 2.2 Means (± standard errors) of EAB larval weight, gallery width, and prothoracic width from caged green and blue ash trees in West Lafayette and Peru Indiana from eggs placed on trees in May/June 2013.

Host	Combined harvests		
	J-shaped larvae weight (mg)	Gallery width (mm)	Prothoracic width (mm)
Green ash	67.9± 1.5	3.90± 0.06	2.94± 0.05
Blue ash	61.9± 1.4	3.42± 0.08	2.62± 0.06
Effect of host	$F_{1,10} = 4.07$	$F_{1,18} = 24.52$	$F_{1,18} = 23.55$
p-value	0.071	<0.001	<0.001

Table 2.3 Survival of emerald ash borer in green and blue ash bolts under laboratory conditions.

Host	Eggs placed	Viable eggs	Larvae collected (% viable eggs)
Green ash	546	502	229 (45.62)
Blue ash	546	502	216 (43.03)
Effect of host			$\chi^2_1 = 0.68$
p- value			0.409

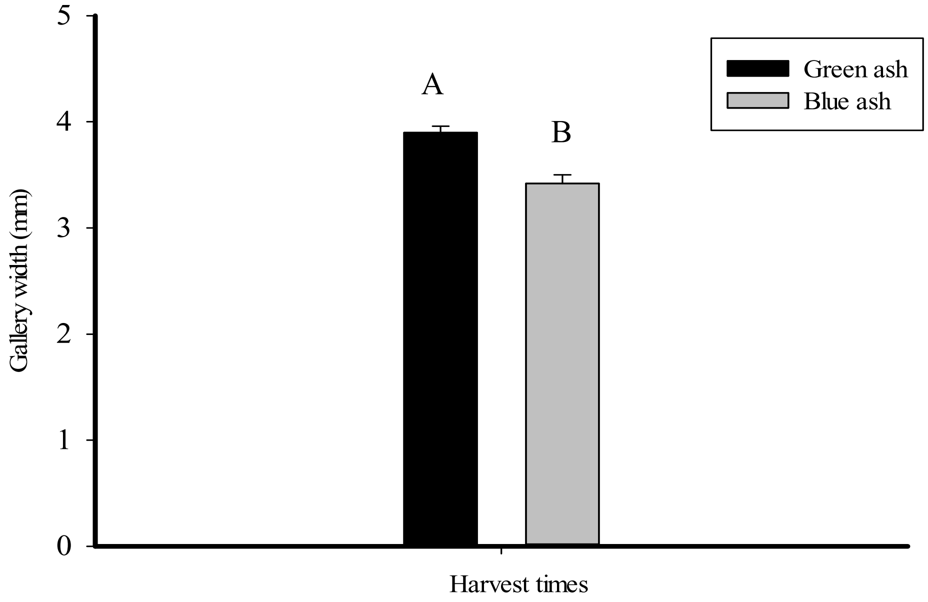

Figure 2.1 Means and standard errors of EAB gallery width collected from caged green and blue ash trees in West Lafayette and Peru, Indiana field sites during 2013 and 2014.

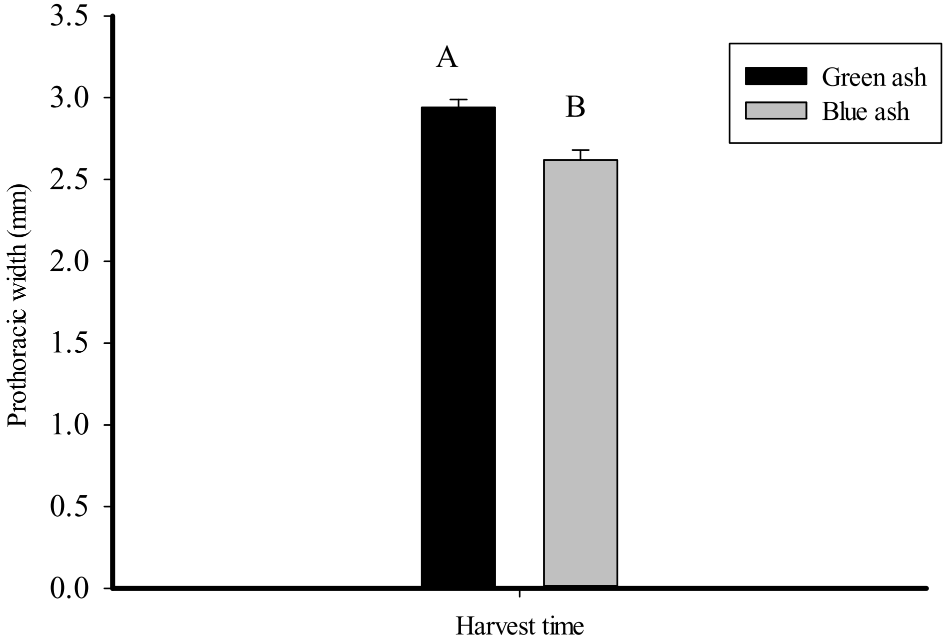

Figure 2.2 Means and standard errors of EAB prothoracic width collected from caged green and blue ash trees in West Lafayette and Peru, Indiana field sites during 2013 and 2014.

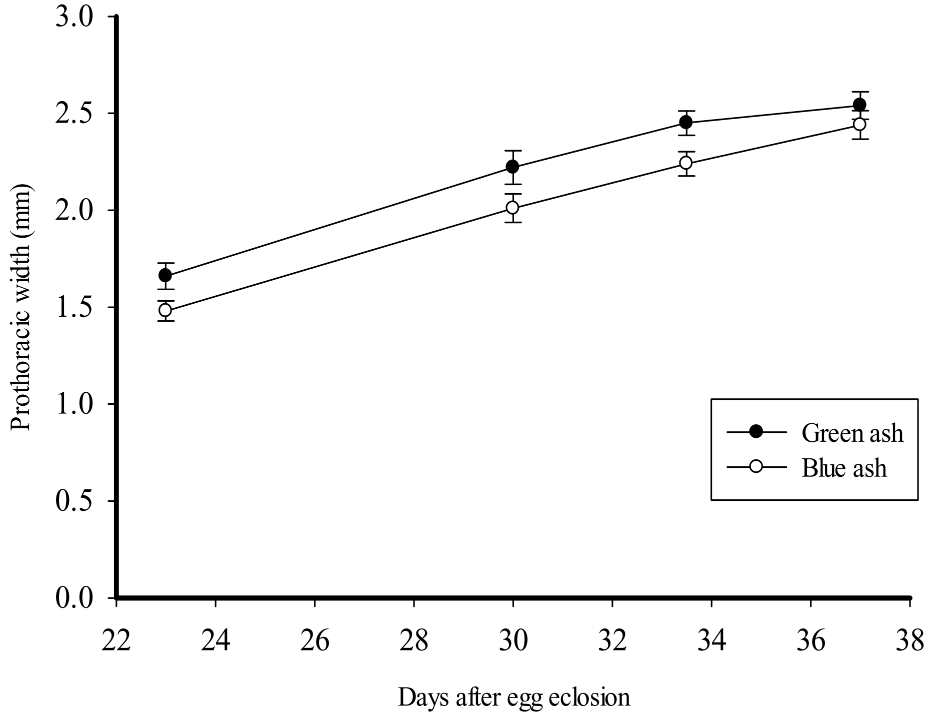

Figure 2.3 EAB prothoracic width means and standard errors of larvae from laboratory green and blue ash bolts collected from West Lafayette and Peru, Indiana field sites in 2014.

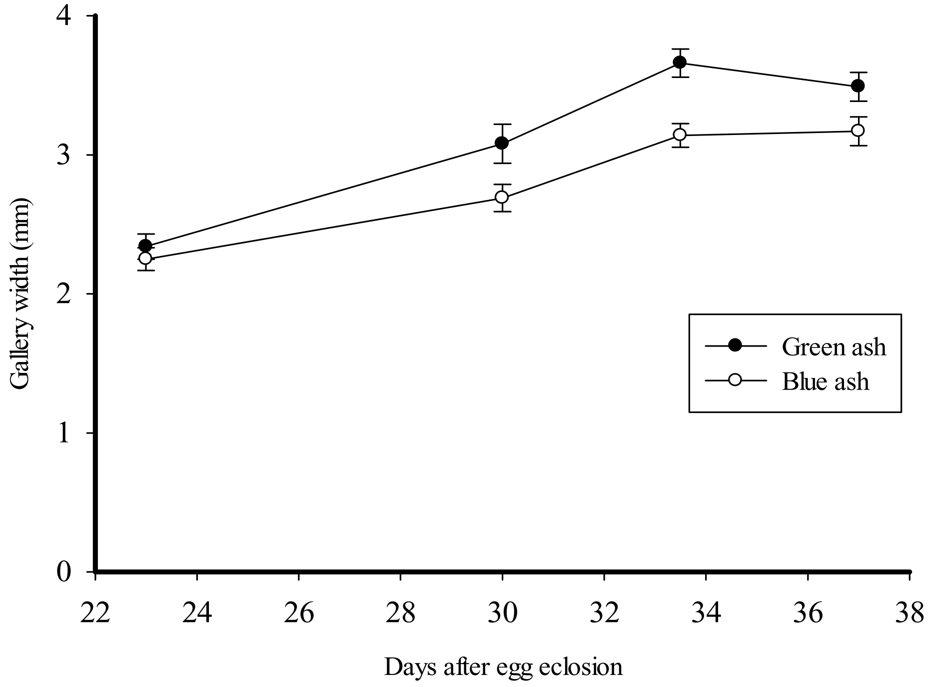

Figure 2.4 EAB gallery width means and standard errors of larvae from laboratory green and blue ash bolts collected from West Lafayette and Peru, Indiana field sites in 2014.

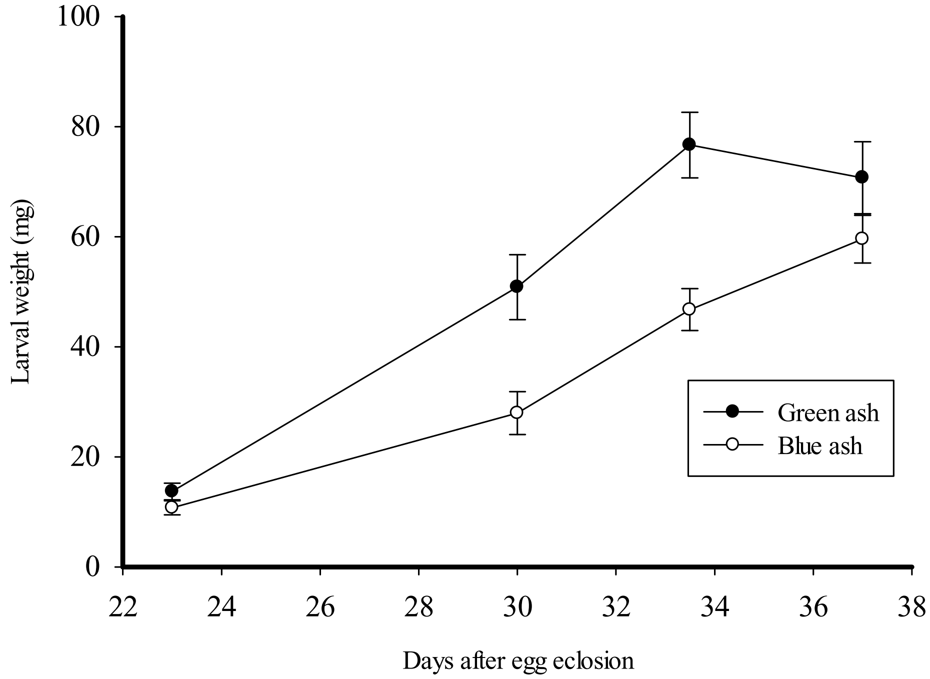

Figure 2.5 Mean EAB weight and standard errors of larvae from laboratory green and blue ash bolts collected from West Lafayette and Peru, Indiana field sites in 2014.

CHAPTER 3. HOST SUITABILITY OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) INFESTED IN BLUE ASH (*FRAXINUS QUADRANGULATA*) AND GREEN ASH (*F. PENNSYLVANICA*) FOR *TETRASTICHUS PLANIPENNISI*: A FIELD AND LABORATORY EXPERIMENT

3.1 Abstract

The larval parasitoid *Tetrastichus planipennisi* was introduced into North America from China to suppress the exotic emerald ash borer (EAB) (*Agrilus planipennis*). Blue ash (*Fraxinus quadrangulata*) is the most resistant and most likely to survive EAB infestation in North America compared to other ash species. If natural enemies can successfully attack and develop on EAB in infested blue ash they may be better able to persist and protect regenerating ash trees. To evaluate this, I determined the capacity of EAB larvae to serve as hosts for *T. planipennisi* when reared in artificially infested blue and green ash (*F. pennsylvanica*) bolts in the field and laboratory. In the field, *T. planipennisi* was able to attack EAB larvae in both blue and green ash. In the laboratory, parasitism rates, parasitoid brood size, sex ratio, and female tibia lengths were measured to compare the relative fitness of EAB as hosts when feeding in green and blue ash trees. No significant differences were found between any of these fitness measures for larvae collected from green and blue ash trees in the laboratory studies. As such, I hypothesize that *Tetrastichus planipennisi* could establish in blue ash forests that have been infested with EAB.

3.2 Introduction

Since its discovery in North America in 2002, the emerald ash borer (EAB), *Agrilus planipennis,* has become a primary pest of ash trees with the potential to cost billions of dollars in losses to urban and rural forests (Kovacs et al. 2010, Mercader et al. 2009). After eradication attempts failed, classical biological control was adopted as a long-term strategy for managing EAB (Herms and McCullough 2014, GAO 2006). The search for natural enemies of EAB began in 2002 in the US and 2003 in China (Liu et al. 2003). Initially, two species of larval parasitoid wasps were found in China, *Spathius agrili* (Hymenoptera: Braconidae) Yang and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) Yang (Liu et al. 2003, Yang et al. 2005, 2006). Later, *Oobius agrili* (Hymenoptera: Encyrtidae) Zhang and Huang, an egg parasitoid, was discovered and described by Zhang et al. (2005).

Tetrastichus planipennisi is a gregarious larval endoparasitoid that has up to four generations a year (Liu et al. 2007). This wasp is a koinobiont, allowing the EAB larva to feed and develop after being parasitized (Alphen and Jervis 1996). In the field in China, this wasp parasitized an average of 22.4% of EAB larvae with rates as high as 40.4% (Liu et al. 2007). This wasp has a high reproductive capacity with an average fecundity of 35 progeny per EAB larva (Liu et al. 2007) and a sex ratio of 3:1 (females to males) (Duan et al. 2012).

In the US, *T. planipennisi* has been successfully introduced and established in Michigan, Maryland, Minnesota, and Indiana (Mapbiocontrol 2014, Duan et al. 2013, Jennings et al. 2013). In the field, parasitism rates of EAB have been reported as high as 28.6% by *T. planipennisi* (Duan et al. 2013). *Tetrastichus planipennisi* is capable of parasitizing EAB larvae in small regenerating ash trees years after high densities of pests destroyed the original forest (Duan et al. 2013, per. comm.). Although this observation occurred in Michigan, follow-up surveys at Huntington, IN in 2013 and 2014 failed to recover *T. planipennisi* from green ash saplings (Mapbiocontrol 2014), even though it had been recovered previously in 2011 (Mapbiocontrol 2014). *Tetrastichus planipennisi* released in 2009 and 2010 persisted in green ash saplings at several Indiana sites (Huntington, Orleans, and Ritchey Woods; Mapbiocontrol 2014); however, these saplings may be insufficient to support EAB and its parasitoids.

In contrast to green ash and other North American ash, blue ash appears to be the most resistant and least preferred North American ash host for EAB (Anulewicz et al. 2007, 2008, Pureswaran and Poland 2009, Tanis and McCullough 2012). Blue ash is an uncommon species of ash that typically grows in limestone outcrops and has square twigs (Sibley 2009). Phylogenetically, blue ash is distantly related to other North American ash species that EAB has encountered in the US (Wallander 2008). Specifically, blue ash is in the Dipetalae clade with its closest relatives which also have square stems, but these ash trees occur in the SW US. In comparison, most US ash trees are within the Melioides clade and appear quite susceptible to EAB. Recent work conducted by Tanis and McCullough (2012) in Michigan demonstrated that blue ash can survive the initial waves of EAB infestation. In a mixed ash forest, up to 71% of the blue ash survived EAB infestations (Tanis and McCullough 2012). In contrast, white ash suffered 100% mortality except for 29 saplings which were too small to be infested at the time of the tree census. Of the surviving blue ash, 80% had signs of infestation which suggests that these trees are able to tolerate and survive EAB attacks (Tanis and McCullough 2012). Similar results have been demonstrated in mixed forests in Canada (Carson 2013), where 82% of blue ash crowns had >90% of their foliage after the initial wave of EAB mortality. In contrast, all but 6.5% of the green ash in the survey had died.

If a resistant species like blue ash are able to maintain low levels of EAB larvae in the forest, more parasitoids should be sustained after the initial wave of ash destruction. Thus, more parasitoids would be available to protect susceptible species of ash in the regrowth forest. My investigation of EAB development in blue ash (Chapter 2) suggests that a higher proportion of larvae have a semivoltine life history. Many larval parasitoids of concealed hosts use feeding vibrations to locate their hosts (Meyhofer and Casas 1999). As such, larval parasitoids, like *T. planipennisi* that use feeding vibrations to find hosts, may have more of an opportunity to find and attack EAB in blue ash because the larval stage takes longer to develop than in more susceptible ash species. This phenomenon would be consistent with the slow-growth-high-mortality hypothesis which predicts greater mortality of herbivores on plants whose resistance involves slowed larval development (Benrey and Denno 1997). If the larvae are suitable hosts for *T. planipennisi*, blue ash could contribute to the persistence of this parasitoid in the forest.

It is unknown whether slowed EAB larval development affects the quality of *T. planipennisi*. Host plant resistance that suppresses larval growth has the potential to reduce the quality of biological control agents. For example, tree species influenced the relative growth, development time, and pupal weight of gypsy moth, which further affected *Cotesia melanoscela* development time and size (Kruse and Raffa 1997). Similarly, *C. melanoscela* survivorship was affected by tree species that the host gypsy moth (*Lymantraia dispar*) consumed (Werren et al. 1992). Host plant effect on parasitoids could be detrimental to biocontrol agents because they would be less fit to attack hosts.

Adult size can be used as a measure of parasitoid quality (Bethke et al. 1991, West et al. 1996). *Tetrastichus planipennisi* hind tibia length is correlated with overall size (Duan and Oppel 2012). Larger adult females have longer ovipositor length which is correlated with larger egg loads and a capacity for wasps to reach EAB larvae through thicker bark. To determine the extent to which EAB larvae in green and blue ash can support *T. planipennisi*, I conducted a field and laboratory study to compare parasitoid adult attack rates and fitness parameters of progeny.

.

3.3 Materials and Methods

3.3.1 Description of field sites and selection of experimental ash trees

In the April 2013, two field sites with existing stands of green (Celery Bog, West Lafayette, Indiana) and blue ash (Mississinewa, Peru, Indiana) were selected as a source of host material for infesting emerald ash borer and adding its natural enemy *Tetrastichus planipennisi*. All ash trees used in this study had a diameter at breast height (DBH) between 8-12 cm. Trees selected for this study had no obvious symptoms or signs of EAB, such as vertical bark splits, epicormic shoots, D-shaped exit holes, canopy dieback, or woodpecker holes.

3.3.2 Field: infestation of standing ash trees with EAB eggs

Experimental trees in each field site were infested with EAB eggs which were acquired from the USDA Forest Service at Michigan State University, East Lansing, Michigan. The eggs had been laid onto coffee filter paper by gravid EAB females as described in Abell et al. (2012). Eggs were incubated at 24°C, 75% humidity, and 16:8 h (light: dark) photoperiod until they were 13 days old, three days before expected eclosion. Eggs were attached to experimental trees using a modified version of Abell et al. (2012) (see Appendix for details) at 54 eggs/m^2 between 28 May to 6 June 2013 along a 1.5 m main stem length. Eggs taken into the field and not transferred to trees were incubated at 24°C in the laboratory and evaluated to determine hatch rate by monitoring for exit holes by neonates for two weeks. The hatch rate was used to estimate the number of viable eggs transferred to ash trees.

3.3.3 Field: release of Tetrastichus planipennisi

Tetrastichus planipennisi were released into 14 green ash cages on 5 September 2013 and 15 blue ash cages on 21 May 2014. Although, my goal was to release parasitoids on same size larvae, I underestimated the development rate of EAB in blue ash and released wasps when most EAB were in pupae or adults. In September, the majority of EAB in green ash were in late instar larval stages, based on dissections of ash logs harvested 14 days after parasitoids were released. In May 2014, only 20.1% of the EAB were in the larval stage in blue ash 14 days after parasitoids were released. The parasitoids were reared and supplied by the USDA EAB Parasitoid Rearing Facility in Brighton, MI. The rearing facility sent wasps in cups containing 50 *T. planipennisi* adult females (1500 total) and at least 5 males per cup.

Wasps were introduced to each experimental tree by placing one cup of 50 females into previously constructed cages (see Appendix for details). Parasitoid release was accomplished by opening cages along a short length of the seams and streaking the bark of trees with a paintbrush to provide adult parasitoids with food. Cups of parasitoids were inserted into the cloth opening of each cages and tapped to agitate wasps to fly onto the trunk. After all wasps were released, cups were removed and inspected, and seams were reclosed and stapled shut. All trees caged with *T. planipennisi* were inspected for living wasps at two or three days, and then seven days after release of wasps (7 and 12 September 2013 for green ash; 24 and 28 May 2014 for blue ash). Honey was applied to the exterior of each cage one week after release as a food resource for *T. planipennisi*.

3.3.4 Field: harvest and dissection of ash tree logs with Tetrastichus planipennisi

On 19 September 2013 and 5 June 2014, green and blue ash trees with *T. planipennisi*, respectively, were felled and transported to the laboratory at Purdue University. The logs were stored in a walk-in cooler set at 5.5°C until they were dissected at which time EAB gallery width, prothoracic width, length, and larval condition were determined (Chapter 2). In addition, pupae and adults were added as categories for EAB condition at the time of dissection. Parasitized larvae were recorded and recognized by a distinct rope-like appearance when filled with numerous parasitoid larvae or appeared as a cluster white pupae that had completely consumed their host. EAB larvae and parasitoids were removed, placed in 24 cell well plates, and covered with parafilm (Bemis Company, Inc., Neenah, WI, US) to reduce desiccation, limit pathogens, and isolate parasitoids. Well plates were monitored for parasitoids for up to seven weeks (25 September-15 November 2013 for green ash; 7 June-26 July 2014 for blue ash). After adult parasitoids emerged, they were sexed, counted, frozen, and then stored in 75% ethanol.

3.3.5 Laboratory: parasitoid development in EAB infesting green and blue ash

A laboratory experiment was conducted to determine the relative suitability of EAB larvae as hosts for *T. planipennisi*. On 11 April 2014, four blue and four green ash trees were cut at Mississinewa and Celery Bog, respectively, and transported back to Purdue University. Ash logs 3.0 - 6.0 cm in diameter were cut into 25 cm long bolts, 13 for green ash and 13 for blue ash. Each bolt was then artificially infested with 13 EAB eggs (338 total) obtained from the USDA ARS Beneficial Insects Introduction Research Unit (Newark, DE) on 15 April 2014 using methods outline in Duan et al. (2011a). Ash bolts were kept inside growth chambers at 27°C 16:8 (light: dark) cycle with 70% humidity.

Each bolt was covered with a two liter plastic bottle to contain released parasitoids. Bottles had two holes (~5 cm in diameter) cut into the side wall that were covered with organdy cloth and sealed with hot glue on the edges to facilitate air circulation. The top two inches of each bottle were removed so they could be flipped upside down and inserted in floral blocks to surround ash bolts (Figure 3.1). In order to minimize variability in the sex ratio, brood size, and tibia length (Duan and Oppel 2012), parasitoid to host ratio was controlled by releasing ten *T. planipennisi* females (260 total) and two males per 2L bottle on 21 May based on the estimate of 10 EAB per ash bolt. Parasitoids were received from the USDA APHIS Brighton Rearing Facility (Brighton, MI). Bottles were then held down on the floral blocks with a strip of duct tape to prevent parasitoid escape. Additionally, honey was added to the organdy cloth holes as food for parasitoids.

Bottles were checked daily for the presence of live females. After two weeks, on 4 June, ash bolts had their bark peeled off with a hand chisel. Female parasitoids (parents) that could be found were removed and put into vials with ethanol (75%). EAB length, prothoracic width, weight, and gallery width were recorded. EAB larvae and parasitoid (progeny) larvae and pupae were then placed in 24 cell well plates. Parasitoids developed to adults in well plates at which point the sex ratio, parasitism rate, and individuals per brood were recorded. First generation (parents) females and recovered brood females (up to five per brood) had their left hind tibia length measured and recorded as previously described.

3.3.6 Data analysis

Field and laboratory data were analyzed using SAS (SAS® 9.4 Institute Inc., Cary, NC). A Chi-squared tests were used to compare the amount of EAB in the larval stage (field only) and the proportion of parasitism by *T. planipennisi* in green and blue ash (field and laboratory). Sex ratio, brood size and female tibia lengths of parasitoids were compared in a mixed model analysis of variance using a PROC GLIMMIX to determine if these parameters were affected by ash species. A mixed model of analysis of variance using PROC GLIMMIX was also used to determine if the brood size of *T. planipennisi* was affected by ash species and the larval instar of its EAB hosts.

3.4 Results

3.4.1 Field study

When green ash logs were harvested in September 2013, 100% of the EAB were in the larval stage (Table 3.1). In contrast, there were significantly less EAB in the larval stage (20.1%) in blue ash logs (χ^2= 223.45; df= 1; p= <0.001; Table 3.1) in June 2014. On average, the brood size was 68.6 *T. planipennisi* per EAB host with an average sex ratio of 4.8:1.0 (female: male). No parasitism was observed on a green ash log with a bark diameter of 3.4 mm or greater. Only one parasitized EAB larva was found in blue ash.

3.4.2 Laboratory study

There was no significant difference in EAB parasitism rates in larvae in blue and green ash (χ^2= 0.46; df= 1; p= 0.498; Table 3.2). From the PROC GLIMMIX, there was no significant difference in average parasitoid brood size (F= 0.39; df = 1, 17; p= 0.541), average parasitoid sex ratio (F = 0.07; df = 1, 17; p= 0.799), and parasitoid tibia lengths (F = 0.62; df = 1, 197; p = 0.433; Table 3.2) in green and blue ash. Brood size of parasitoids was not significantly affected by ash species (F= 0.07; df= 1, 17; p= 0.795) or the interaction between ash species and EAB larval stage (F= 0.04; df= 1, 6; p= 0.848). However, brood size of parasitoids was significantly greater on the 4th instars when compared to those in the 3rd instar (F= 6.30; df= 1, 6; p= 0.046).

3.5 Discussion

This study demonstrates that EAB larvae can be attacked by *T. planipennisi* in both green and blue ash in the field and laboratory conditions and is the first and only documentation of parasitism of EAB larvae by this wasp in blue ash trees. In the field, EAB larval parasitism was low at 11.3% in green ash, but similar to the 13% rate observed for *T. planipennisi* in field collected EAB larvae from green ash (Duan et al. 2011b). Under laboratory conditions, *T. planipennisi* parasitized EAB larvae at the same rate in both green ash (48.39%) and blue ash (54.72%). A similar laboratory study conducted with green and tropical ash (*F. uhdei*) also failed to find tree host to affect the rate at which EAB larvae were attacked by *T. planipennisi* (Duan and Oppel 2012) suggesting that some host plant species may not affect parasitism rates in the laboratory. Parasitism rates observed here were under no-choice conditions and may be artificially high. Parasitism by *T. planipennisi* should be further explored in the field to determine the extent to which host plant choice affects oviposition behavior.

In the field study only one EAB larva was parasitized by *Tetrastichus planipennisi* in blue ash. This low incidence could reflect the low proportion of feeding EAB larvae (20.1%) available when *T. planipennisi* was released compared to 100% in green ash. The low incidence of feeding larvae is important because *T. planipennisi* only attack feeding larvae (Ulyshen et al. 2010). Nevertheless the many observations in the laboratory and single observation of EAB parasitism in the field in blue ash indicates that parasitism can occur in the field. In the laboratory, EAB larvae were at susceptible stages of development when parasitoids were released on each ash host. As such, these findings better represent the capacity of *T. planipennisi* to parasitize hosts in green and blue ash logs than the field study.

Smaller EAB instars produced smaller broods of parasitoids in the lab (Table 3.3). Additionally, the mean brood size of *T. planipennisi* were similar across EAB third and fourth instars from blue and green ash (Table 3.3). *Tetrastichus planipennisi* brood size from EAB larvae in green ash was similar to a study by Ulyshen et al. (2010).

In the laboratory, there was no significant difference in sex ratios or tibia lengths between parasitoids emerging from EAB larvae reared in either ash species. The sex ratios observed in my study are similar to those observed from green ash in other studies (Liu and Bauer 2007, Duan et al. 2011b, Duan et al. 2012) suggesting that blue and green ash do not influence the quality of EAB larvae as hosts for *T. planipennisi*. More studies are needed to directly measure the effects of ash host on the fitness of this parasitoid.

Although I observed a parasitoid to host ratio of 2.1:1 in green ash and 2.5:1 in blue ash, it is unlikely that the difference between numbers of available hosts could have affected my results because both ratios are similar to the 2:1 ratio in green ash in studies conducted by Duan and Oppel (2012) where, the average sex ratios, brood sizes, and tibia lengths of *T. planipennisi* in my study were similar to those reported in green ash trees.

In conclusion, there was no host effect on *T. planipennisi* fitness parameters reared from green and blue ash. This parasitoid has similar sex ratios, brood sizes, and female tibia lengths when emerging from EAB larvae in blue and green ash hosts. Similar parasitoid fitness parameters suggest that in blue ash, EAB could be more susceptible to larval parasitoids since they take more time to develop to adults (Chapter 2). As such, parasitoid deployment strategies for EAB parasitoids should take into account rates of larval development in surviving host ash species and the composition of blue ash in the local ash community.

3.6 Literature cited

Abell, K., J. Duan, L. Bauer, J. Lelito, and R. Van Driesche. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Colep: Buprestidae). Biological Control 63: 320-325.

Alphen, J. J., and M. A. Jervis. 1996. Foraging behavior, pp. 26. *In* M. A. Jervis and N. Kidd. (Eds.). Insect Natural Enemies (1st ed.). New York, New York: Chapman and Hall.

Anulewicz, A., D. McCullough, and D. Cappaert. 2007. Emerald Ash Borer (*Agrilus planipennis*) Density and Canopy Dieback in Three North American Ash Species. Arboriculture & Urban Forestry 33: 338-349.

Anulewicz, A., D. McCullough, D. Cappaert, and T. Poland. 2008. Host range of the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in North America: Results of multiple-choice field experiments. Environmental Entomology 37: 230-241.

Benrey, B., and R. Denno. 1997. The slow-growth-high-mortality hypothesis: a testing using the cabbage butterfly. Ecology 78: 987-999.

Bethke, J. A., G. S. Nuessly, T. D. Paine, and R. A. Redak. 1991. Effect of host insect-host plant associations on selected fitness components of *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). Biological Control 1: 164-169.

Carson, S. L. 2013. Spatiotemporal dynamics and host selection of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae, at Point Pelee National Park, Ontario, Canada. PhD dissertation.

Duan, J., and C. Oppel. 2012. Critical rearing parameters of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) as affected by host plant substrate and host-parasitoid group structure. Journal of Economic Entomology 105: 792-801.

Duan, J. J., T. Watt, and C. Oppel. 2011a. An alternative host plant-based method for laboratory rearing of emerald ash borer to produce larval parasitoids for biological control, p. 3. *In* G. Parra, D. Lance, V. Mastro, R. Reardon, and C. Benedict (eds.), Proceedings of Emerald Ash Borer National Research and Technology Development Meeting, October 12 & 13, 2011 Wooster, Ohio. USDA Technology Transfer FHTET-2011-06, Raleigh, North Carolina.

Duan, J. J., C. B. Oppel, M. D. Ulyshen, L. S. Bauer, and J. LeLito. 2011b. Biology and life history of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a larval endoparasitoid of emerald ash borer (Coleoptera: Buprestidae). Florida Entomologist 94: 933-940.

Duan, J., L. Bauer, K. Abell, and R. Van Driesche. 2012. Population responses of hymenoptera parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in the north central United States. BioControl 57:199-209.

Duan, J., L. Bauer, K. Abell, J. Lelito, and R. V. Driesche. 2013. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 106: 1145-1154.

Government Accountability Office (GAO). 2006. Invasive forest pests: Lessons learned from three recent infestations may aid in managing future efforts. Rep. US GAO, GAO-06–353. http://www.gao.gov/assets/250/249776.pdf

Herms, D., and D. McCullough. 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. Annual Review of Entomology 59: 13-30.

Jennings, D., J. Gould, J. Vandenberg, J. Duan, and P. Shrewsbury. 2013. Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (*Agrilus planipennis*). PLOS ONE 8: 1-9.

Kovacs, K., R. Haight, D. G. McCullough, R. Mercader, N. Siegert, and A. Liebhold. 2010. Cost of potential emeralds ash borer damage in U.S. communities, 2009-2019. Ecological Economics 69: 569-578.

Kruse, J. J., and K. F. Raffa. 1997. Effects of selected Midwestern larval host plants on the performance by two strains of the gypsy moth (Lepidoptera: Lymantriidae) parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). Environmental Entomology 26: 1155-1166.

Liu, H., L. Bauer, R. Gao, T. Zhao, T. Petrice, and R. Haack. 2003. Exploratory survey for the emerald ash borer *Agrilus planipennis* (Coleopetera: Buprestidae), and its natural enemies in China. The Great Lakes Entomologist 36: 191-204.

Liu, H., L. Bauer, D. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42: 61-71.

Liu, H., and L. Bauer. 2007. *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a gregarious larval endoparasitoid of emerald ash borer from China. *In* V. Mastro, D. Lance, R. Reardon, and G. Parra (Compliers), Proceedings, Emerald ash borer and Asian longhorned beetle research and technology development meeting 2006; 29 October – 2 November 2006, Cincinnati, OH: U.S. USDA FS , FHTET-2007-04, 2007: 61-62.

Mapbiocontrol. 2014. Parasitoid Recoveries. EAB Biocontrol. http://www.mapbiocontrol.org/eab/recovery/

Mercader, R., N. Siegert, A. Liebhold, and D. McCullough. 2009. Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. Agricultural and Forest Entomology 11: 421-424.

Pureswaran, D., and T. Poland. 2009. Host Selection and Feed Preference of *Agrilus planipennis* (Coletoptera: Burprestidae) on Ash (*Fraxinus spp.*). Environmental Entomology 38: 757-765.

Sibley, D. 2009. Olive family: ashes, pp. 379. *In* D. Sibley, The Sibley Guide to Trees. Alfred Knopf, Inc., New York, USA.

Tanis, S., and D. G. McCullough. 2012. Differential persistence of blue ash and white ash following emerald ash borer invasion. Canadian Journal for Forest Research 42: 1542-1550.

Ulyshen, M D., J. J. Duan, L. S. Bauer, and I. Fraser. 2010. Suitability and accessibility of immature *Agrilus planipennis* (Coleoptera: Buprestidae) stages to *Tetrastichus planipennisi* (Hymenoptera: Eulophidae). Journal of Economic Entomology 103: 1080-1085.

West, S. A., K. E. Flanagan, and H. C. J. Godfray. 1996. The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). Journal of Animal Ecology 65: 631-639.

Werren, J. H., M. J. Raupp, C. S. Sadof, and T. M. Odell. 1992. Host plants used by gypsy moths affect survival and development of the parasitoid *Cotesia melanoscela*. Environmental Entomology 21: 173-177.

Yang, Z.-Q., J. Strazanac, P. Marsh, C. Van Acterber, and W.-Y Choi. 2005. First recorded parasitoid from China of *Agrilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 95: 636-642.

Yang, Z.-Q., J. Strazanac, Y.-X. Yao, and X.-Y. Wang. 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). Proceedings of the Entomological Society of Washington 108: 550-558.

Zhang, Y., D. Huang, T. Zaho, H. Liu, and L. Bauer. 2005. Two new species of egg parastioids (Hymenoptera: Encyrtidae) of wood-boring beetle pests from China. Phytoparasitica 33: 253-260.

Table 3.1 EAB larval parasitism rates by *Tetrastichus planipennisi* in green ash and blue ash as well as mean (±standard errors) brood size and sex ratio in the field.

Host	Number of EAB recovered (Larvae-adult stages)	Number of EAB larvae recovered (% larvae)	Larvae parasitized (% parasitized)	Parasitoid brood size	Sex ratio (F:M)
Green ash (Sept. 2013)	151	151 (100%)	17 (11.3%)	68.6±12.9	4.8:1 ±1.4
Blue ash (May 2014)	210	43 (20.5%)	1 (2.3%)	No data[1]	No data
Effect of host $\chi^2_1=$		223.45			
p value		<0.001			

---

[1] *T. planipennisi* desiccated before fully developing.

Table 3.2 EAB larval parasitism rates by *Tetrastichus planipennisi* in green and blue ash bolts as well as mean (±standard errors) tibia lengths, brood sizes, and sex ratio in a laboratory study in 2014.

Host	Larvae recovered	Larvae parasitized (% parasitized)	Tibia length (mm)	Parasitoid brood size	Sex ratio (F:M)
Green ash	62	30 (48.39)a[1]	0.614± 0.0077a	36.62± 4.52a	3.04± 0.74a
Blue ash	53	29 (54.72)a	0.603± 0.0086a	33.04± 3.52a	2.81± 0.49a
Effect of host		χ^2= 0.4583	$F_{1, 197}$= 0.62	$F_{1, 17}$= 0.39	$F_{1, 17}$= 0.07
p values		0.498	0.433	0.541	0.799

[1] Means followed by the same letter are not significantly different, p <0.05.

Table 3.3 Mean (± standard errors) of *Tetrastichus planipennisi* brood size emerging from second, third, and fourth EAB larval instars in a laboratory study in 2014.

Hosts	Second Instar (n) `	Third Instar (n)	Fourth Instar (n)
Green Ash	No data (0)	22.20±3.80 (5)	40.84± 5.73 (19)
Blue Ash	12.5± 0.50 (2)	22.83±2.85 (6)	38.72± 4.35 (18)
Effect of host		$F_{1,17}$= 0.07	
p value		0.7946	
Interaction of host and instar		$F_{1,6}$ = 0.04	
p value		0.848	

Figure 3.1 Ash bolts in floral foam with two liter bottles in the laboratory 2014.

CHAPTER 4. SUMMARY

Emerald ash borer developed more slowly in blue ash than in green ash (Chapter 2). The mechanism of slowed development is unclear, but literature suggests it may be due to nutritional differences within the host phloem layer. Nitrogen limitation in the phloem layer is not likely to be involved because a majority of amino acids and total percentage of nitrogen were demonstrated to be the same in susceptible black ash and resistant Manchurian ash (Hill et al. 2012). In contrast, secondary metabolites, like phenolics and lignans, are more likely to affect EAB development because there is interspecific variation among resistant and susceptible ash species (Eyles et al. 2007, Cipollini et al. 2011, Whitehill et al. 2011, 2012, Chakraborty et al. 2014). Several coumarins have been found to be distinct or at significantly higher levels in blue ash compared to other ash species (Whitehill et al. 2012). Coumarins can inhibit the development or affect adult fertility in a number of insect taxa (Meisner and Skatulla 1975, Mansour 1981, Fung and Herrebout 1987, Silva et al. 2006). Thus, I would hypothesize that differences in the secondary metabolite profile contributes to the slowed EAB development observed in blue ash.

The slowed development of EAB larvae increases the risk of being attacked by natural enemies that specialize on larvae such as woodpeckers (Jennings et al. 2013); the native parasitoids *Atanycolus* spp. and *Phasgnophora sulcata*, (Duan et al. 2012); and the introduced parasitoids *T. planipennisi* and *Spathius agrili* (Gould et al. 2013).

Tetrastichus planipennisi, in particular*,* should be better able to find and attack EAB because they only attack feeding larvae (Ulyshen et al. 2010), and it is suspected that movement and feeding vibrations are cues used to detect hosts (Ulyshen et al. 2011).

In contrast to EAB larvae, *T. planipennisi* was apparently unaffected by blue ash (Chapter 3) as supported by similar rates of parasitism, brood sizes, sex ratios, and tibia lengths. This phenomenon has also been observed in another plant insect system. For example, *Pieris brassicae* (Lepidoptera: Pieridae) was shown to develop slower and have lower pupal mass when reared on the host plant *Sinapis arvensis* compared to *Brassica nigra* (Gols et al. 2008). The gregarious koinobiont endoparasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) had the same brood size, parasitism, sex ratio, and parasitoid mass when reared from *P. brassicae* larvae feeding on different host plants. However in other plant insect systems, host plants that have affected herbivores also affect their parasitoids. For example, gypsy moth (*Lymantraia dispar*) relative growth, development time and pupal weight were influenced by tree species (Kruse and Raffa 1997). Tree species also influenced the development time, size, and survivorship of the parasitoid *C. melanosela* (Kruse and Raffa 1997, Werren et al. 1992). Similarly, *Manduca sexta* had slowed development and consequently its larval parasitoid *C. congregata* had increased mortality on tobacco plants compared to tomato plants (pers. comm. Mike Garvey). These findings suggest host plant effects on parasitoids can be difficult to predict. Thus, more studies are needed to determine the extent to which host plants can affect the fitness of *T. planipennisi* and other larval parasitoids of EAB.

Efficacy of a classical biological control program may also benefit from slowed EAB larval development in blue ash. Nearly 100% of ash trees that EAB encounters are killed in Eastern North American (Klooster et al. 2014). Biological control may be threatened because it will be more difficult for parasitoids to persist with such high ash tree mortality. Blue ash is the only native species that is able to survive EAB infestation (Tanis and McCullough 2012, Carson 2013) to date. Thus, blue ash resistance could complement biological control of EAB by serving as a refuge for parasitoids. This is supported by *T. planipennisi* ability to parasitize and completely develop on EAB larvae in blue ash with no apparent fitness loss (Chapter 3) when compared to green ash. *Tetrastichus planipennisi* could persist on blue ash when other ash species are in decline. When susceptible ash trees begin to reestablish in our forests, *T. planipennisi*, and possibly other larval biological control agents, would be readily available to protect susceptible ash trees by suppressing EAB populations.

Tetrastichus planipennisi capacity to protect ash trees is limited by its ovipositor length. This wasp can only parasitize larvae that are beneath bark <3.2 mm or in branches with diameters up to 11.2 cm (Abell et al. 2012). The ovipositor limitation was also observed in my study in green ash from the field (Chapter 3). However, if approved for release, *Spathius galinae* may contribute to the protection of larger ash trees because this larval parasitoid has three times the body size and twice the ovipositor length as *T. planipennisi* (Yang et al. 2006, Belokobylskij et al. 2012, Yang et al. 2012). Benefits of blue ash are limited to its range in Midwestern North America (Figure 4.1, NPGS 2014). In spite of this, blue ash and other EAB resistant trees, such as Manchurian ash, could be planted to benefit EAB parasitoids in urban and rural forests.

In conclusion, blue ash appears not to affect *T. planipennisi*, but does slow the development of EAB larvae. The slowed EAB development provides a longer opportunity for natural enemies to attack larvae in blue ash which would be consistent with the slow-growth-high-mortality hypothesis (Benrey and Denno 1997), but this needs be further investigated. Blue ash may also be able to serve as a refuge for released parasitoids which would complement the biological control program for EAB. Blue ash may affect other fitness parameters of *T. planipennisi* such as development time or fecundity and they should be further explored.

.

4.1 Literature cited

Abell, K., J. Duan, L. Bauer, J. Lelito, and R. Van Driesche. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Colep: Buprestidae). Biological Control 63: 320-325.

Belokobylskij, S. A., G. I. Yurchenko, J. S. Strazanac, A. Zaldivar-Riveron, and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Annuals of the Entomological Society of America 105: 165-178.

Benrey, B., and R. Denno. 1997. The slow-growth-high-mortality hypothesis: a testing using the cabbage butterfly. Ecology 78: 987-999.

Carson, S. L. 2013. Spatiotemporal dynamics and host selection of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae, at Point Pelee National Park, Ontario, Canada. PhD dissertation.

Chakraborty, S., J. Whitehill, A. Hill, S. Opiyo, D. Cipollini, D. Herms, and P. Bonello. 2014. Effects of water availability on emerald ash borer larval performance and phloem phenolics of Manchurian and black ash. Plant, cell, & environment DOI: 10.1111/pce.12215.

Chen, Y., M. D. Ulyshen, and T. M. Poland. 2012. Differential utilization of ash phloem by emerald ash borer larvae: ash species and larval stage effects. Agricultural and Forest Entomology 14: 324-330.

Cipollini, D., Q. Wang, J. Whitehill, J. Powell, P. Bonello, and D. Herms. 2011. Distinguishing defensive characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. Journal of Chemical Ecology 37: 450-459.

Duan, J. J., L. S. Bauer, K. J. Abell, and R. van Driesche. 2012. Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States. BioControl 57:199-209.

Eyles, A., W. Jones, K Riedl, D. Cipollini, S. Schwartz, K. Chan, D. Herms, and P. Bonello. 2007. Tree phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). Journal of Chemical Ecology 33: 1430-1448.

Fung, S.-Y., and W. M. Herrebout. 1987. Coumarins in *Prunus mahaleb* and its herbivore, the small ermine moth *Yponomeuta mahalebellus*. Journal of Chemical Ecology 13: 2041-2047.

Gols, R., L. M. A. Witjes, J. J. A. van Loon, Maarten A. Posthumous, M. Dicke, and J. A. Harvey. 2008. The effect of direct and indirect defenses in two wild brassicaceous plant species on a specialist herbivore and its gregarious endoparasitoid. Entomologia Experimentalis et Applicata 128: 99-108.

Gould, J. S., L. S. Bauer, J. Lelito, and J. Duan. 2013. Emerald ash borer biological control release and recovery guidelines. USDA, APHIS, FS Northern Research Station, and Agricultural Research Service. pp. 1-76.

Hill, A. L., J. G. Whitehill, S. O. Opiyo, P. L. Phelan, and P. Bonello. 2012. Nutritional attributes of ash (*Fraxinus* spp.) outer bark and phloem and their relationships to resistance against the emerald ash borer. Tree physiology 32: 1522-1532.

Jennings, D. E., J. R. Gould, J. D. Vandenberg, J. J. Duan, and P. M. Shrewsbury. 2013. Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (*Agrilus planipennis*). PLOS One 8: e83491.

Klooster, W. S., D. A. Herms, K. S. Knight, C. P. Herms, D. G. McCullough, A. Smith, K. J. K. Gandhi, and J. Cardina. 2014. Ash (*Fraxinus* spp.) mortality, regeneration, and seed bank dynamics in mixed hardwood forests following invasion by emerald ash borer (*Agrilus planipennis*). Biological Invasions 16: 859-873.

Kruse, J. J., and K. F. Raffa. 1997. Effects of selected Midwestern larval host plants on the performance by two strains of the gypsy moth (Lepidoptera: Lymantriidae) parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). Environmental Entomology 26: 1155-1166.

Mansour, M. H. 1981. Efficiency of two allelochemics on the conversion of ingested and digested food into the body tissues of *Spodoptera littoralis* (Boisd.) (Lepid., Noctuidae). Journal of Applied Entomology 92: 493-499.

Meyhofer, R., and J. Casas. 1999. Vibratory stimuli in host location by parasitic wasps. Journal of Insect Physiology 45: 967-971.

Meisner, J., and U. Skatulla. 1975. Phagostimulation and phagodeterrency in the larva of the gypsy moth, *Porthetria dispar* L. Phytoparasitica 3: 19-26.

NPGS Ash Conservation Project, The. 2014. United States Department of Agriculture (USDA) Agricultural Research Service http://www.ars.usda.gov/sp2UserFiles/Place/36251200/Ash_Project/MapPage.html#nogo

Silva, M.C.P., W.R. Terra, and C. Ferreia. 2006. Absorption of toxic beta-glucosides produced by plants and their effect on tissue trehalases from insects. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 143: 367-373.

Tanis, S., and D. G. McCullough. 2012. Differential persistence of blue ash and white ash following emerald ash borer invasion. Canadian Journal for Forest Research 42: 1542-1550.

Ulyshen, M.D., J. J. Duan, and L. S. Bauer. 2010. Interaction between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae). Biological Control 52: 188-193.

Ulyshen, M. D., R. W. Mankin, Y. Chen, J. J. Duan, T. M. Poland, and L. S. Bauer. 2011. Role of emerald ash borer (Coleoptera: Buprestidae) larval vibrations in host-quality assessment by *Tetrastichus planipennisi* (Hymenoptera: Eulophidae). Journal of Economic Entomology 104: 81-86.

Werren, J. H., M. J. Raupp, C. S. Sadof, and T. M. Odell. 1992. Host plants used by gypsy moths affect survival and development of the parasitoid *Cotesia melanoscela*. Environmental Entomology 21: 173-177.

Whitehill, J., A. Popova-Butler, K. Green-Church, J. Koch, D. Herms, and P. Bonello. 2011. Interspecific proteomic comparisons reveal ash phloem genes potentially involved in constitutive resistance to the emerald ash borer. PLOS One DOI: 10.1371/journal.pone.0024863.

Whitehill, J., S. Opiyo, J. Koch, D. Herms, D. Cipollini, and P. Bonello. 2012. Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to emerald ash borer. Journal of Chemical Ecology 38: 499-511.

Yang, Z.-Q., J. Strazanac, Y.-X. Yao, and X.-Y. Wang. 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). Proceedings of the Entomological Society of Washington 108: 550-558.

Yang, S., J. J. Duan, T. Watt, K. J. Abell, and R. Van Driesche. 2012. Response of an idiobiont ectoparasitoid, *Spathius galinae* (Hymenoptera: Braconidae), to host larvae parasitized by the koinobiont endoparasitoid *Tetrastichus planipennisi* (Hymenoptera: Eulophidae): implications for biological control of emerald ash borer (Coleoptera: Buprestidae). Environmental Entomology 41: 925-932.

Figure 4.1 Distribution of blue ash (*Fraxinus quadrangulata*) in North America (USDA 2014).

APPENDIX

APPENDIX

Field: infestation of standing ash trees with EAB eggs

Two egg cohorts 3-6 and 4-7 days old were received 23 and 31 May 2013, respectively from the USDA Forest Service at Michigan State University, East Lansing, Michigan. After incubation of eggs to maturity, they were taken to field sites and attached to eleven blue ash and eleven green ash. The first egg cohort was transferred to ash trees between 28-30 May, while the second cohort was transferred to ash trees from 5-6 June following a modified procedure described by Abell et al. (2012). The same number of blue and green ash trees were infested with eggs on alternate days during the set up. The target egg density transferred on all trees was 54 EAB/m^2 on a 1.5 m main stem length (cage area) beginning 0.3 m above the soil line (Table Appendix I). Densities were achieved by grafting a set number of eggs on each main stem based on the surface area (diameter at breast height (DBH) and 1.5 m main stem length).

Beginning at 0.30 m above soil line, four eggs were transferred, one in each cardinal direction, onto ash trees at 0.19-0.30 m (Table Appendix II) above the bottom of the cage area, which created an egg band. Cotton balls and tree warp (Jobe’s Tree Wrap, Easy Gardener Products Inc., Waco, TX) were used to snuggly hold individual eggs against tree bark and protect eggs from predation (Abell et al. 2012). Tree wrap was attached to trees with staples. Individual ash trees had at least five egg bands per tree (Table Appendix II) dependent on diameter at breast height (DBH). The last egg band varied in the egg quantity between 1-4 eggs based on amount of eggs per tree (Table Appendix II).

Field: building cages around trunks of trees

On 8-12 July 2013, tree wrap, coffee filter paper, and EAB eggs were removed from study trees four weeks after eggs were transferred to ash trees (Figure I). Egg location on ash stems was marked with paint. Recovered EAB eggs were inspected for emergence holes underneath a dissecting scope at our lab in order to estimate an egg hatch rate. Cages were then constructed around ash trees to prevent natural enemies (woodpeckers, parasitoids, etc.) from consuming or parasitizing EAB larvae. A band of silicone was applied around the circumference of trees and a strip of foam was used to cover the silicone 0.3 m below the lowest band and above the highest egg band (Figure Appendix I). The silicone and foam filled in cracks and crevices in bark and firmly secure cages to trees (Abell et al. 2012). Six, 12.5 gauge galvanized bailing wire were cut into 0.45 m lengths and bent into D-shapes for each tree. The three pairs of D-rings were divided, spread, and stapled evenly between the foam strips to provide a frame for cages. Organdy cloth was stapled around the trunk of trees and duct taped down to foam strips (Figure Appendix II). Additionally, the duct tape was stapled to the tree to prevent tape from peeling off. Cloth ends were folded onto themselves and stapled along the seam to enclose cages.

Literature cited

Abell, K., J. Duan, L. Bauer, J. Lelito, and R. Van Driesche. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). Biological Control 63: 320-325.

Table Appendix I Number of eggs and bands per tree based on tree diameter and area of 1.5 m section for green and blue ash infestation May and June 2013.

Tree Diameter (DBH) (cm)	Area of 1.5 m section (m^2)*	EAB Eggs**	Bands***
8	0.377	20	5
9	0.424	23	6
10	0.471	25	7
11	0.518	28	7
12	0.564	30	8

*= (Diameter/100)*3.14*1.5 m, **=Area*54 EAB/m^2, ***= EAB Eggs per tree/4 (eggs per band)

Table Appendix II Distance between egg bands and band height (m) starting at 0.3 m on green and blue ash for egg infestation May and June 2013.

Number of Bands	**Distance between bands (m)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
5	0.30	0.60	0.90	1.20	1.50	1.80	-	-	-
6	0.25	0.55	0.80	1.05	1.30	1.55	1.80	-	-
7	0.21	0.51	0.72	0.93	1.14	1.35	1.56	1.77	-
8	0.19	0.49	0.68	0.87	1.06	1.25	1.44	1.63	1.82

Figure 4.2 Figure Appendix I Silicone and foam addition and egg band removal on a green ash tree at Celery bog, July 2013.

Figure 4.3 Figure Appendix II Completed cage on a green ash tree at Celery bog, July 2013.