ABSTRACT

Title of Thesis: EFFECT OF HABITAT FACTORS AND HOST PLANT ON NATURAL ENEMIES OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) AND IMPLICATIONS FOR BIOLOGICAL CONTROL

Jacqueline N. Hoban, Master of Science, 2017

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In an effort to manage the invasive wood-boring buprestid, emerald ash borer (*Agrilus planipennis*), biological control efforts have resulted in the release of four classical biological control agents. Emerald ash borer is known to infest ash trees (*Fraxinus spp.*) in both urban and natural habitats and, recently emerald ash borer has expanded its host range to include white fringetree (*Chionanthus virginicus*). While these parasitoids have been studied in forested habitats, little is known about their efficacy in urban landscapes and in white fringetree. Here, I evaluated the efficacy of *Tetrastichus planipennisi* an introduced, larval endoparasitoid, at parasitizing emerald ash borer in white fringetree. Additionally, I released *T. planipennisi* at sites along an urbanization gradient in Maryland and northern Virginia and evaluated habitat factors for their effect on emerald ash borer and its natural enemies. From my results,

implications for biological control and management of emerald ash borer are discussed.

EFFECT OF HABITAT FACTORS AND HOST PLANT ON NATURAL ENEMIES OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) AND IMPLICATIONS FOR BIOLOGICAL CONTROL

by

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Introduction

Invasive species, organisms either intentionally or inadvertently introduced into novel environments in which they thrive, threaten ecosystems across the globe (Mack et al. 2000). Due to the increased globalization of anthropogenic activities and commerce, instances of the establishment of invasive species have increased rapidly over the last century (Mack et al. 2000). Invasive biota are able to thrive in their new environments due to factors such as a lack of top-down and bottom-up control mechanisms resulting from an unshared co-evolutionary history (Mack et al. 2000). Insects and other arthropods make up a large portion of invasive organisms across the globe (Pimentel et al. 2001) and can have significant detrimental effects on their invaded ecosystems (Kenis et al. 2009).

Considered to be one of the most destructive forest pests in North America, the emerald ash borer (*Agrilus planipennisi* Fairmaire), an Asian wood-boring beetle (Coleoptera: Buprestidae), was first discovered decimating ash trees near Detroit, Michigan in 2002 (Haack et al. 2002). Emerald ash borer was inadvertently introduced from northeast Asia via wooden packing material into the Detroit area and has since spread to 31 states within the U.S. and 2 Canadian provinces. Emerald ash borer is responsible for the death of hundreds of millions of ash trees (*Fraxinus spp.*) in North America (Herms and McCullough 2014). Since its discovery, many institutions have participated in comprehensive investigation efforts to mitigate the spread and damage of emerald ash borer. These research efforts have improved our understanding of emerald ash borer's biology and life history, both in its original host range in Asia and in its new range in North America.

During early summer months, emerald ash borer adult females lay their eggs in the bark cracks of ash trees (*Fraxinus spp.*). Upon hatching, the larvae bore into the tree and feed on the phloem tissue. Larval feeding causes damage in the form of frass-packed galleries that effectively girdle the trees causing crown dieback, and at high infestation levels, tree death (Poland and McCullough 2006). Due to a shared co-evolutionary history and evolved resistance, Asian ash trees (i.e. *Fraxinus chinensis*, *Fraxinus mancshurica*) are typically only susceptible to damage by emerald ash borer if the trees are unhealthy, because their defenses against herbivory have been lowered (Liu et al. 2007, Rebek et al. 2008, Cipollini et al. 2011, Poland et al. 2015, Villari et al. 2015). Unlike Asian ash trees, healthy North American ash trees, especially green ash (*F. pennsylvanica*) and white ash (*F. americana*), are highly susceptible to emerald ash borer infestation and subsequent damage (Rebek et al. 2008, Poland et al.

2015, Villari et al. 2015). Emerald ash borer infestation in vulnerable North American ash trees can result in tree mortality in as a little as one year (Poland and McCullough 2006). In addition to the lack of bottom up control, emerald ash borer has been geographically isolated from parasitism and predation by its co-evolved, specialist natural enemies (enemy release hypothesis), although some indigenous, generalist parasitoids and predators have been observed attacking emerald ash borer in North America (Bauer et al. 2005, Cappaert et al. 2005, Kula et al. 2010, Duan et al. 2013a, 2014, 2015, Bauer et al. 2015). As a result, emerald ash borer populations have grown and spread rapidly in its novel host range.

In an effort to reduce population growth and spread, both chemical and biological control methods have been implemented against emerald ash borer. Chemical control methods have proven to be effective against emerald ash borer and have been utilized heavily in urban landscapes (McCullough et al. 2011, Herms et al. 2014, Poland et al. 2016), but are impractical in forests, where the majority of ash trees are located (Poland and McCullough 2006). As a result, classical biological control, the introduction and establishment of natural enemies from the invader's native host range, has been implemented against the emerald ash borer. Four parasitoid species have been identified in Northeast Asia as host-specific, promising classical biological control agents of emerald ash borer (Liu et al. 2003, 2007; Duan et al. 2012, Belokobylskij et al. 2012). These include *Spathius agrili* Yang (Hymenoptera: Braconidae), *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae), *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae). Following extensive host-specificity studies and environmental impact assessment, the U.S. regulatory agency approved the environmental release of the three Chinese parasitoids in 2007 and *S. galinae* in 2015 in continental U.S. (Bauer et al. 2005, 2008, 2015, Duan et al. 2014, 2015, 2017). Of the four introduced biological control agents, *T. planipennisi* has thus far demonstrated the most success in terms of establishment, recovery, and dispersal, having been recovered consistently year to year and in control plots several kilometers away from release plots (Bauer et al. 2015, Duan et al. 2015, Jennings et al. 2016b). *Tetrastichus planipennisi* is a gregarious, larval endoparasitoid, which specializes in parasitism of third and fourth instar emerald ash borer larvae. The release and recovery of these classical biological control agents have been a major part of ongoing research into evaluating the spread, growth, and impact of emerald ash borer populations.

Despite over a decade of research, it was not until the summer of 2014 in Ohio that emerald ash borer was discovered infesting native North American white fringetree (*Chionanthus virginicus* L.), the first tree species outside of the genus *Fraxinus* known to host emerald ash borer in North America (Cipollini 2015). Both host plant genera are in the family Oleaceae and are closely related (Wallander and Albert 2000), but have different growth habits. The traditional host, *Fraxinus spp*., grows as a typical tree, while white fringetree tends to be more shrub-like and multi-stemmed. White fringetree's native range extends from Massachusetts to Texas, but it is planted in the urban landscape outside of this eastern distribution, overlapping with the range of various ash species and emerald ash borer's current distribution (Thiemann et al. 2016). Since the initial discovery in Ohio, emerald ash borer has

been confirmed infesting white fringetrees in Illinois, Indiana, Pennsylvania (Cipollini and Rigsby 2015, Thiemann et al. 2016, Peterson and Cipollini 2017, Rutledge and Arango-Velez 2017), and most recently Maryland (personal observation) and North Carolina and Michigan (personal communication Cipollini).

While emerald ash borer is able to complete its development in this newly acquired host both in the lab and in the field, little emerald ash borer-caused mortality has been observed in white fringetree in the field (Cipollini and Rigsby 2015). Emerald ash borer has been shown to develop more slowly and experience higher mortality in white fringetree than in ash trees (Cipollini and Rigsby 2015, Rutledge and Arango-Velez 2017). Interestingly, emerald ash borer is unable to develop in the Chinese fringetree (*Chionanthus retusus*) from its native range, under any conditions (Cipollini and Rigsby 2015), which may relate to their shared evolutionary history.

When an organism shifts to a novel host (host shift or host expansion), it is hypothesized that they create enemy free space (EFS) defined as "ways of living that reduce or eliminate a species' vulnerability to one or more species of natural enemies" (Jeffries and Lawton 1984). To date no emerald ash borer larvae have been recorded as parasitized from white fringetree in the field (personal communication, Cipollini). With that in mind, I ask the question: when emerald ash borer has had no co-evolutionary history with this newly acquired host plant genera, will its native, specialist natural enemy introduced from Asia, be able to detect white fringetree as an emerald ash borer host plant and if so, successfully attack emerald ash borer larvae at similar rates to emerald ash borer in green ash? If not, then emerald ash borer may be able to utilize white fringetree as a novel host plant to escape parasitism and create enemy free space.

To address this question, I designed laboratory choice and no-choice host plant assays to evaluate the attack rate of emerald ash borer larvae infesting the two different host plant species by the introduced biocontrol agent *T. planipennisi.* I first conducted a preliminary no-choice exposure assay to demonstrate the ability of *T. planipennisi* to parasitize and develop in emerald ash borer larvae infesting white fringetree. I then conducted a choice bioassay to further examine the host finding behavior and attack (parasitism) rate of *T. planipennisi* when given a choice between emerald ash borer-infested green ash versus white fringetree. Additionally, a second choice assay was performed in which I compared the behaviors, performance, and parasitism rates of *T. planipennisi* when given a choice between emerald ash borer infested host plants, green ash and white fringetree, where I controlled for emerald ash borer larval development.

Materials and Methods

Parasitoids

Tetrastichus planipennisi used in this study were produced and supplied by the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) EAB Parasitoid Rearing Facility in Brighton, MI. The parasitoids were shipped as late stage larvae or pupae

within EAB-infested bolts of ash. The wasps were reared out from the ash bolts in environmental chambers at 30 °C. The emerged wasps were maintained at 25 °C, provided honey on the screens of containment cups, and held for at least one week to ensure mating prior to exposure to host larvae. Naive, adult females one to three weeks of age were used in exposures during bioassay experiments.

Host Plants

Small branches or "sticks" were cut from EAB host trees and used in the lab studies. Sources of white fringetree were obtained from trees purchased from Stadler Nurseries in Laytonsville, MD. Plants were maintained in pots outside at the University of Maryland (UMD) Research Greenhouse facility and provided water and fertilizer as needed. Sources of green ash were from trees grown outside of the Beneficial Insect Research Lab in Newark, DE and from small ash trees on the UMD campus. To remove any potential pathogens or other pests from the sticks, each stick was scrubbed under running cold water and then soaked in a 10% bleach solution for 15 minutes and rinsed thoroughly. Cut sticks were kept in containers with a shallow level of water to retain moisture throughout the experiment.

No-Choice and Choice Assay Experimental Procedures and Data Collection

I presented *T. planipennisi* to emerald ash borer infested sticks (exposure) of both white fringetree and green ash separately (no choice) and together (choice). In these studies green ash and white fringetree sticks were between 1.27-2.54 cm diameter and cut into 18 cm lengths. These sticks were then each infested with five to seven emerald ash borer eggs that were on pieces of coffee filter paper. Paper was grafted onto the bark with parafilm according to the protocol by Duan et al. (2013b). The logs were then placed in a 30 ^{o}C chamber with florafoam and water to allow emerald ash borer larvae to develop to late instars (L3 and L4), the stages most susceptible to *T. planipennisi*. Since emerald ash borer developmental rates are known to differ between host plants (slower on white fringetree than ash) (Cipollini and Rigsby 2015, Rutledge and Arango-Velez 2017), an attempt was made to standardize the larval life stages to which *T. planipennisi* were exposed. For the no-choice assay, sticks of ash and white fringetree were infested at the same time and exposed to the parasitoids at two time steps 7 days apart. In the subsequent choice assay I infested the white fringetree sticks 10 days before the green ash sticks. At the exposure time for both assays, subsets of the sticks were destructively sampled to quantify emerald ash borer larval stages.

Exposures to *T. planipennisi* adults were performed in exposure cages that were constructed from the bottoms of two crisper boxes where one box served as the bottom and the other the top of the exposure cage. Boxes were sealed with tape and parafilm to prevent wasp escape and each cage had small mesh ventilation windows. Sticks were wrapped in wet paper towel at the base and parafilmed in small petri dishes to retain moisture. Individual sticks were placed in the center of each half of a cage in the choice assays and directly in the center during the no choice assays.

Wasps were added to each exposure cage at approximately a 1:1 host-to-parasitoid ratio based on estimates of the number of hosts available by counting emerald ash borer egg hatching rates in the sticks. After five days, the wasps were removed and each stick was relocated into individual emergence tubes. Emergence of wasps from each stick was monitored and recorded. Emerged wasps were preserved in 70% ethanol for fitness measurements. After a minimum of 8 weeks post-exposure, the sticks were then debarked and the stages (L1, L2, L3, L4, JL) and fates (dead, alive, parasitized, diseased) of the emerald ash borer larvae were recorded. There were 15 replicates of the no choice assays performed at two time steps, which were eventually pooled to amount to 30 replicates. There were 15 replicates of the choice assay.

Choice and Behavioral Observation Assay Experimental Procedure

and Data Collection

A second choice assay was performed to compare the ability of *T. planipennisi* to detect and parasitize emerald ash borer larvae when given a choice in emerald ash borer host plant. Additionally, to determine if emerald ash borer host plant affected the behavior of *T. planipennisi*, behaviors were observed and quantified during the choice assay. To standardize emerald ash borer larval size between host plant treatments, larvae were reared in tropical ash (*Fraxinus udheii*) sticks in a 30 ^{o}C environmental chamber (ARB-366, Percival Scientific Inc., Perry, Iowa, USA) until they reached third and fourth instars (~three to four weeks), the preferred stages for parasitism by *T. planipennisi* (Ulyshen et al. 2010a). They were then inserted into artificial grooves in the two host plant types (treatments), green ash and white fringetree, using methods outlined in Ulyshen et al. (2010b). Each stick contained one emerald ash borer larvae enclosed in the grooves with parafilm. Both species of sticks were previously cut to approximately 12.7 cm in length and were between 1.27 cm and 2.54 cm in diameter. The sticks infested with emerald ash borer larvae were then placed in florafoam and water in a 30 ^{o}C environmental chamber for five days. After five days, the parafilm was removed to check for host feeding by the emerald ash borer larvae and only those sticks with evidence of feeding (frass-packed grooves (Fig. 1.1) were used. Sticks were resealed with a thin layer of parafilm (maximum of two wraps around) and prepared for exposure to *T. planipennisi*. Investigations have shown that *T. planipennisi* readily parasitize through thin layers of parafilm (personal communication, Duan). For each tree species, three sticks were wrapped in paper towel at the base and placed in urine sample cups filled with water and rockwool and then wrapped in parafilm to maintain moisture. Each set of 3 sticks (experimental unit) of each of the 2 tree species (treatment) were placed at opposite corners of a parasitoid exposure container (replicate). A total of 20 replicates were performed among three time blocks.

Figure 1.1. A. Emerald ash borer larva inserted into artificial gallery in white fringetree stick prior to feeding. **B.** Frass-packed artificial gallery in white fringetree stick as evidence of feeding by emerald ash borer larvae five days post insertion.

Parasitoid exposures to emerald ash borer larvae were performed in cubic boxes that were constructed using 2-mm thick clear acrylic sheets (30 × 30 × 30 cm). Every cage had three mesh ventilation windows (10 × 10 cm) and one door (20 × 20 cm) which was sealed with multipurpose plastic wrap (Press'n Seal, Glad, Broadway, CA, USA). For behavioral observation purposes, the tops of the boxes were divided into six equally wide sections along the diagonal of the box using a black marker and a ruler to create "zones" numbered one to six, corner to corner (Fig. 1.2). The cages were arranged in the environmental chambers such that the ventilations windows from one cage were not directly facing the next cage's ventilation windows to reduce cross contamination of treatments.

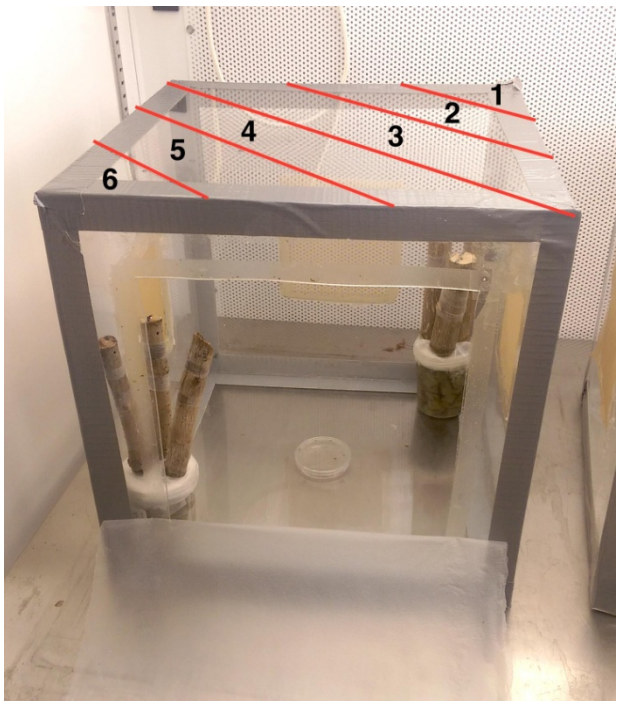


Figure 1.2. Exposure cage set up with "zones" enhanced in red for visual purposes.

Each exposure cage held the three sticks of each tree species, each with one emerald ash borer totaling six emerald ash borer larvae per cage. The green ash cluster was placed in the corner of the cage zoned number 1 and the white fringetree cluster was placed in the corner zoned number 6. Six *T. planipennisi* females (host: parasitoid 1:1) and one male were placed in Falcon dishes and released into the center of each cage. The cages were then observed over time to track female parasitoid movement and activity. At each time interval, each female was recorded as to which zone she appeared in (one to six) and her location such as on green ash stick or white fringetree stick or other (in a zone but not on a stick). These observations were made four times during the first hour post release, twice per hour for the next two hours, and twice per day for the four following days, for a total of five full exposure days. For each observation, if a female was not found in six minutes, the wasp was considered not observed for that time period. If a female wasp was found dead, a new female was added to maintain a 1:1 H:P ratio.

The exposure cages were held in environmental chambers (ARB-366, Percival Scientific Inc., Perry, Iowa, USA) to provide standardized conditions of 25 ^{o}C, 16:8 light to dark photoperiod, and approximately 60 percent relative humidity. A food solution of 50 % honey water was provided in small solo cups with cotton wick when observations were not being made and were removed at least fifteen minutes prior to observations.

The wasps were removed after five days of exposure to the sticks with emerald ash borer larvae. The stick bundles (3 sticks combined) were then placed in their own emergence tubes. The tubes were monitored for parasitoid emergence and both male and female progeny were counted and recorded. After a minimum of eight weeks post-exposure, the sticks were then debarked and the stages (L3, L4, JL) and fates (dead, alive, parasitized, diseased) of the emerald ash borer larvae were recorded.

Statistical Analysis

The preliminary choice and no-choice parasitism data were analyzed using PROC GLIMMIX as binomial distributions in SAS® Studio 3.6 University Edition as a part of SAS Institute® to determine if there were differences in parasitism between host plant types. Replicates were considered a random effect for both assay types, and the no-choice was blocked by exposure time. Additionally in SAS® University Edition, a TTEST was performed to determine if there were differences in the number of wasp progeny emerged from select replicates in the choice assay in which parasitism occurred in pairs of both host plant species. Emerald ash borer larval weights assessed at the time of exposure were analyzed in SAS University Edition as two-way ANOVAs.

The choice and observation assay analyses were performed in SAS® Studio 3.6 University Edition as a part of SAS Institute® using PROC GLIMMIX as binomial distributions for parasitism and all observation data, with the exception of

observations over time. Observation analyses were performed on all zones, but were not significant and were then combined to create three zones: GA (zones 1 & 2), WF (zones 5 & 6), and the middle, larger buffer zone (zones 3 & 4). All multiple mean comparison test p-values were adjusted with Tukey-Kramer HSD. Observations made over time were analyzed using JMP Pro 13.01 as a part of SAS Institute® in a mixed linear model with replicates treated as random effect and observation number treated as a repeated measure. The proportion of the test wasps (n=6 per replicate) landing on green ash sticks or white fringetree sticks were Arcsine transformed. Analysis tested for the main effects of treatment (host plant), and observation day, and any interaction between treatment and observation day.

Results

No-Choice and Choice Assays

In the no-choice assay, there were significant differences in emerald ash borer larval weight between treatment types at the time of exposure to parasitoids, with higher weight in larvae reared in green ash than white fringetree ($F_{1,46}$ $p<0.0008$) (Fig. 1.3). Emerald ash borer larvae in both white fringetree and green ash were parasitized, and parasitism rate was not significantly different among the two host plant treatments ($F_{1,28}$ 1.95, $P<0.1736$) (Fig. 1.4).

The choice assay showed similar results in regards to emerald ash borer larval weight at the time of exposure to parasitoids, resulting in significantly higher larval weight in green ash than white fringetree ($F_{1,15}$ $p<0.0017$) (Fig. 1.3). When given a choice of emerald ash borer infested host plant, however, the mean percentage of parasitism was significantly higher in green ash than white fringetree ($F_{1,24}$ 4.41, $P<0.0464$) (Fig. 1.4). From the replicates that had parasitism in larvae from both green ash and white fringetree, there were numerically more progeny produced per parasitized emerald ash borer larvae in green ash than white fringetree, but not significantly so ($F_{3,3}$ $P<0.2347$) (Fig. 1.5).

Choice and Observation Assay

When *T. planipennisi* was given a choice of emerald ash borer larvae of standardized size (L3 and L4) in green ash compared to white fringetree sticks, there were significant differences in overall parasitism rates between the host plant types ($F_{1,36}=15.21$ $P<0.0004$), with higher parasitism in green ash (48.33%) compared to white fringetree (8.33%) (Fig. 1.6). Parasitized green ash larvae produced a mean of 46.13 *T. planipennisi* adult progeny per larva whereas white fringetree produced a mean of 25.60 *T. planipennisi* adult progeny per larva (Fig. 1.7), although the difference was not significant ($F_{1,18}=2.86$ $P<0.1078$).

For the behavioral data analysis, the mean total number of observations of wasps present in each zone (WF, GA, and buffer) was significantly different, ($F_{2,55}$ = 8.14 p<0.0008), although pairwise comparison analyses revealed no significant difference between GA and WF zones (adj Tukeys P<0.9935) (Fig. 1.8). Similar results were observed for observation data from day 1 only, with an overall significant difference between the zones ($F_{2,55}$ = 6.36, P<0.0033), but no significant difference between GA and WF zones (adj Tukeys P<0.9801) (Fig. 1.9).

The mean overall frequency of wasp observations only on green ash sticks (9.55) compared to white fringetree sticks (6.05) was not significantly different ($F_{1,36}$ P<0.1050) (Fig. 1.10). However, analysis of mean proportions of observations of responders (wasps on sticks) on host plants over time (days 1 – 6) showed a significant difference between host plant type ($F_{1, 682}$ 6.56 P<0.0107), where wasps on white fringetree sticks were observed significantly less frequently than on green ash sticks (Fig. 1.11). The main effect of day was also significant ($F_{5, 682}$ 4.15 P<0.0010). The interaction between treatment (host plant) and day was not significant ($F_{5, 682}$ 6.56 P<0.1195). Numerically the mean frequency of wasps observed on each stick type for each observation was higher on green ash than white fringetree at every observation time except observations 14, 15, and 17 (Fig. 1.11).

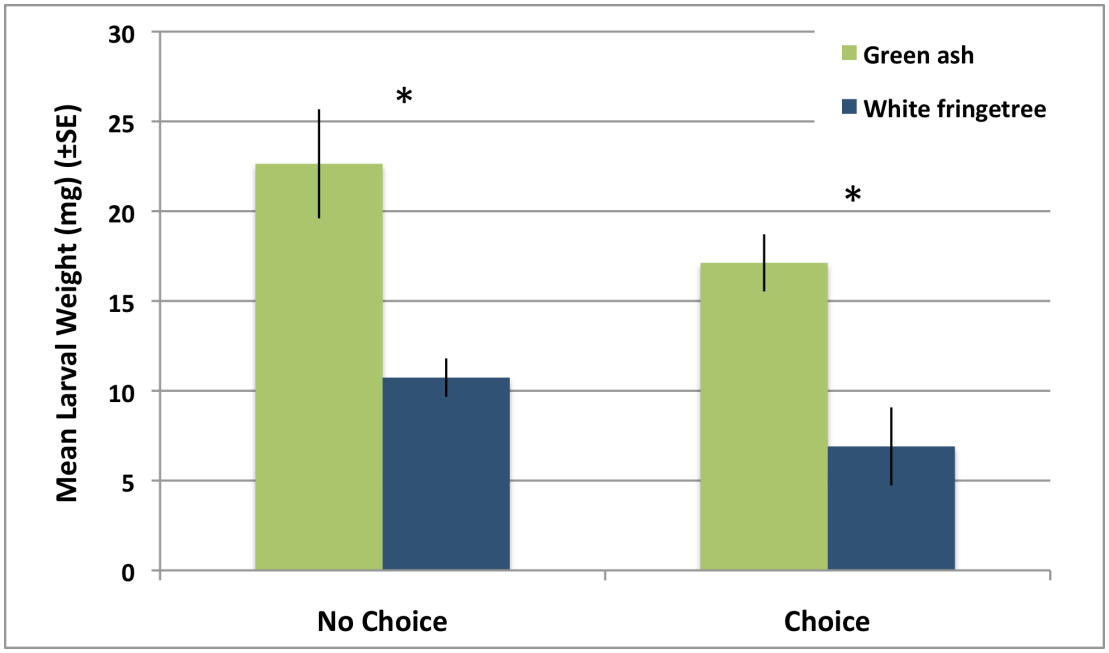


Figure 1.3. Mean (±SE) emerald ash borer larval weight between host plants by assay type during destructive sampling at the time of exposure of emerald ash borer infested sticks to parasitoids. No Choice GA (n=25). No Choice WF (n=23). Choice GA (n=12). Choice WF (n=6). Bars with "*" indicate significant differences between treatments (*P*<0.05).

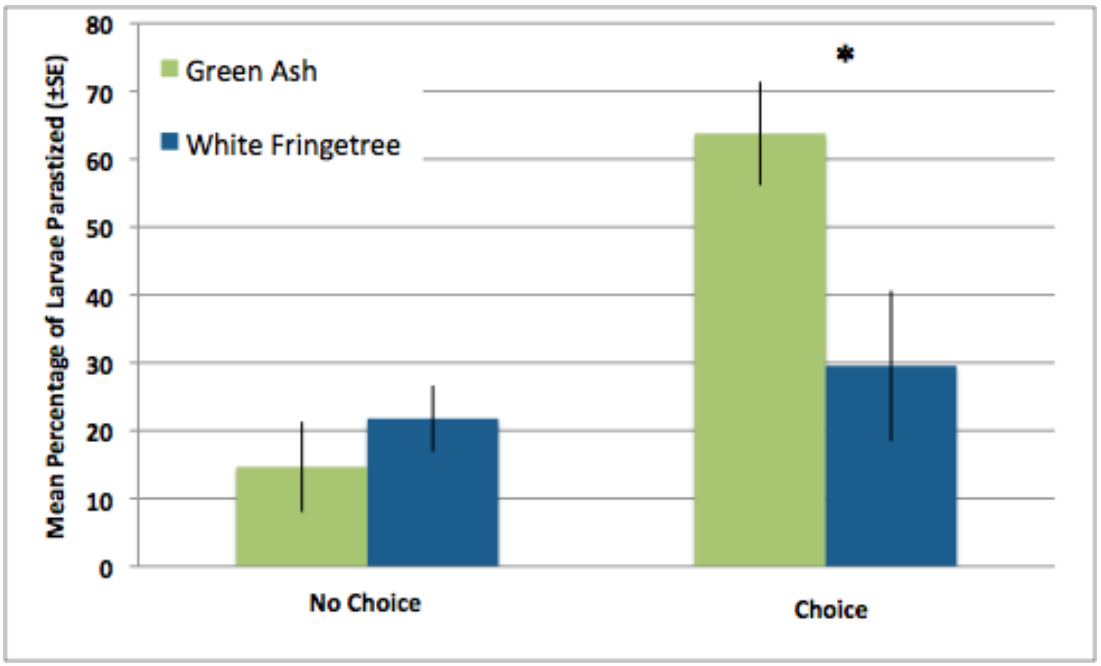


Figure 1.4. Mean (±SE) parasitism rate (%) of emerald ash borer between host plant by assay type. No Choice (n=30). Choice (n=15). Bars with "*" indicate significant differences between treatments (P<0.05).

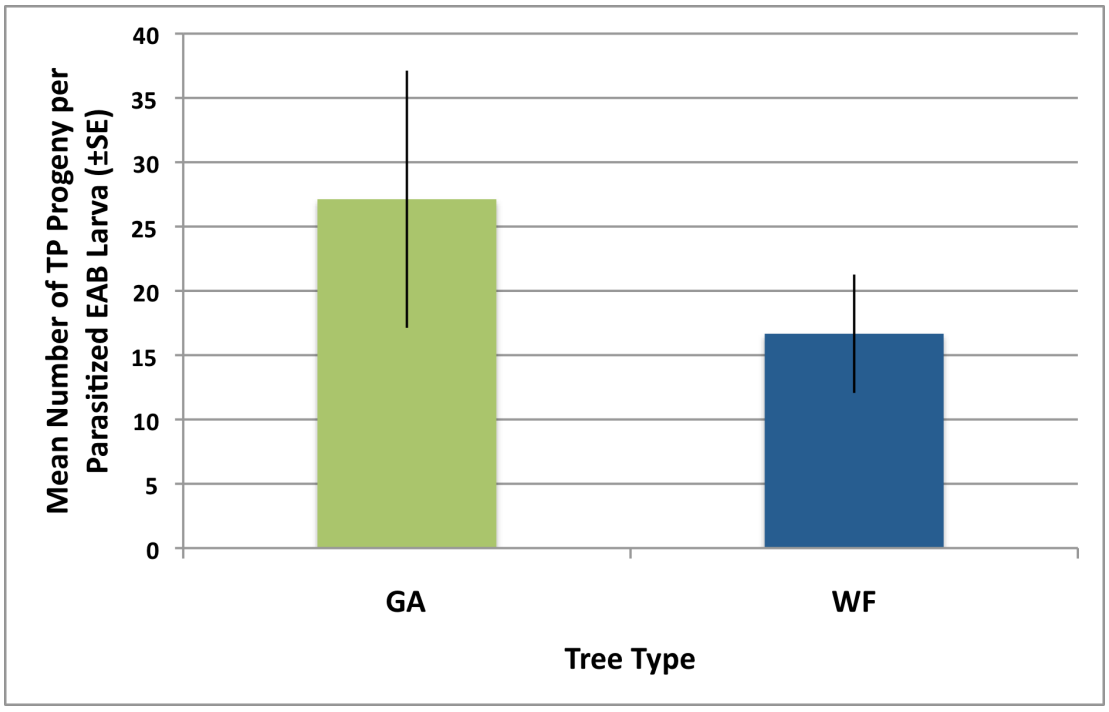


Figure 1.5. Mean (±SE) number of *Tetrastichus planipennisi* (TP) progeny emerged per parasitized emerald ash borer (EAB) larva between hosts in replicates of choice assay that had parasitism in both White fringetree (WF) and Green ash (GA). (n=4). Bars with "*" indicate significant differences between treatments (P<0.05).

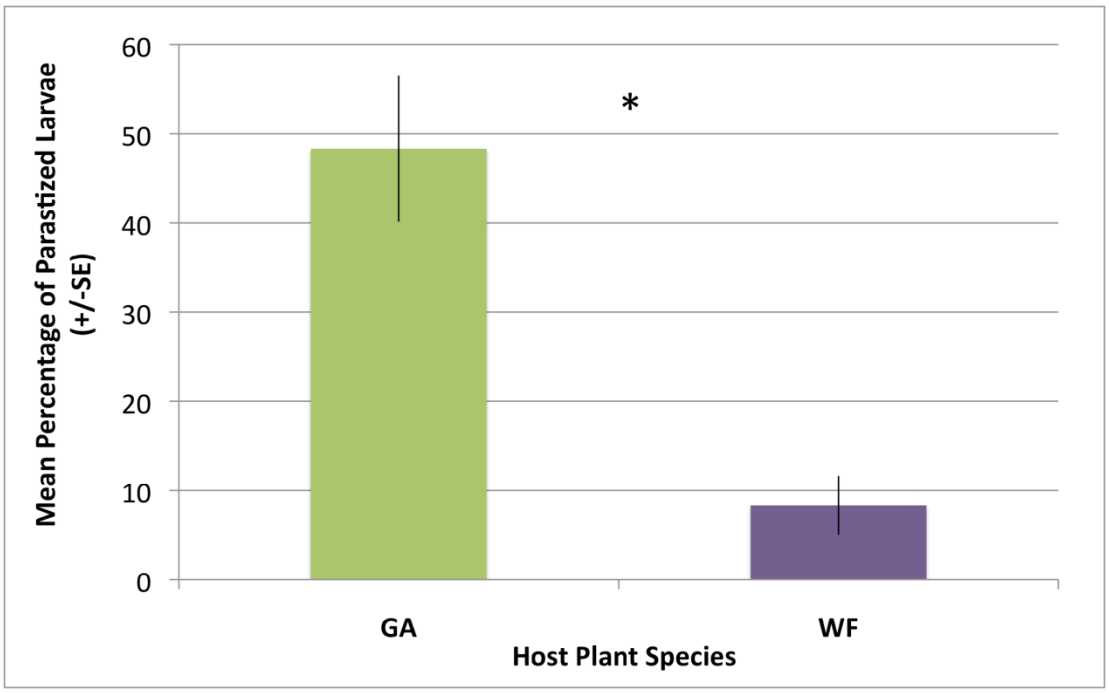


Figure 1.6. Choice/Observation: Mean (±SE) percentage of parasitized emerald ash borer larvae by host plant (GA=green ash; WF=white fringetree). Bars with "*" indicate significant differences between treatments ($P<0.05$).

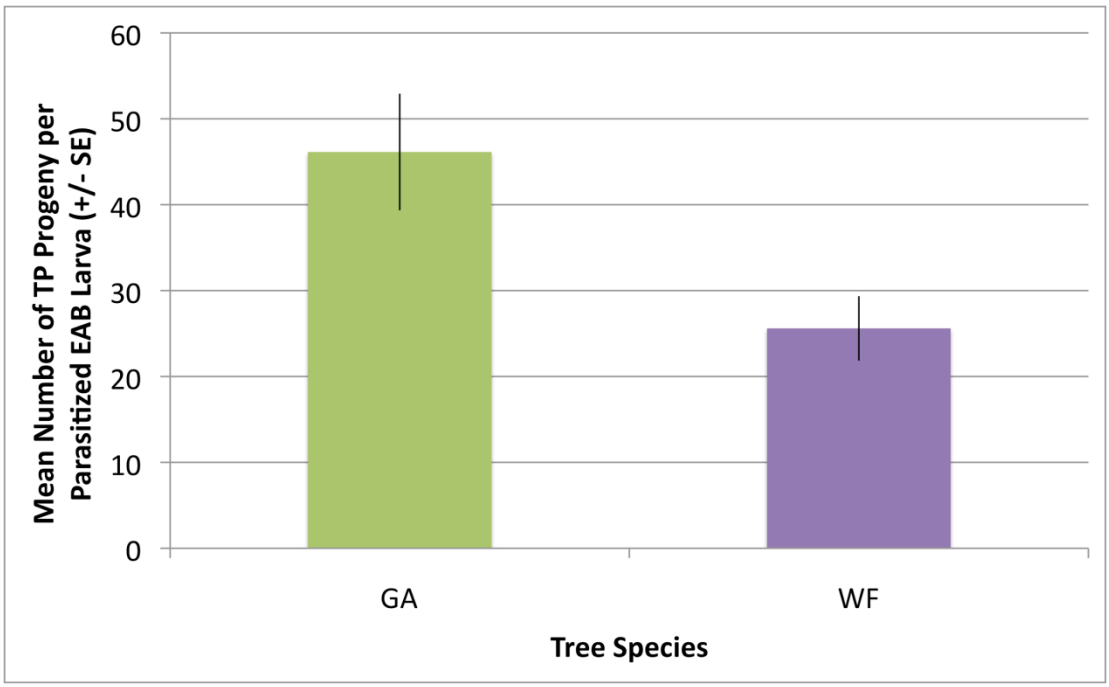


Figure 1.7. Choice/Observation: Mean (±SE) number of *Tetrastichus planipennisi* (TP) progeny per parasitized emerald ash borer larva by host plant (GA=green ash; WF=white fringetree). Bars with "*" indicate significant differences between treatments ($P<0.05$).

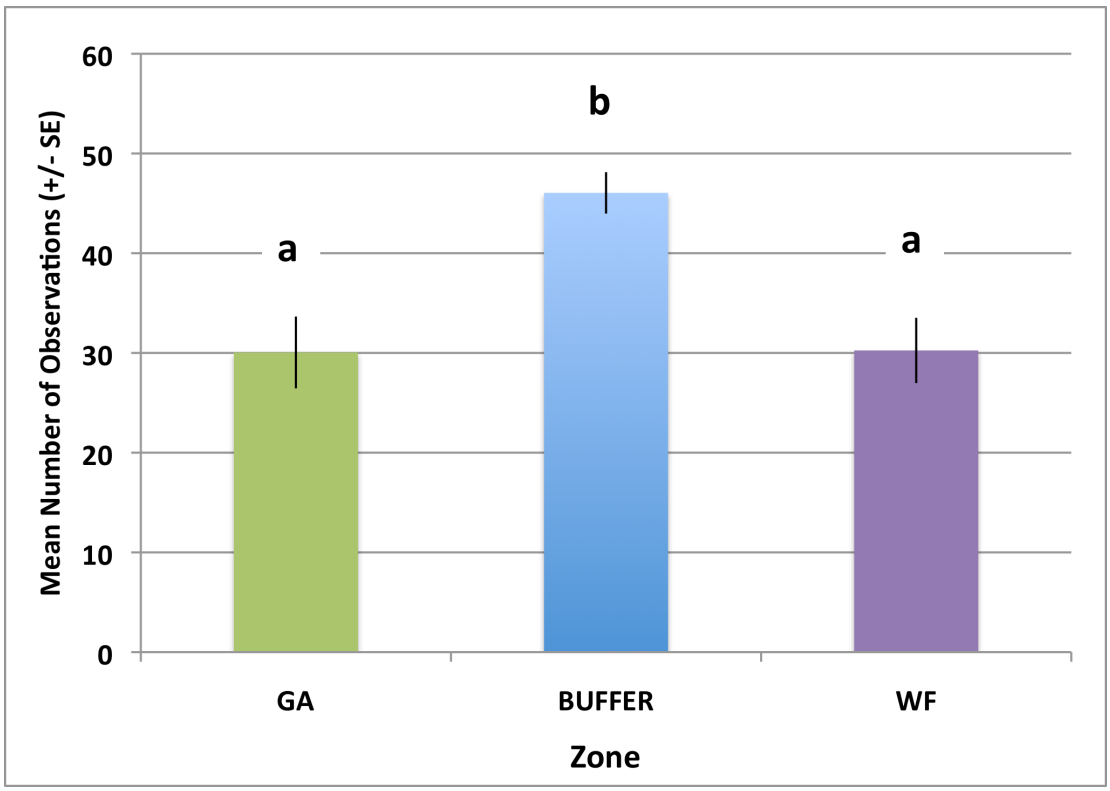


Figure 1.8. Mean (±SE) number of total observations of *Tetrastichus planipennisi* found in each zone (GA=green ash; WF=white fringetree; Buffer=area in cage between host stick areas) pooled over time. Different letters indicate significant differences between treatments ($P<0.05$, adj. Tukey-Kramer).

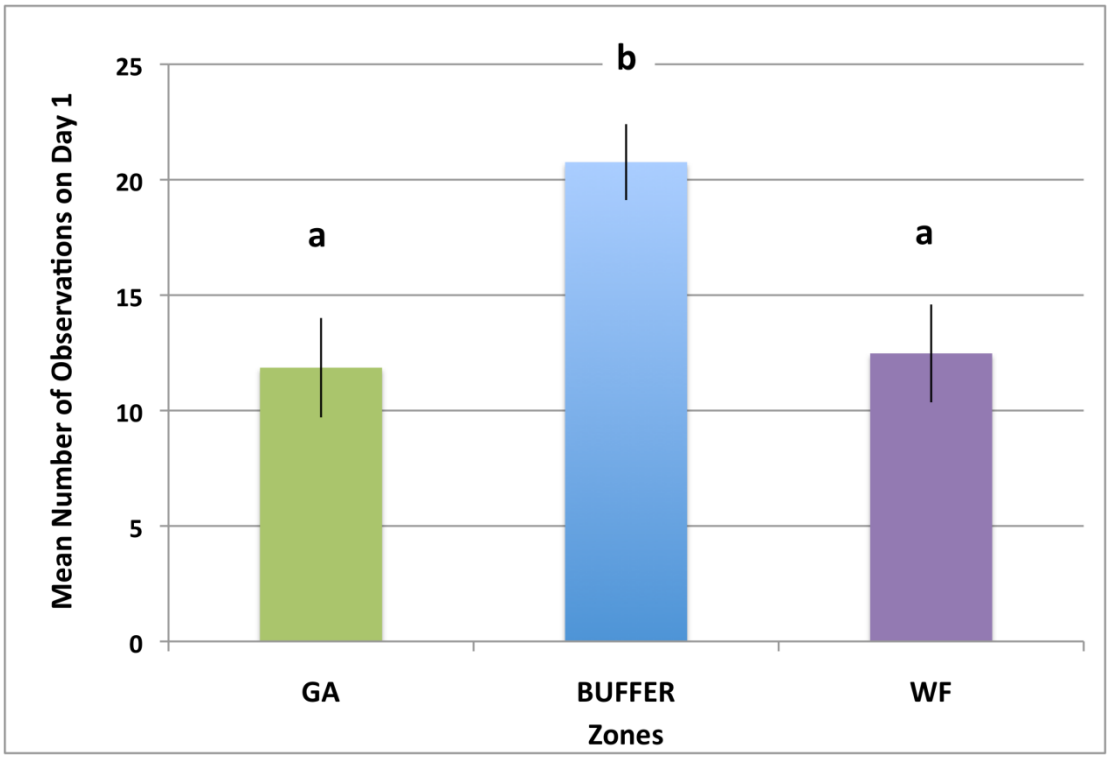


Figure 1.9. Mean (±SE) number observations of *Tetrastichus planipennisi* found in each zone (GA=green ash; WF=white fringetree; Buffer=area in cage between host stick areas) over all day 1 observations. Different letters indicate significant differences between treatments ($P<0.05$, adj. Tukey-Kramer).

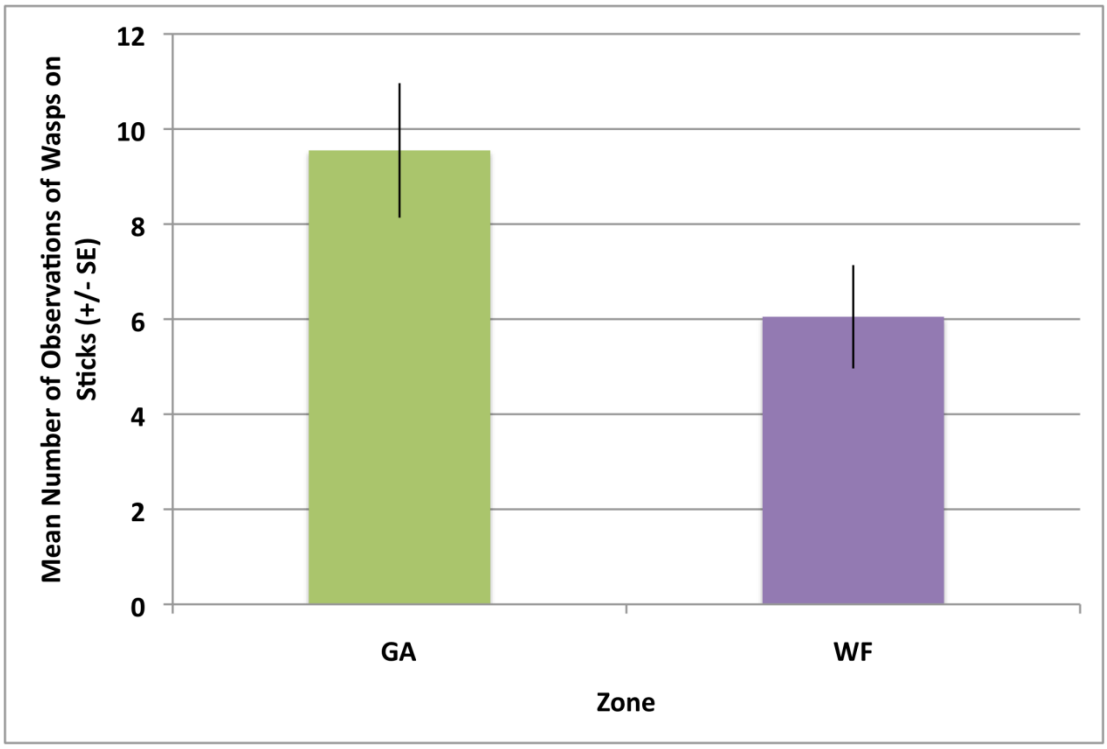


Figure 1.10. Mean (±SE) number of observations of *Tetrastichus planipennisi* wasps on sticks of each tree species (GA=green ash; WF=white fringetree) over all observations. Bars with "*" indicate significant differences between treatments ($P<0.05$).

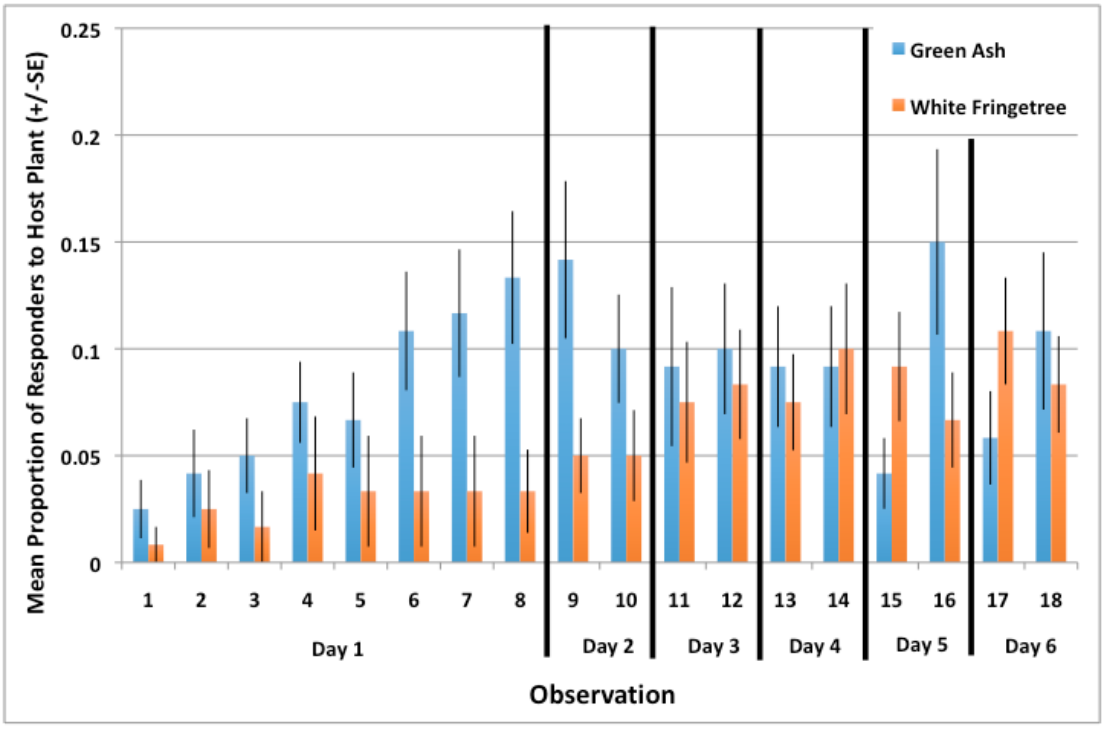


Figure 1.11. The mean (±SE) The proportion of the test wasps (n=6 per replicate) landing on green ash sticks or white fringetree sticks observed over time (observation #). Non-responders were excluded from the figure (wasps not on green ash or white fringetree sticks). Observations were made 4x in the first hour of the exposure, 2x per hour the following two hours, and every 12 hours thereafter. Mixed Linear ANOVA Model performed on Arcsine transformed data with replicate treated as a random effect. The interaction between treatment (host plant) and day was not significant ($F_{5, 682}$ 6.56 P<0.1195). Main treatment (host plant) effect: ($F_{1, 6820}$ 6.56 P<0.0107). The main effect of day was also significant ($F_{5, 682}$ 4.15 P<0.0010).

Discussion

The evidence provided from the preliminary no-choice assay is the first to demonstrate that *T. planipennisi* is able to detect, parasitize, and successfully develop within emerald ash borer larval hosts reared within the novel host plant, white fringetree. Furthermore in the first choice assay, *T. planipennisi* parasitized emerald ash borer larvae in white fringetree when in the presence of emerald ash borer infested green ash in the lab. Although *T. planipennisi* was able to attack emerald ash borer larvae in white fringetree, rates of parasitism were significantly lower in white fringetree than green ash. These findings suggest that emerald ash borer may experience partial enemy free space by utilizing white fringetree as an alternative host to ash. Jeffries and Lawton (1984) suggest that by expanding their host range or shifting hosts, herbivores may benefit by what is known as “enemy free space” (Jeffries and Lawton 1984, Schiers and Bruyn 2002). However, research is mixed in support of the hypothesis that “enemy free space” is generated through host shifting alone (Heard et al. 2006).

The first choice assay found greater parasitism in emerald ash borer larvae in ash than in white fringetree (Fig. 1.4). However, despite experimental attempts, larval size could not be standardized between larvae in the different host plants in both of the preliminary no choice and choice assays (Fig. 1.3). Additionally, the no choice assay had some methodological issues that resulted in low survival of emerald ash borer in green ash, which affected parasitism rates. Emerald ash borer larvae have been shown to develop faster and are larger in ash than white fringetree (Cipollini and Rigsby 2015). Therefore, due to known larval size preferences of *T. planipennisi* (Ulyshen et al. 2010a), from the original choice assay alone it cannot be determined if the parasitoids preferred green ash as a host plant over white fringetree, or rather preferred the larger larvae that developed in green ash.

However, in the second choice assay emerald ash borer larval size in both ash and white fringetree was standardized at the time of exposure to *T. planipennisi* by inserting susceptible stages of emerald ash borer larval hosts (L3 and L4 instars) into the two treatment host plants. Under these conditions significantly higher parasitism by *T. planipennisi* occurred in green ash than white fringetree (Fig. 1.6). Anecdotally, while dissecting the sticks 8 weeks after the exposure to determine parasitism rates, it was noted that the larvae in green ash sticks created longer galleries and were likely developing faster than the larvae present in white fringetree sticks. It is unlikely developmental differences in emerald ash borer larvae were present at the time that *T. planipennisi* were making oviposition choices. It was only 10 days from the time larvae were inserted into the host sticks and the termination of exposure of emerald ash borer to wasps (5 days feeding to establish that larvae took to the transfer and 5 days of exposure to *T. planipennisi*). Moreover, the behavioral observations also indicated a preference for green ash in that *T. planipennisi* were observed more frequently on green ash than white fringetree sticks (Fig. 1.11).

Also of interest is that numerically more *T. planipennisi* progeny emerged from emerald ash borer larvae in green ash than white fringetree. This is not surprising given that emerald ash borer has been shown to develop faster and survive better in ash than white fringetree in laboratory studies (Cipollini and Rigsby 2015, Rutledge and Arango-Velez 2017) and also emerald ash borer in this assay produced longer galleries. It is likely that larvae from ash are healthier and better hosts for *T. planipennisi* than those from white fringetree.

Overall, results of these studies demonstrated that the introduced biological control agent, *T. planipennisi* is able to detect and develop successfully in emerald ash borer larvae in its novel host plant, white fringetree, and likely prefers and performs better in larvae in green ash to white fringetree. The observed decrease in parasitism and reduced frequency of wasps associated with emerald ash borer from white fringetree over green ash indicates that white fringetree may be a source of enemy free space for emerald ash borer. These findings along with a lack of parasitism observed in the field (Peterson and Cipollini 2017, personal communication Cipollini) further support the hypothesis that host shifting or host expansion by a phytophagous insect could create enemy free space from natural enemies. A review of papers examining enemy free space including those as a result of host shifts from 1988 to 2002 by Heard et al. (2006) found similar results in support of enemy free space resulting from host shifting in 9 out of 13 studies.

However, evidence for enemy free space was not consistent across time and space (Heard et al. 2006).

Enemy free space created by a host shift or expansion is hypothesized to be a temporary state as natural enemies adapt to the novel host plant over time (Grosman et al. 2005, Heard et al. 2006). Although emerald ash borer may not have a high impact on white fringetree, as little mortality has been observed in the field (Peterson and Cipollini 2017), its expansion onto white fringetree should be continuously observed. Field observations of emerald ash borer and its natural enemies associated with white fringetree could provide insight into the tri-trophic temporal response to enemy free space associated with a host shift, as studies related to enemy free space hypothesis are often performed long after the host shift has taken place and it is not often clear when or to which host plant the shift or expansion occurred (Heard et al. 2006).

Here all studies, and those of Cipollini (Cipollini and Rigsby 2015, Rutledge and Arango-Velez 2017) that indicated emerald ash borer perform better in ash than white fringetree, were conducted under laboratory conditions. Conditions in the field may alter the behavior of *T. planipennisi* compared with lab conditions. It is important that follow up with field studies or field observations to confirm that these laboratory findings translate into the field. In addition, assays should be performed with emerald ash borer's other introduced larval and egg parasitoids (*Spathius agrili*, *Spathius galinae*, *Oobius agrili*). Further field observations may also provide insight on other indigenous natural enemy induced mortality of emerald ash borer in white fringetree.

Tri-trophic interactions in the field will have other influential factors not considered in these controlled lab studies. For example, the slow growth – high mortality (SGHM) hypothesis predicts that with increased larval development time, there is an increase in the rate of mortality by natural enemies, and has been supported by several studies with free-living hosts (Benrey and Denno 1997, Uesudi 2015). With slower growth of emerald ash borer in white fringetree, susceptible larval stages would be available for a longer period of time and may result in an increase in the mortality rate of emerald ash borer larvae due to parasitism. Although, as previously stated, parasitism of emerald ash borer has not been observed in the field to date, although few field studies have been performed (Peterson and Cipollini 2017, personal communication Cipollini).

Also of interest is that research into alternative host plants for emerald ash borer has yielded yet another species of host plant in an additional genus of the same family capable of successfully hosting emerald ash borer in lab studies, the cultivated olive tree, *Olea europea* (Oleaceae) (Cipollini et al. 2017). Although no evidence for emerald ash borer infestation in olive in the field has yet been identified, the ability of emerald ash borer to utilize another host plant, especially an economically important food crop such as olives, poses an increased threat by this invasive beetle. If emerald ash borer emerge from olive trees in field conditions, investigations into the response of emerald ash borer's natural enemies to alternate host plants will become more urgent.

Chapter 2: Effect of biotic and abiotic factors along an urbanization gradient on emerald ash borer (*Agrilus planipennis*) and its indigenous and introduced natural enemies

Introduction

Emerald ash borer, *Agrilus planipennisi* Fairmaire (Coleoptera: Buprestidae), is an invasive forest pest in North America. Emerald ash borer was initially discovered as the cause of widespread ash tree decline (*Fraxinus spp.*) in Windsor, Ontario and the Detroit, Michigan area in 2002 (Haack et al. 2002). Dendrochronological reconstruction suggests that emerald ash borer was introduced into North America via wood-packing material in the early 1990s (Siegert et al. 2014) and has since spread to 31 U.S. states and 2 Canadian provinces (Emeraldashborer.info). Hundreds of millions of ash trees have died in both forests and urban landscapes across North America as a result of emerald ash borer infestation (Herms and McCullough 2014). The full ecological consequences of this invasion are still being monitored and investigated (Poland and McCullough 2006, Herms and McCullough 2014, Duan et al. 2017).

Research pertaining to the management of emerald ash borer has been ongoing since its discovery in North America (Herms and McCullough 2014). Chemical control has proven effective in ash trees treated prior to heavy emerald ash borer infestation, although insecticide treatments need to be reapplied annually or biennially (McCullough et al. 2011, Herms et al. 2014, Poland et al. 2016). Insecticide treatments have mainly been applied to ash trees in the managed landscape as it would not been economically or ecologically feasible to treat entire forested stands of ash (Poland and McCullough 2006).

After eradication efforts of emerald ash borer were unsuccessful, parasitoids from its native range in northeast Asia were surveyed for and imported to the U.S. for further investigation and release as classical biological control agents (Bauer et al. 2005, 2008, 2015). Four hymenopteran parasitoids have since been released in the U.S. and Canada in forests infested with emerald ash borer: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae), *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Liu et al. 2003, 2007, Bauer et al. 2005, 2008, 2015, Belokobylskij et al. 2012, Duan et al. 2014, 2015, 2017). Recovery and release programs report varying degrees of success in terms of establishment and effectiveness of the introduced biological control agents (Bauer et al. 2015). *Tetrastichus planipennisi*, an introduced, gregarious, larval endoparasitoid has successfully established and dispersed in several locations including Maryland, Michigan, and Wisconsin (Duan et al. 2012, 2013c, Bauer et al. 2015, Jennings et al. 2016b, Duan et al. 2017, Jennings et al. 2017). Recently, evidence of the importance of *T. planipennisi* and its effectiveness at parasitizing emerald ash borer larvae in saplings has been exhibited in Michigan (Duan et al.

2017). *Tetrastichus planipennisi* may prove to protect young ash trees after emerald ash borer has eliminated many of the larger trees in a given area, potentially increasing sapling survival and creating a natural population balance in the future (Duan et al. 2017).

Oobius agrili an introduced, solitary egg parasitoid has been recovered in multiple locations and has been observed dispersing to control plots in Michigan (Duan et al. 2011, Abell et al. 2014, Duan et al. 2014, Jennings et al. 2014, Bauer et al. 2015, Abell et al. 2016, Parisio et al. 2017). Additionally, both *O. agrili* and *T. planipennisi* were recovered from ash trees that were also treated with imidacloprid indicating that the two methods of emerald ash borer management are compatible (Davidson and Rieske 2016). Life table analysis suggests that emerald ash borer mortality caused by both native and non-native parasitoids have reduced emerald ash borer's population growth rate in forests (Duan et al. 2014), although despite this populations are still increasing and spreading rapidly. In addition to emerald ash borer mortality induced by parasitism, North American woodpeckers significantly impact emerald ash borer survivorship (Cappaert et al. 2005, Jennings et al. 2013, Duan et al. 2014, MacQuarrie and Sharbach 2015, Jennings et al. 2016a).

Ash trees are not only abundant and ecologically important in forested habitats, but are widely planted street and landscape trees (McFarlane and Meyer 2005) in urban environments. Green ash (*Fraxinus pennsylvanicus*) is especially prevalent in the landscape due to its tolerance for anaerobic soils, fast growth habit, and yellow autumn aesthetic. Emerald ash borer infestations in urban ash trees result in extreme economic impacts where tree removal, replacement, and insecticide treatments have been estimated to cost $10.7 billion dollars over a ten year time span across infested regions in the U.S. (Kovacs et al. 2010). Despite the overwhelming presence and impact emerald ash borer has had in the urban landscape, few studies have investigated the ecological significance of emerald ash borer in our urban forests and green spaces.

Understanding emerald ash borer and its associated natural enemies along the urbanization gradient is important and greatly understudied. One study quantified emerald ash borer mortality factors in three urban sites in Canada using a life-table approach and compared them with previous research performed in forested habitats (Macquarrie and Scharbach 2015). Their findings, which they compared with previous investigations performed in forested habitats, link the infestation of urban ash trees with increased emerald ash borer dispersal to more urban and forested habitats and helped to explain emerald ash borer outbreak dynamics in forested habitats. Based on this information, Macquarrie and Sharbach (2015) suggest that management of emerald ash borer in urban landscapes with systemic insecticide applications, tree removal, and augmentation of egg and larval parasitoid populations may reduce emerald ash borer outbreaks in other urban and natural habitats. While biological control efforts have resulted in the release of the four introduced parasitoids in forests across emerald ash borer infested areas of the U.S., no information is available on their establishment or effectiveness in urban landscapes as a means of emerald ash borer population control. Additionally, very little is known about the impact of natural enemies in the urban landscape in general (Raupp et al. 2010).

Studies have observed that while urbanization negatively affects biodiversity (McKinney 2000, 2001), parasitoid populations can respond positively to changes in vegetation diversity in the form of increased plant species richness (Raupp et al. 2001, Raupp et al. 2010), vegetation complexity (Shrewsbury and Raupp 2006), and pervious surface area (Dale et al. 2016). Dale and Frank (2017) determined that multiple factors associated with impervious surfaces found in urban localities worked together to additively increase pest fitness. Understanding the relationship of biotic and abiotic factors associated with emerald ash borer and its natural enemies along the urbanization gradient may inform emerald ash borer management strategies as well as increase our understanding of pest populations and their natural enemies along an urbanization gradient.

Understanding the habitat factors that affect arthropod communities can aid in the conservation of biodiversity, pest management, and the increase of ecosystem services in the urban landscape (McIntyre 2000, McKinney 2002, 2006, Raupp et al. 2010, Jones & Leather 2012). The urbanization gradient provides a unique opportunity to evaluate the effects of multiple landscape factors that vary along the gradient on organisms of interest. Urbanization itself is difficult to quantify, although previous research oftentimes associates impervious surface cover with the physical gradient of urbanization (McKinney 2002). Additionally, habitat loss is viewed as another variable that changes along the gradient, as habitats become more fragmented in more urbanized areas and is associated with diversity changes as well (McKinney 2002). In this study, multiple methods were utilized to evaluate field sites along the gradient and measure both biotic and abiotic factors that may influence emerald ash borer and its natural enemies.

Here, parasitoid releases were conducted at 9 sites in Maryland and Northern Virginia. Sites represented varying levels of urbanization related factors along a gradient from more natural forest patch to more urbanized street tree habitats. To determine if the assemblage and impact of introduced and native parasitoids varied in emerald ash borer infested ash trees along the urbanization gradient, ash trees were debarked at each site to quantify emerald ash borer mortality factors, including parasitism by the released larval parasitoid *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) and indigenous parasitoids.

I predict that natural enemies, as indicated by parasitism and predation, will be more abundant in sites at the more natural end of the urbanization gradient (i.e. less impervious surface) than the more urban localities (i.e. more impervious surface) (Raupp et al. 2010, MacQuarrie and Sharbach 2015). Additionally, I predict that arthropods and hymenopteran parasitoids will be more associated with habitat measurements at two local spatial scales (75 m and 150 m radius), which has been previously observed (Philpott et al. 2014, Burks and Philpott 2017). In contrast, I expect that woodpeckers will respond to habitat factors at the landscape level (1000 m radius) more so than the local spatial scales. I predict that emerald ash borer densities will be higher in more urban sites as previous research suggests that pest densities tend to be higher in more urban localities and increase with increased impervious surface cover (Raupp et al. 2010, Meineke et al. 2014, Dale and Frank 2014, 2017a, 2017b).

Results from this study may help to infer best practices in urban landscapes to improve natural enemy presence and emerald ash borer management strategies as well as determine ash tree localities that are better suited for parasitoid releases. Additionally, understanding other mortality factors associated with emerald ash borer in these varying habitat types may improve our ability to manage emerald ash borer and other invasive pests along the urbanization gradient.

Materials and Methods

Parasitoids

The classical biological control agent released in this study, *T. planipennisi,* were produced and supplied from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) EAB Parasitoid Rearing Facility in Brighton, MI. *Tetrastichus plainpennisi* were shipped as late stage larvae or pupae within bolts of ash. The parasitoids were reared out to adults in environmental chambers at 30 °C and then counted and placed in release cups made from 9 oz, opaque plastic cups. Release cups had 2.54 cm diameter mesh screens glued onto the lids and a twist tie hot glued onto the back of the cup for hanging the release cup from trees. Each cup contained 25 female parasitoids and between 5 and 15 male parasitoids along with a small piece of Kimwipe taped inside the cup to provide cover. Release cups were maintained in environmental chambers at 25 °C with honey for nutrition. *Tetrastichus planipennisi* were released weekly or as available based on emergence.

Study Sites and Trees and Habitat Quantification

Factors influencing emerald ash borer mortality were investigated using nine study sites located across Maryland and Northern Virginia with known emerald ash borer populations. To qualify as a study site the location needed to have at least four living green ash trees (within 75 m radius) with visible signs of emerald ash borer infestation (ex. woodpecker damage, exit holes, canopy dieback, epicormic shoots). Four living green ash trees within a 75 m radius and each with a DBH between 5.08 and 25.4 cm at each site were chosen. Sites that met the above requirements were specifically selected that represented the spectrum of habitats along an urbanization gradient from natural/forested to highly urbanized.

To quantify the degree of urbanization and the habitat factors that vary along the gradient that may affect arthropod and avian natural enemies, I used satellite imagery via Geographic Information Systems (GIS) mapping software in ArcGIS v. 10.4 (ESRI) to determine the land-cover types at all sites. I utilized the Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD) (2011) to calculate the percentage of land-cover types within buffer zones at varying spatial scales surrounding the center points of each study site calculated from the central most point among the 4 trees sampled at each site using

www.geomidpoint.com. I categorized the classes of land-cover into 6 classifications **1) open water**, **2) natural habitat** [deciduous, evergreen and mixed forest, and woody wetland and emergent herbaceous wetlands], **3) developed open space**, **4) developed areas** [including low, medium, and high intensity], **5) grassland**, and **6) agricultural land** [including pasture/hay and cultivated crops] (modified from Philpott et al. 2014) (Fig. 2.1) (see Appendix A for NLCD value coding). To characterize our study sites, I calculated the percent of each land cover type at increasing spatial scales in 75 m, 150 m, and 1000 m radius buffers around each study site using spatial statistics tools in ArcGIS v. 10.4 (ESRI) (Fig. 2.2). To calculate raw impervious surface cover separately from the other land cover types, I used the NLCD 2001 Percent Developed Imperviousness (2011 Edition) map layer at each of the spatial scales. At the end of the season I assessed the crown condition (1-to-5, 1= no dieback, 5 = total canopy death) of each of the 4 study trees (Smith 2006, Flowers et al. 2013) and performed pace-to-plant surveys to estimate impervious surface land cover at the individual tree level (Dale et al. 2016).

In addition to the ArcGIS land cover and impervious surface measurements, I performed transects at the smallest spatial scale (75 m radius) during the summer of 2016. At each site, I quantified and identified all of the living hardwood trees that were at least 2.54 cm DBH in two crossing 10 m wide transects running 150 m north to south and 150 m east to west. Each tree was identified to the genus level (species when possible) and I recorded their DBH and GPS location and whether or not each tree was naturally occurring or had been planted/managed. All ash tree species (*Fraxinus spp.*) were counted and identified even if they were dead, as long as at least half of the tree remained standing. *Fraxinus spp*. also underwent additional measurements including characterization of the crown condition (1-to-5, 1 = no dieback, 5 = total canopy death) (Smith 2006, Flowers et al. 2013) and number of emerald ash borer exit holes and woodpecker holes on the bottom 2 m of the trunk. From all of these transect measurements we calculated Shannon-Weiner Diversity Index for tree genera, percent ash composition, the number of ash trees, the total number of trees, tree richness, and the average ash crown condition at each site.

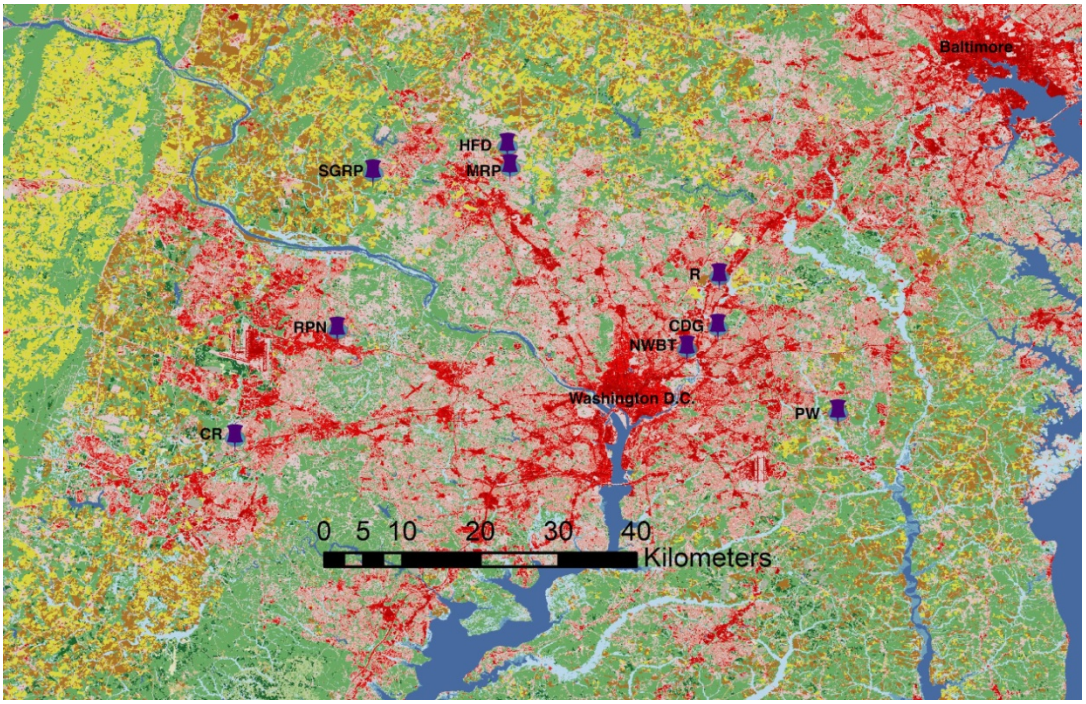


Figure 2.1. Distribution of field sites (9) across Maryland and Northern Virginia in ArcGIS v. 10.4 (ESRI) showing Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD) (2011). See Appendix A for NLCD value coding.

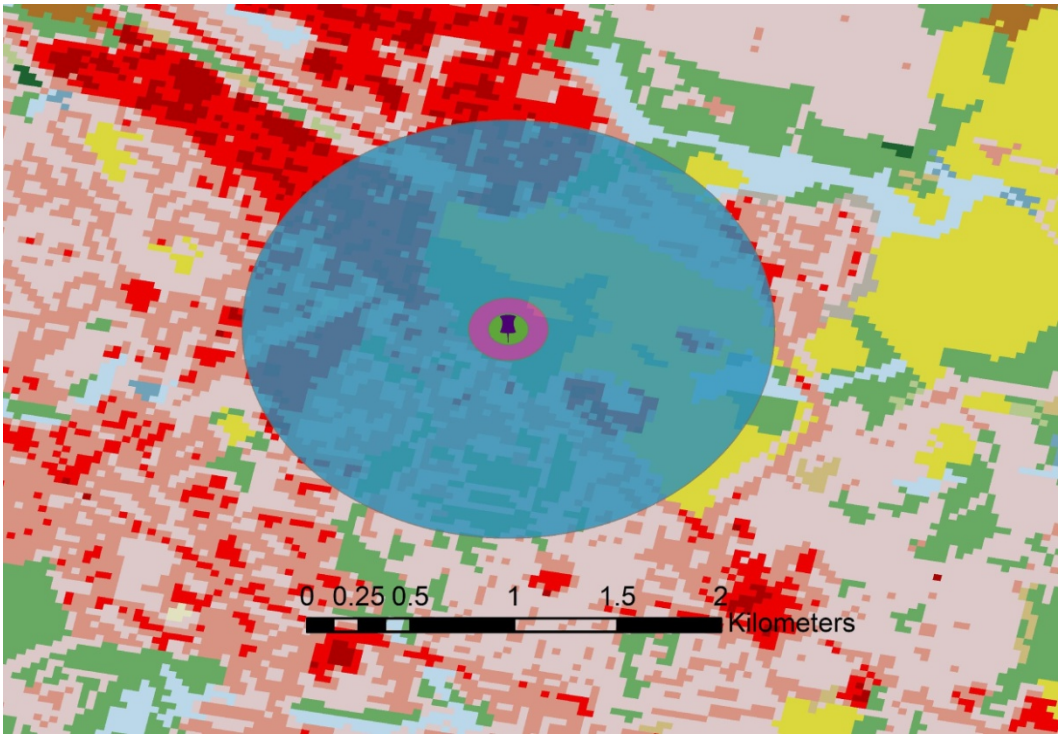


Figure 2.2. Example (site ID: MRP) of buffers at 75m radius, 150 m radius, and 1000 m radius around a field site in ArcGIS v. 10.4 (ESRI) showing Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD) (2011). See Appendix A for NLCD value coding.

Experimental Procedure

Starting in late July, *T. planipennisi* were released at each site as parasitoids became available (emerging from bolts in the lab through August 26th). Each release cup (described above) contained 25 female *T. planipennisi* and several males to ensure mating. The release cups were hung from nails at approximately 2 m in height to allow the parasitoids to disperse passively (Fig. 2.3). Each site received 510 ± 5 *T. planipennisi* females over the season, dispersed between the four study trees at each site *[approximately 125 female parasitoids per tree with a few exceptions]*.

Figure 2.3. *Tetrastichus planipennisi* release cup with parasitoids visible at time of release.

Data Collection

The winter (January – March 2017) following the parasitoid releases, the four trees at each study site were sampled for emerald ash borer and its natural enemies. Each of the four trees at each site was entirely debarked using draw-knives to assess emerald ash borer larval fate and for natural enemy activity. In several cases, release trees had become heavily infested by emerald ash borer during the summer and resulted in no surviving emerald ash borer and complete tree death. In such cases, the trees were discarded and, if available, adjacent ash trees were sampled for emerald ash borer larvae. To assess the emerald ash borer larvae, the bark was carefully removed from the study trees, meter-by-meter up the bole and all living branches of at least 2.54 cm in diameter using a drawknife. Each emerald ash borer gallery encountered was visually traced to reveal the stage (L1, L2, L3, L4, JL, PP, or A) and fate (alive, undetermined dead, emerged, diseased, woodpecker predation, or parasitized) of each larva. All living and parasitized larvae were placed in cell plates and brought back to the lab where they were held in environmental chambers at 25 °C where they were monitored for parasitoid emergence. Emerged parasitoids were placed in 70 % ethanol for further identification.

Statistical Analysis

From the debarking data, we consolidated the responses into four response variables: **1) emerald ash borer density per m^2 phloem area** which was calculated

using the phloem area calculation formula from McCullough and Siegert (2007), **2) woodpecker predation rate** ((# EAB woodpecked/(# EAB woodpecked+#EAB alive+#EAB exited))*100), **3) parasitism rate** ((# EAB parasitized/(# EAB parasitized+#EAB alive+#EAB exited))*100), **4) undetermined mortality rate** which included larvae killed by tree defense, fungus, pathogens or other indiscernible causes ((# EAB undetermined/(# EAB undetermined+#EAB alive+#EAB adults exited))*100).

To determine which factors best correlated with the above four response variables I performed a Pearson's correlation test using Statistix10 ® analysis software (Analytical Software ®). The explanatory variables included NLCD land cover measurements, NLCD impervious surface cover measurements, impervious surface measurements from Pace-to-Plant survey (Dale and Frank 2017), and habitat characteristics quantified from transects. I then performed individual linear regressions (Statistix10 ®) on factors that had strong correlations (greater than 0.50 R^2 or the highest correlation) with the response variables of interest, as well as explanatory variables I predicted may be pertinent to the response variables. I performed best subset regressions (Statistix10 ®) on non-collinear, explanatory variables of interest against individual response variables to see if multiple factors could explain more of the variation, but none were successful.

To determine if there were differences in each of the response variables between trees that were planted (managed trees in more urban landscapes, typically cultivars/grafted) or naturally occurring (straight species occurring naturally, not planted) One-way ANOVAs were performed. Proportion parasitism and proportion undetermined mortality response variables were Arcsine transformed to meet normality assumptions for all statistical analyses.

Results

The varying factors that indicate level of urbanization and that may influence emerald ash borer populations and their natural enemies were quantified and summarized at 9 field sites. Table 2.1 summarizes each site including, site descriptions, number of ash trees sampled, type of ash trees sampled (planted/managed or naturally occurring) average phloem area, Pace-to-Plant impervious surface estimates, and habitat characteristic data collected using transects (75 m radius) (number and diversity index of hardwood tree genera and number and percent ash tree composition). The site types included parking lot, residential, street, woodlot, stream valley, soccer field, and a disc golf course. The sites varied in their habitat measurements, for example they displayed variable numbers of hardwood tree genera ranging from 2 to 18 and % ash compositions ranging from 2% to 60%, although the site with 60% ash composition was a soccer field in which only 5 trees were encountered total.

Tables 2.2-2.4 represent the NLCD land cover percentages for each of the 6 land cover types described above at each of the three spatial scales of interest. Percent land cover of both Grassland (71) and Open Water (11) were very low among all three spatial scales, but were included to fully represent each site's total composition.

Open Water was not included in analyses due to low prevalence and little ecological significance to this study system. Table 2.5 includes NLCD impervious surface measurements at three spatial scales which ranged from 0% to 45% of impervious surface cover across all spatial scales.

I determined the relationships between the explanatory abiotic and biotic habitat factors and the response variables of interest (emerald ash borer density, woodpecker predation rate, parasitism rate, and undetermined mortality rate). From the Pearson's correlation matrix I identified the factors that correlated the strongest with each of the response variables (Table 2.6). Factors that strongly correlated with response variables (Table 2.6) were then tested using linear regressions to determine if the relationships were significant.

Relationships between response variables (emerald ash borer density, woodpecker predation rate, parasitism rate, and undetermined mortality rate) and sites varied. For example, woodpecker predation rates were variable and ranged from averages of 6% to 73% of predation of emerald ash borer larvae across sites (Table 2.8). Woodpecker predation rates did not have very strong correlations with any of the explanatory variables, although the strongest correlation visible from the Pearson's correlation matrix was with natural land cover at 75 m radius with a positive R^2 value of 0.5058 (Table 2.6 and 2.7). The linear regression determined that the relationship between woodpecker predation rate and natural land cover at 75 m radius was not significant ($F_{1,7}$ 3.52, $P<0.1648$) with an R^2 of 0.2559.

Overall, parasitism was low and varied between sites (Table 2.8). Mean parasitism rates ranged from 0% to 5.04% across the 9 sites (Table 2.8). Two broods of *T. planipennisi* were recovered at MRP and four broods were recovered at SGRP (Table 2.8). Parasitism rate (Arcsine transformed) shows a strong correlation with several explanatory variables from the Pearson correlation matrix (Tables 2.6 and 2.7). Parasitism rate (Arcsine transformed) had significant negative relationships with % developed land cover at 150 m (D150) ($F_{1,7}$ 7.19, $P<0.0315$) with an R^2 of 0.5068 (Fig. 2.4A), percent impervious surface cover at 75 m (IMP75) ($F_{1,7}$ 7.61, $P<0.0281$) with an R^2 of 0.5209 (Fig. 2.4B), and percent impervious surface cover at 150 m (IMP150) ($F_{1,7}$ 5.73, $P<0.0480$) with an R^2 of 0.4499 (Figure 2.4C).

Mean undetermined mortality rates varied from 5% to 43% between sites (Table 2.6). The strongest correlation and only significant linear relationship was with number of tree genera (Treegener) ($F_{1,7}$ 5.98, $P<0.044$) with an R^2 of 0.4609 (Fig. 5)

Mean emerald ash borer densities varied from 6.1 to 39.4 EAB per m^2 phloem area between the 9 sites (Table 2.8). Mean emerald ash borer density had positive correlations with agricultural land cover at 75 m spatial scale (A75) and 150 m spatial scale (A150), grassland at all three spatial scales (G75, G150, G1000), and developed land at 150 m spatial scale (D150) according to the Pearson correlation matrix, although only agricultural land cover at 150 m spatial scale (A150) had a significant linear relationship ($F_{1,7}$ 8.60, $P<0.0220$) with an R^2 of 0.5512 (Appendix B) however, only 3 of the 9 sites had any agricultural land cover.

Examining differences in explanatory variables between planted and naturally occurring trees found the mean emerald ash borer density in naturally occurring trees was 19.6 EAB per m^2 phloem area and in planted trees was 15.3 EAB per m^2 phloem area, with no significant difference ($F_{1,7}$ 0.23, $P<0.6438$) (Fig. 2.6A). The mean

woodpecker predation rate in natural trees was 48.8% and the mean woodpecker predation rate in planted trees was 32.7%, with no significant difference ($F_{1,7}$ 1.22, P<0.3067) (Fig. 2.6B). The mean parasitism rate (untransformed) in natural trees was 2.3% and the mean parasitism rate in planted trees (untransformed) was 0.4%, although the difference was not significant ($F_{1,7}$ 3.60, P<0.0997) (Fig. 2.6C). The mean mortality rate by undetermined factors (untransformed) in natural trees was 26.7 % and the mean mortality rate by undetermined factors (untransformed) in planted trees was 5.6% and was significantly different (transformed) ($F_{1,7}$ 7.61, P<0.0282) (Fig. 2.6D).

Table 2.1. Site habitat characteristics summary including quantified transect data. Site description is a subjective definition to provide insight into site type**.** Average phloem area was calculated using McCullough and Siegert (2007) formula for phloem area estimation. Pace2Plant is an average estimate of percent impervious surface cover at the tree level scale and was developed by Dale and Frank (2016). Transect data were collected from two intersecting transects that ran 150 m north to south and 150 m east to west and all hardwood trees were quantified and consolidated into the data below.

SITE ID	Site Description	Number of ash trees sampled	Trees Planted (P) or Naturally Ocurring (N)	Average Phloem area (m^2) ± SE	PACE2PLANT - Average estimated % impervious surface around tree base (Dale & Frank 2016)	TRANSECT DATA (75m radius)					
						% Ash Composition	Total number of trees observed	Number of Tree genera observed	Number of ash trees observed	Shannon-Weiner Index of Tree Genus Diversity	Average Crown Condition of observed ash trees
CR	Stream Valley	4	N	5.01 ± 1.77	3 ± 0	15%	297	18	46	2.128	4.15
MRP	Woodlot	4	N	2.87 ± 0.31	0 ± 0	31%	361	11	111	1.880	3.17
NWBT	Woodlot	4	N	4.60 ± 0.58	9.75 ± 0.95	23%	112	10	26	1.819	4.10
CDG	Disc Golf Course	3	N	2.95 ± 0.31	0 ± 0	8%	126	13	10	2.115	2.00
PW	Residential	3	P	8.25 ± 0.71	52.67 ± 1.45	6%	69	12	4	2.134	3.00
SGRP	Soccer Field	4	P	7.66 ± 0.89	3.75 ±	60%	5	2	3	0.673	1.33
HFD	Residential	3	P	12.63 ± 2.22	53 ± 3.51	10%	30	9	3	1.699	3.00
RI	Street	4	P	10.76 ± 1.31	57 ± 6.67	2%	180	11	4	1.315	3.33
RPN	Parking Lot	4	P	12.71 ± 1.01	52.75 ± 6.28	31%	16	6	5	1.717	3.60

Table 2.2. Percent land cover measurements at 75 m radius buffer by site using Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD) (2011 Edition) map layer in ArcGIS v. 10.4 (ESRI). The six land cover types are comprised of one or more NLCD values (modified from Philpott et al. 2014) as follows 1. open water (NLCD value 11), 2. natural habitat [NLCD values: deciduous (41), evergreen (42) and mixed forest (43), and woody wetland (90) and emergent herbaceous wetlands (95)], 3. developed open space (NLCD value 21), 4. developed areas [NLCD values: low (22), medium (23), and high intensity (24)], 5. grassland (NLCD value 71), and 6. agricultural land [NLCD values: pasture/hay (82) and cultivated crops (83)]. See Appendix A for NLCD value coding.

	NLCD % LAND COVER MEASUREMENTS - 75m radius					
SITE ID	% Natural	% Open Space	% Agriculture	% Developed	% Grassland	% Open Water
CR	74%	26%	0%	0%	0%	0%
MRP	100%	0%	0%	0%	0%	0%
NWBT	10%	70%	0%	20%	0%	0%
CDG	69%	25%	0%	6%	0%	0%
PW	10%	35%	15%	40%	10%	0%
SGRP	0%	75%	0%	25%	0%	0%
HFD	14%	14%	0%	71%	0%	0%
RI	0%	16%	0%	84%	0%	0%
RPN	0%	32%	0%	68%	0%	0%

Table 2.3. Percent land cover measurements at 150 m radius buffer by site using Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD) (2011 Edition) map layer in ArcGIS v. 10.4 (ESRI). The six land cover types are comprised of one or more NLCD values (modified from Philpott et al. 2014) as follows 1. open water (NLCD value 11), 2. natural habitat [NLCD values: deciduous (41), evergreen (42) and mixed forest (43), and woody wetland (90) and emergent herbaceous wetlands (95)], 3. developed open space (NLCD value 21), 4. developed areas [NLCD values: low (22), medium (23), and high intensity (24)], 5. grassland (NLCD value 71), and 6. agricultural land [NLCD values: pasture/hay (82) and cultivated crops (83)]. See Appendix A for NLCD value coding.

	NLCD % LAND COVER MEASUREMENTS - 150m radius					
SITE ID	% Natural	% Open Space	% Agriculture	% Developed	% Grassland	% Open Water
CR	53%	35%	0%	13%	0%	0%
MRP	96%	0%	4%	0%	0%	0%
NWBT	18%	68%	0%	14%	0%	0%
CDG	41%	22%	0%	38%	0%	0%
PW	10%	32%	15%	43%	6%	0%
SGRP	0%	60%	10%	30%	0%	0%
HFD	20%	21%	0%	59%	0%	0%
RI	1%	28%	0%	71%	0%	0%
RPN	3%	34%	0%	63%	0%	0%

Table 2.4. Percent land cover measurements at 1000 m radius buffer by site using Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD) (2011 Edition) map layer in ArcGIS v. 10.4 (ESRI). The six land cover types are comprised of one or more NLCD values (modified from Philpott et al. 2014) as follows 1. open water (NLCD value 11), 2. natural habitat [NLCD values: deciduous (41), evergreen (42) and mixed forest (43), and woody wetland (90) and emergent herbaceous wetlands (95)], 3. developed open space (NLCD value 21), 4. developed areas [NLCD values: low (22), medium (23), and high intensity (24)], 5. grassland (NLCD value 71), and 6. agricultural land [NLCD values: pasture/hay (82) and cultivated crops (83)]. See Appendix A for NLCD value coding.

	NLCD % LAND COVER MEASUREMENTS - 1000m radius					
SITE ID	% Natural	% Open Space	% Agriculture	% Developed	% Grassland	% Open Water
CR	52%	30%	0%	18%	0%	0%
MRP	17%	23%	21%	40%	0%	0%
NWBT	7%	22%	0%	71%	0%	0%
CDG	36%	21%	0%	43%	0%	0%
PW	11%	32%	14%	43%	1%	0%
SGRP	23%	24%	40%	12%	0%	0%
HFD	17%	35%	10%	36%	0%	0%
RI	7%	27%	1%	64%	0%	0%
RPN	12%	28%	0%	59%	0%	1%

Table 2.5. Impervious surface cover at 3 spatial scales by site using NLCD 2001 Percent Developed Imperviousness (2011 Edition) map layer in ArcGIS v. 10.4 (ESRI).

	GIS IMPERVIOUS SURFACE MEASUREMENTS		
SITE ID	% impervious surface cover - 75m radius	% impervious surface cover - 150m radius	% impervious surface cover - 1000m radius
CR	1.950	7.342	7.833
MRP	0.000	0.000	21.840
NWBT	6.947	7.731	36.997
CDG	12.450	25.078	23.237
PW	18.000	17.273	21.261
SGRP	11.211	17.038	6.551
HFD	32.632	24.291	16.588
RI	40.650	44.923	41.292
RPN	38.947	36.039	29.742

Table 2.6. Correlation matrix including all response (highlighted) and explanatory values. "*" indicates strong relationship and was further investigated using linear regressions.
EXPLANATORY VARIABLE KEY: Pace2Plant = estimated % impervious surface around tree base (Dale and Frank 2017a), % Ash = % ash composition from transect habitat quantification, TreeGenera = tree richness from habitat quantification transects, # Trees = number of trees counted from transect habitat quantification, SWI = Shannon-Weiner Diversity Index of tree genera from transect habitat quantification, Crown = average crown condition of ash trees encountered in transect habitat quantification, IMP75 = % impervious surface cover at 75 m radius spatial scale, IMP150 = % impervious surface cover at 150 m radius spatial scale, IMP1000 = % impervious surface cover at 1000 m radius spatial scale, N# = % natural land cover at spatial scales 75 m, 150, m, 1000 m radii, A# = % agricultural land cover at spatial scales 75 m, 150, m, 1000 m radii, G# = % grassland land cover at spatial scales 75 m, 150, m, 1000 m radii, D# = % developed land cover at spatial scales 75 m, 150, m, 1000 m radii, OS# = % open space land cover at spatial scales 75 m, 150, m, 1000 m radii, RESPONSE VARIABLE KEY: WP = woodpecker predation rate, PARA~ = Parasitism rate (Arcsine transformed), UNDETMORT~ = undetermined mortality rate (Arcsine transformed), EABDEN = emerald ash borer density per m^2 phloem area.

	PACE2PLANT	%ASH	#TREES	TREEGENERA	#ASH	SWI
PACE2PLANT	1.0000					
%ASH	-0.4364	1.0000				
#TREES	-0.4631	-0.1985	1.0000			
TREEGENERA	-0.1661	-0.7166	0.6755	1.0000		
#ASH	-0.5454	0.1548	0.8571	0.3331	1.0000	
SWI	-0.0422	-0.6549	0.3986	0.7953	0.2937	1.0000
CROWN	0.2070	-0.4055	0.4005	0.5328	0.2791	0.5239
IMP75	0.8872	-0.2975	-0.5413	-0.3314	-0.6431	-0.2567
IMP150	0.7104	-0.3084	-0.4854	-0.2558	-0.7015	-0.2914
IMP1000	0.4088	-0.4187	-0.0084	0.0105	-0.1147	0.0963
WP	-0.2996	-0.2405	0.0629	0.2866	0.1751	0.4745*
EABDEN	-0.2261	0.1556	0.2161	0.0559	0.4164	0.1986
UNDETMORT~	-0.4824	-0.2488	0.3647	0.6789*	0.1366	0.5736*
PARA~	-0.4655	-0.0802	0.5292*	0.5582*	0.4019	0.3503
N75	-0.6637	-0.0975	0.8229	0.6057	0.8082	0.5314
OS75	-0.2503	0.5589	-0.5405	-0.4872	-0.4178	-0.4584
A75	0.3761	-0.3087	-0.1906	0.1493	-0.2044	0.3282
G75	0.3761	-0.3087	-0.1906	0.1493	-0.2044	0.3282
D75	0.9247	-0.2646	-0.5371	-0.3713	-0.6114	-0.3324
N150	-0.6015	-0.0332	0.8476	0.5094	0.9248	0.4829
OS150	-0.1241	0.4135	-0.5084	-0.3757	-0.4790	-0.4210
G150	0.3761	-0.3087	-0.1906	0.1493	-0.2044	0.3282
A150	0.0756	0.2462	-0.2392	-0.2561	-0.0993	-0.1620
D150	0.8552	-0.3561	-0.6084	-0.2821	-0.7631	-0.2319
N1000	-0.5702	0.0011	0.3720	0.5226	0.1837	0.2765
OS1000	0.6748	-0.3455	-0.2517	0.1262	-0.3307	0.1187
G1000	0.3761	-0.3087	-0.1906	0.1493	-0.2044	0.3282
A1000	-0.2479	0.7220	-0.1669	-0.6087	0.1225	-0.6381
D1000	0.4167	-0.4128	-0.0814	0.0121	-0.1239	0.2141

	CROWN	IMP75	IMP150	IMP1000	WP	EABDEN
CROWN	1.0000					
IMP75	0.0278	1.0000				
IMP150	-0.1298	0.9230	1.0000			
IMP1000	0.4039	0.4614	0.4665	1.0000		
WP	-0.2125	-0.3462	-0.4005	-0.3945	1.0000	
EABDEN	0.0151	-0.5865*	-0.6770*	-0.1212	0.0362	1.0000
UNDETMORT~	0.4693	-0.5316*	-0.3703	0.0274	0.0438	0.1049
PARA~	0.4320	-0.7218*	-0.6708*	-0.0650	-0.0939	0.6240
N75	0.0808	-0.6793	-0.5974	-0.3088	0.5058	0.1705
OS75	-0.2231	-0.2060	-0.1437	-0.1031	-0.2868	0.2002
A75	-0.0308	-0.0021	-0.0704	-0.0492	-0.0024	0.6614*
G75	-0.0308	-0.0021	-0.0704	-0.0492	-0.0024	0.6614*
D75	0.0793	0.9673	0.8325	0.4544	-0.3825	-0.4579
N150	0.1707	-0.6700	-0.6777	-0.2359	0.4723	0.2881
OS150	-0.0060	-0.0813	-0.0365	0.0211	-0.3879	0.0627
G150	-0.0308	-0.0021	-0.0704	-0.0492	-0.0024	0.6614*
A150	-0.4379	-0.2002	-0.2299	-0.3549	-0.0942	0.7424*
D150	-0.1144	0.9696	0.9491	0.3644	-0.2611	-0.5882*
N1000	-0.0707	-0.4836	-0.3100	-0.6814	0.3651	-0.2423
OS1000	0.2259	0.4420	0.2123	-0.2883	0.0990	-0.1184
G1000	-0.0308	-0.0021	-0.0704	-0.0492	-0.0024	0.6614*
A1000	-0.6866	-0.2831	-0.3193	-0.5682	-0.0065	0.4287
D1000	0.4802	0.4307	0.3865	0.9728	-0.3003	-0.0733

	UNDETMORT~	PARA~	N75	OS75	A75	G75
UNDETMORT~	1.0000					
PARA~	0.7343	1.0000				
N75	0.3525	0.3185	1.0000			
OS75	0.2223	0.2635	-0.5286	1.0000		
A75	0.0045	0.2810	-0.2004	0.0368	1.0000	
G75	0.0045	0.2810	-0.2004	0.0368	1.0000	1.0000
D75	-0.5909*	-0.6244*	-0.7556	-0.1418	0.0572	0.0572
N150	0.2219	0.3247	0.9589	-0.5457	-0.1969	-0.1969
OS150	0.3023	0.2994	-0.6079	0.9580	-0.0294	-0.0294
G150	0.0045	0.2810	-0.2004	0.0368	1.0000	1.0000
A150	-0.2406	0.2136	-0.2010	0.2952	0.8027	0.8027
D150	-0.4802	-0.7119*	-0.6769	-0.1633	0.0949	0.0949
N1000	0.5102*	0.2317	0.6082	-0.1230	-0.2441	-0.2441
OS1000	-0.2886	-0.1915	-0.3087	-0.2769	0.3845	0.3845
G1000	0.0045	0.2810	-0.2004	0.0368	1.0000	1.0000
A1000	-0.5475*	-0.0773	-0.0666	0.3146	0.1284	0.1284
D1000	0.0851	-0.0524	-0.3175	-0.0576	0.0019	0.0019

	D75	N150	OS150	G150	A150	D150
D75	1.0000					
N150	-0.6942	1.0000				
OS150	-0.0050	-0.6228	1.0000			
G150	0.0572	-0.1969	-0.0294	1.0000		
A150	-0.1099	-0.1741	0.1252	0.8027	1.0000	
D150	0.9169	-0.7232	-0.0612	0.0949	-0.1071	1.0000
N1000	-0.5929	0.4134	-0.1329	-0.2441	-0.1996	-0.3734
OS1000	0.5208	-0.2796	-0.1666	0.3845	0.1649	0.4585
G1000	0.0572	-0.1969	-0.0294	1.0000	0.8027	0.0949
A1000	-0.1813	0.0099	0.1120	0.1284	0.6757	-0.2583
D1000	0.4220	-0.2328	0.0620	0.0019	-0.3552	0.3266

	N1000	OS1000	G1000	A1000	D1000
N1000	1.0000				
OS1000	0.0208	1.0000			
G1000	-0.2441	0.3845	1.0000		
A1000	-0.0811	-0.0526	0.1284	1.0000	
D1000	-0.6880	-0.2379	0.0019	-0.6197	1.0000

Table 2.7. Correlation table consolidated showing correlations between response variables and explanatory variables of interest. "*" indicates strong relationship and was further investigated using linear regressions.
EXPLANATORY VARIABLE KEY: TreeGener = tree richness from habitat quantification transects, Trees = number of trees counted from transect habitat quantification, IMP75 = % impervious surface cover at 75 m radius spatial scale, IMP150 = % impervious surface cover at 150 m radius spatial scale, N75 = % natural land cover at spatial scales 75 m radius, A# = % agricultural land cover at spatial scales 75 m, 150, m, 1000 m radii, G# = % grassland land cover at spatial scales 75 m, 150, m, 1000 m radii, D# = % developed land cover at spatial scales 75 m, 150, m, 1000 m radii, OS# = % open space land cover at spatial scales 75 m, 150, m, 1000 m radii, RESPONSE VARIABLE KEY: WP = woodpecker predation rate, PARA~ = Parasitism rate (Arcsine transformed), UNDETMORT~ = undetermined mortality rate (Arcsine transformed), EABDEN = emerald ash borer density per m^2 phloem area.

	TREES	TREEGENER	IMP75	IMP150	D75	D150
PARA~01	0.5292*	0.5582*	-0.7218*	-0.6708*	-0.6244*	-0.7119*
OTHER~01	0.3647	0.6789*	-0.5316*	-0.3703	-0.5909*	-0.4802
EABDEN	0.2161	0.0559	-0.5865*	-0.6770*	-0.4579	-0.5882*
WP	0.0629	0.2866	-0.3462	-0.4005	-0.3825	-0.2611

	A1000	N75	N1000	G150	A75	G75
PARA~01	-0.0773	0.3185	0.2317	0.2810	0.2810	0.2810
OTHER~01	-0.5475*	0.3525	0.5102*	0.0045	0.0045	0.0045
EABDEN	0.4287	0.1705	-0.2423	0.6614*	0.6614*	0.6614*
WP	-0.0065	0.5058*	0.3651	-0.0024	-0.0024	-0.0024

	G1000	PARA~01	OTHER~01	EABDEN	WP
PARA~01	0.2810	1.0000			
OTHER~01	0.0045	0.7343	1.0000		
EABDEN	0.6614*	0.6240	0.1049	1.0000	
WP	-0.0024	-0.0939	0.0438	0.0362	1.0000

Table 2.8. The mean percentage of EAB mortality factors and EAB density (±SE) by site organized from lowest to highest percent impervious surface at 150 m radius spatial scale.
Mortality response calculation: ((#mortality type / (#mortality type+#alive+#adult))*100) EAB exited. Percent parasitism and percent undetermined mortality are displayed as true percentages, but were Arcsine transformed for all data analyses to meet normality assumptions. EAB density was calculated using McCullough and Siegert (2007) phloem area estimation method.

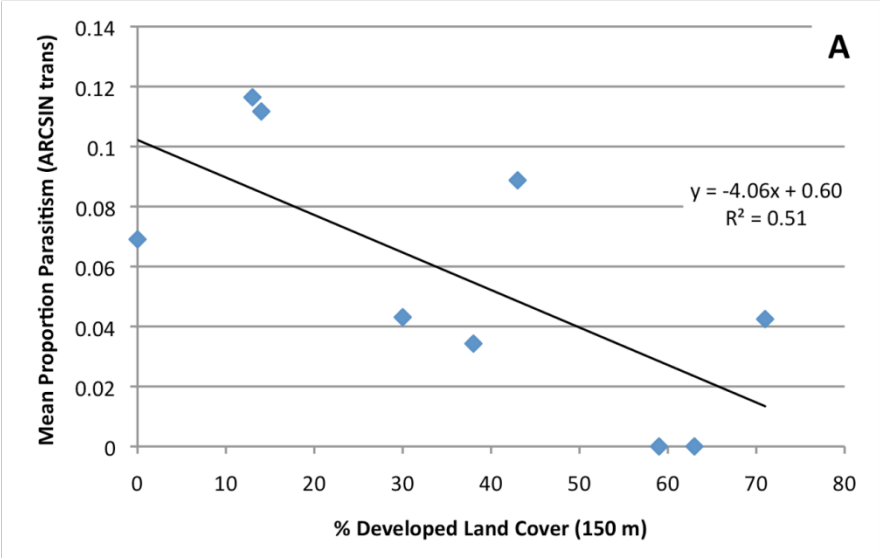
SITE	% Woodpecker Predation (±SE)	% Parasitism (±SE)	% Undetermined Mortality (±SE)	Mean EAB Density per m^2 (±SE)
MRP	49.7 ± 8.9	1.1 ± 0.9	5.0 ± 2.0	31.7 ± 9.1
CR	40.1 ± 20.7	5.0 ± 5.0	42.1 ± 22.4	13.3 ± 8.4
NWBT	36.1 ± 15.6	2.6 ± 1.8	34.1 ± 13.8	25.5 ± 13
SGRP	26.9 ± 3.6	0.4 ± 0.3	3.1 ±1.3	19.7 ± 4.1
PW	39.8 ± 12.8	0.9 ± 0.5	12.6 ± 7.4	39.4 ± 11.5
HFD	73.1 ± 5.1	0.0 ± 0.0	1.3 ± 1.3	6.2 ± 2.9
CDG	69.4 ± 7.7	0.5 ± 0.5	25.6 ± 10.6	8.0 ± 2.7
RPN	17.8 ± 7.0	0.0 ± 0.0	6.8 ± 1.8	5.7 ± 0.3
RI	6.3 ± 2.1	0.71 ± 0.71	4.9 ± 0.6	5.5 ± 2.3

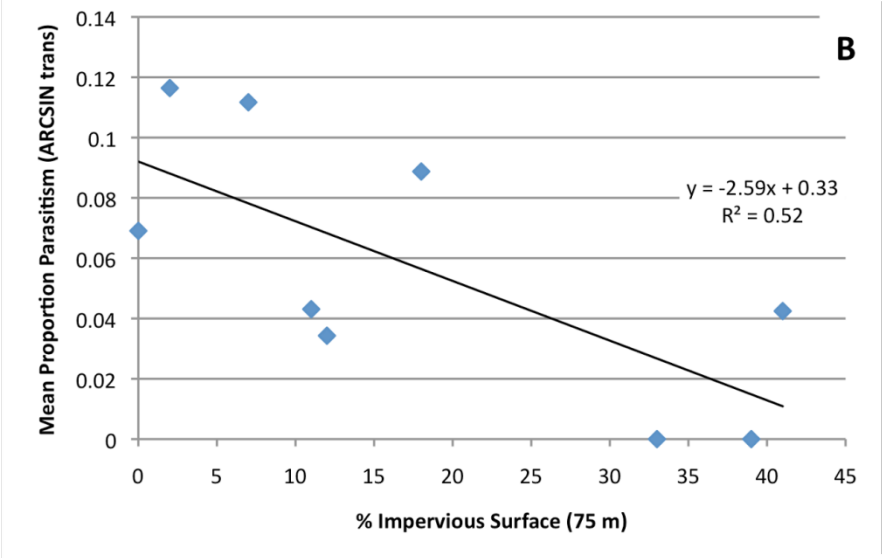
Table 2.9. Identified indigenous and introduced parasitoids recovered and from which field sites they were recovered.

Family	Species	Indigenous or Introduced	Sites
Braconidae	*Leluthia sp.*	Indigenous	CDG, CR
Braconidae	*Spathius sp.*	Indigenous	CR, NWBT, PW
Braconidae	*Atanycolous sp.*	Indigenous	CR, MRP, NWBT, PW
Eulophidae	*Tetrastichus planipennisi*	Introduced	MRP, SGRP
Eupelmidae	*Balcha indica*	Indigenous[1]	NWBT
Eupelmidae	*Eupelmus sp.*	Indigenous	CR, MRP
Bethylidae	*Sclerodermus sp.*	unknown	NWBT
unknown	*unknown*[2]	unknown	CDG, CR, MRP, NWBT, PW, RI

[1]Inadvertently introduced from southeast Asia and naturalised in the eastern United States of America (Bauer et al. 2015).

[2]Many parasitoids could not be identified due to mite (*Tyrophagus sp*) infestation in lab cultures


A
Mean Proportion Parasitism (ARCSIN trans)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
y = -4.06x + 0.60
R² = 0.51
0
10
20
30
40
50
60
70
80
% Developed Land Cover (150 m)


B
Mean Proportion Parasitism (ARCSIN trans)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
y = -2.59x + 0.33
R² = 0.52
0
5
10
15
20
25
30
35
40
45
% Impervious Surface (75 m)

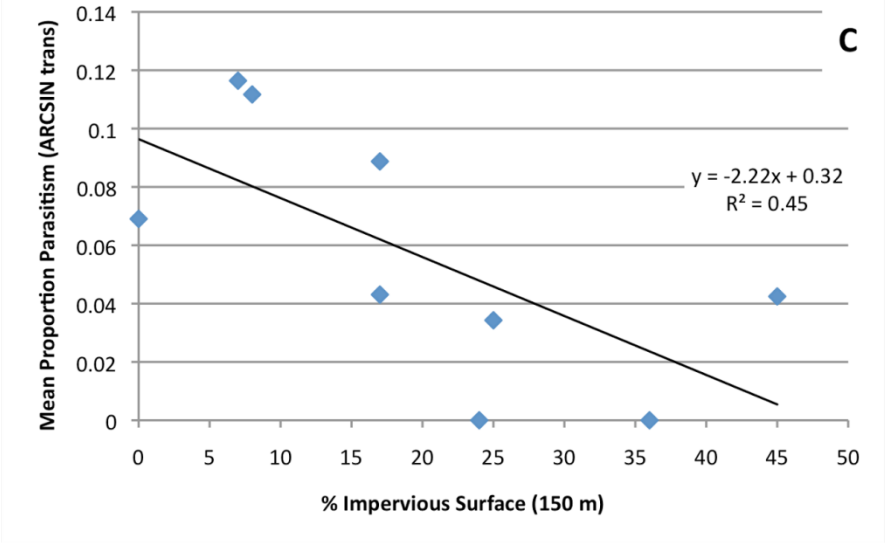

C
Mean Proportion Parasitism (ARCSIN trans)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
y = -2.22x + 0.32
R² = 0.45
0
5
10
15
20
25
30
35
40
45
50
% Impervious Surface (150 m)

Figure 2.4: A. Mean proportion parasitism (Arcsine transformed) and % developed land cover at 150 m radius spatial scale, B. Mean proportion parasitism (Arcsine transformed) and % impervious surface at 75 m radius spatial scale, C. Mean proportion parasitism (Arcsine transformed) and % impervious surface at 150 m radius spatial scale.

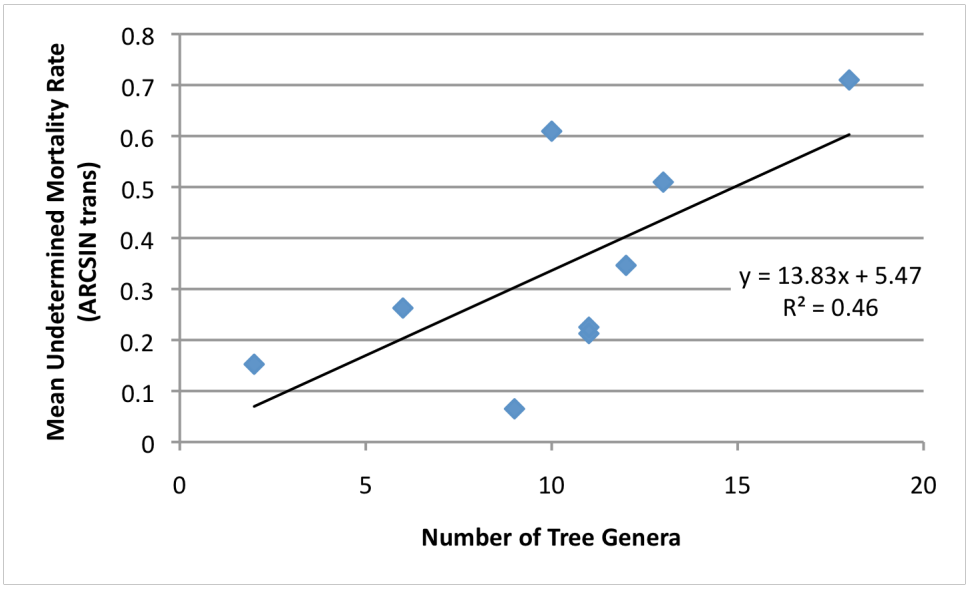


Figure 2.5: Mean undetermined mortality rate (Arcsine transformed) and number of tree genera.

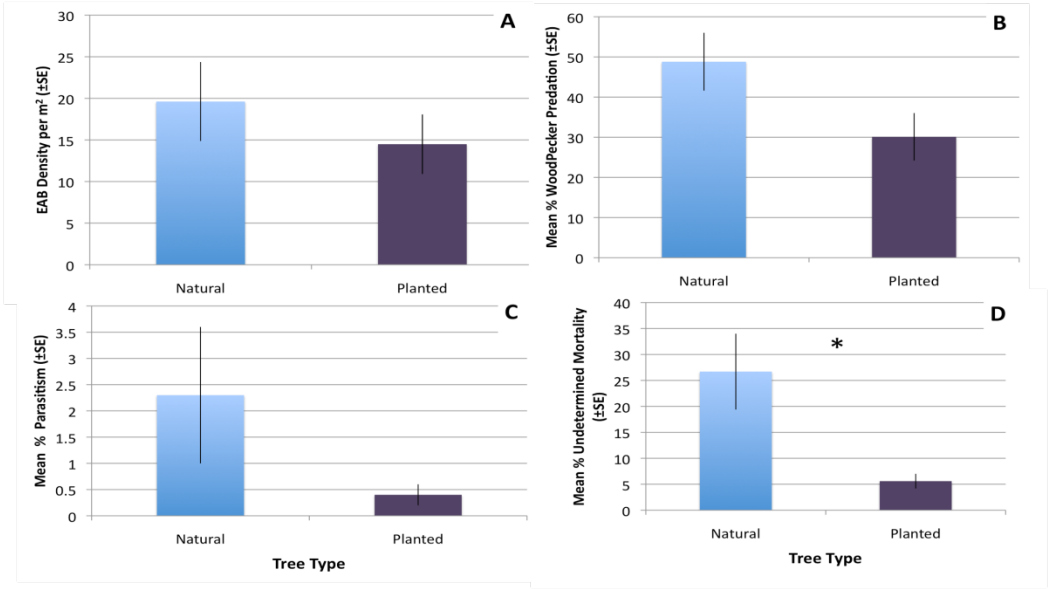


Figure 2.6: Response variables [A. Mean EAB Density, B. Mean Woodpecker Predation Rate, C. Mean Parasitism Rate (Untransformed), and D. Mean Undetermined Mortality Rate (untransformed)] (±SE) comparisons between planted and naturally occurring trees among sites.”*” indicates significant difference between tree types (P<0.05).

Discussion

In general, parasitism was higher in less urban habitats, which supported my predictions, but emerald ash borer abundance was greater in less urban habitats counter to my predictions. Overall, emerald ash borer mortality was lower in more urban habitats and in planted trees over naturally occurring trees, although each of the various mortality factors (parasitism, predation, and undetermined) were associated with different habitat factors. Parasitism was negatively related to imperviousness, while undetermined mortality was positively related to tree richness. Woodpecker predation was unexplained by any habitat factors measured here. EAB density and parasitoid induced mortality were more correlated with local spatial scale habitat measurements, which aligned with my predictions, as well.

Parasitoids did attack emerald ash borer larvae in most study sites. Overall, we recovered few classical biological control agents among all of our sites, regardless of habitat type. While a one-year release - recovery survey does not often result in much recovery, we performed these releases to augment native and introduced parasitoid populations, as biological control agents have been released and recovered across Maryland since 2009 (Jennings et al. 2016a). The two sites at which *T. planipennisi* was recovered (MRP and SGRP) (Table 2.9) were sites with the highest percent ash compositions of 31% and 60% respectively (Table 2.1).

Native parasitoids that I recovered included: *Spathius sp*., *Leluthia sp*., *Antaycolous sp*., *Balcha indica* (naturalised, non-native), and *Eupelmus sp*. (Table 2.9). I recovered two broods of *Sclerodermus sp*. (Hymenoptera: Bethylidae) at NWBT (Table 2.9), which to my knowledge is the first bethylid discovered attacking emerald ash borer larvae in North America. *Sclerodermus pupariae* have been known to attack emerald ash borer in its native range in China and under laboratory conditions (Yang et al. 2012, Wang et al. 2016). I collected the adult *Sclerodermus sp.* and set them up in exposure assays to confirm their ability to reproduce on emerald ash borer larvae and we were able to produce several generations under laboratory conditions. Further research into the identification and possible use as a biological control agent for emerald ash borer control should be pursued. Additionally, awaiting identification confirmation, I can record first reports of *Leluthia sp.* attacking emerald ash borer larvae in Maryland (Kula et al. 2010, Bauer et al. 2015). Many parasitized emerald ash borer larvae that we recovered during debarking did not complete development in the lab due to a heavy mite (*Tyrophagus* sp.) infestation and therefore parasitoids could not be identified and were labeled "unknown". Despite this, I am confident that all *Tetrastichus planipennisi* were identified, as they are easily identifiable in their larval and pupal forms at the time of tree debarking.

It has been hypothesized that parasitism would be reduced as one moves along the urbanization gradient from natural, more vegetative diverse habitats into more urban/impervious habitats, although it has been relatively understudied (Shrewsbury and Raupp 2000, 2006, Raupp et al. 2010, Meineke et al. 2014, Macquarrie and Sharbach 2015). Our results support this hypothesis. Here, I determined that parasitism had a significant negative association with percent impervious surface at both 75 m and 150 m spatial scales, as well as, percent developed land cover at 150 m spatial scale (Fig 2.4). These results indicate that as we move along the urbanization gradient into more urban localities as defined by increased impervious surface cover, we see less mortality due to parasitism.

Other factors associated with urbanization may also affect natural enemy impacts. I observed parasitism of emerald ash borer to be numerically reduced in planted/managed trees over naturally occurring trees, but due to high variation, this was not significant (Fig. 2.6C). Planted trees are usually cultivars of ash that tend to be shorter and broader in habit than naturally occurring species, which tend to be tall and straight. Interestingly, we sampled one site with planted/managed trees that were not cultivars (PW site), and had the typical growth habit associated with the naturally occurring trees, but were clearly planted and in a managed system as street trees. When we place these trees (PW site) in with the naturally-occurring category, we do achieve a significant difference in parasitism between the two tree types ($F_{1,7}$ 9.59 $P<0.0174$). This indicates that trees that are straight species, even in managed systems may be more suitable for parasitism than cultivar, grafted ash trees. Anecdotally, we observed thicker bark in cultivar green ash, which is known to affect parasitism by *T. planipennisi*, which require thinner bark for parasitism (Abell et al. 2012). We did not measure the difference in bark thickness and this should be investigated further before conclusions can be drawn between the tree types. Another possible explanation for higher parasitism rate at sites with less impervious surface may be that parasitoids are

numerically responding to the higher densities of emerald ash borer that are associated with more natural habitats.

I acknowledge that there are many other factors that might explain the negative correlation between parasitism and increased impervious surface. Research shows that urban systems may act as predictive models for climate change scenarios with increased temperatures and CO^2 levels causing many disruptions among the trophic levels (Youngsteadt et al. 2015). Increased temperatures often associated with increased impervious surface may make the habitat less suitable for beneficial insects (Dale and Frank 2017a). Temperature also affects arthropod development and the warmer temperatures associated with higher impervious surfaces may cause a phenological mismatch which might reduce host availability (Meineke et al. 2014). It is possible that pollution in urban systems may interrupt parasitoid host finding abilities and arthropod communication systems in polluted systems should be studied further (Gish et al. 2015).

Woodpecker predation was responsible for the highest mean rate of mortality among all of the sites, and its variation was not justified by any of the explanatory variables we investigated. This finding is unsurprising as a previous study in Michigan examining factors affecting woodpecker predation on emerald ash borer similarly could not explain woodpecker predation with habitat factors, due to high variation in attack rates within sites (Lindell et al. 2008). Lindell et al. (2008) saw reduced woodpecker predation in more forested sites, although this explained very little of the variation. Results here suggested the opposite, with woodpecker predation increased at natural land cover at 75 m radius, although this result was not significant. Previous research has shown that avian nest survival is correlated with habitat measurements along the urbanization gradient at the 1000 m spatial scale (Ryder et al. 2010). Although I predicted habitat factors at a larger spatial scale (1000 m) would be more highly correlated with woodpecker predation rate, I did not see a notable increase in R^2 values at this spatial scale compared to local scale values. This suggests that there are other, unmeasured drivers at play in the woodpecker ecosystem. Anecdotally, I observed bird feeders and cached seeds from bird feeders in the ash trees we sampled in some of the more urban sites that were part of residential areas (HFD site and PW site). I hypothesize that bird feeders may have increased the woodpecker's association with these more urban trees in neighborhoods.

Undetermined mortality rate was highly variable among the 9 sites. Tree richness was significantly related to undetermined mortality rate, which increased with increased number of tree genera. Undetermined mortality included several types of mortality that were difficult to tease apart including larvae killed by tree defenses, various pathogens, disease, unsuccessful parasitism and other such forms of mortality. While it is unclear why tree richness would relate to this grouping of mortality factors, other complexities associated with increased tree genera that were not measured here, may provide an explanation for this relationship.

A limitation of this study was that tree removals in urban sites limited this study to one year, as municipalities could not guarantee sites would retain ash trees year to year (tree removal is a standard emerald ash borer / hazard tree management practice) which prevented multiple year sampling. It may be helpful to perform

parasitoid releases in urban sites that can be sustained for several years prior to sampling to further elucidate parasitoid establishment and parasitism rates. Additionally, future research to examine emerald ash borer parasitism rates in response to floral resource availability warrants consideration as it has been shown that, in general, parasitoid populations have been positively correlated with floral resource availability on local scales even in highly urbanized (highly impervious) locations (Bennett and Gratton 2012, Burks and Philpott 2017), which could prove beneficial when considering parasitoid release locations.

I predicted that emerald ash borer densities would be higher in more urban habitats than natural. However, here I found higher emerald ash borer densities associated with factors related to more natural habitats. I observed generally lower mean emerald ash borer density among planted trees than naturally occurring trees, although not significantly so (Fig. 2.6A). This appears to contradict previous studies' results that indicate higher pest densities in urban habitats (Raupp et al. 2010, Meineke et al. 2014, Dale and Frank 2014, 2017a, 2017b). Higher emerald ash borer density has been linked with increased mortality (Duan et al. 2010, MacQuarrie and Sharbach 2015), which could explain the results I see here between habitat types, as there was a numerically lower mean EAB density in urban sites (Fig 2.6A). Additionally, I suspect that due to ash tree removal practices in municipalities, the trees available for observation may be skewed toward lower EAB density populations. Alternatively, I hypothesize that the method by which we calculated EAB density may have underestimated emerald ash borer density in urban trees. The formula provided by McCullough and Siegert (2007) estimates tree phloem area using DBH alone, which was higher in our urban trees, although these trees were generally cultivars with a growth habit shorter and wider than straight species in the naturally occurring trees. This formula was created using only naturally occurring trees, therefore I believe that this overestimated the phloem area given that our natural trees were tall and thin (small DBH) and the urban trees were short and wide (large DBH). Interestingly, I anecdotally observed higher densities of non-emerald ash borer pests including bark beetles (Coleoptera: Curculionidae: Scolytinae), clearwing borers (Lepidoptera: Sesiidae), and round-headed borers (Coleoptera: Cerambycidae) known to infest ash trees in the planted/managed trees.

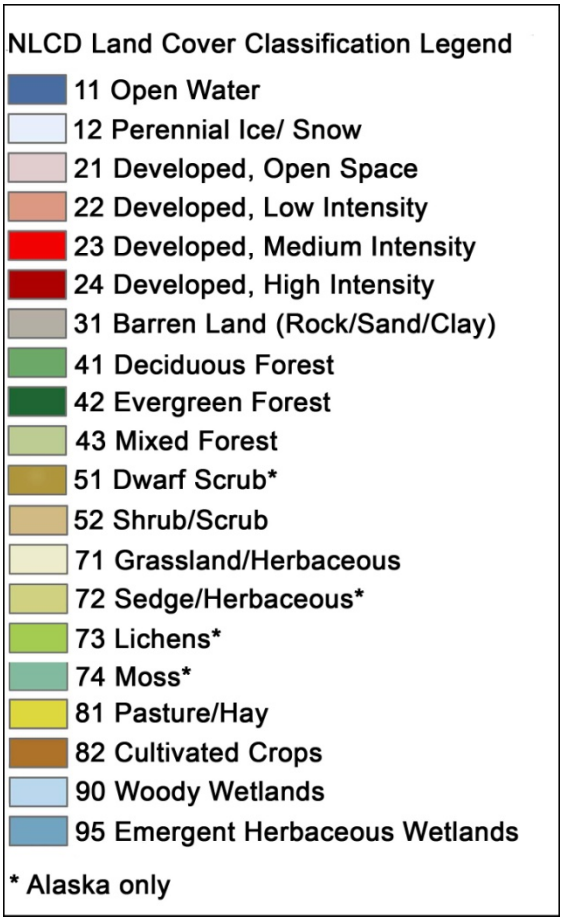
Emerald ash borer density had a significant, positive linear relationship with percent agricultural land cover at 150 m spatial scale (Appendix B). It is unclear as to what would be the cause of this phenomenon, although this could be a result of low sample size as only three sites had agricultural land cover at that spatial scale.

Urbanization greatly affects ecological processes and arthropod communities and have specifically been observed becoming more destabilized as one moves along the urbanization gradient (Raupp et al. 2010). Here I recognize similar results with generally lower emerald ash borer mortality in planted trees over natural trees and reduced rates of pest mortality due to parasitism (Fig. 2.6C) and undescribed mortality factors (Fig 2.6D) in planted/managed ash trees. My results support previous results found by MacQuarrie and Sharbach (2015) that looked at emerald ash borer survival in urban systems and determined that emerald ash borer suffered low mortality due to biotic factors. Overall, parasitism was responsible for low rates of emerald ash borer mortality regardless of site type, and were likely not affecting

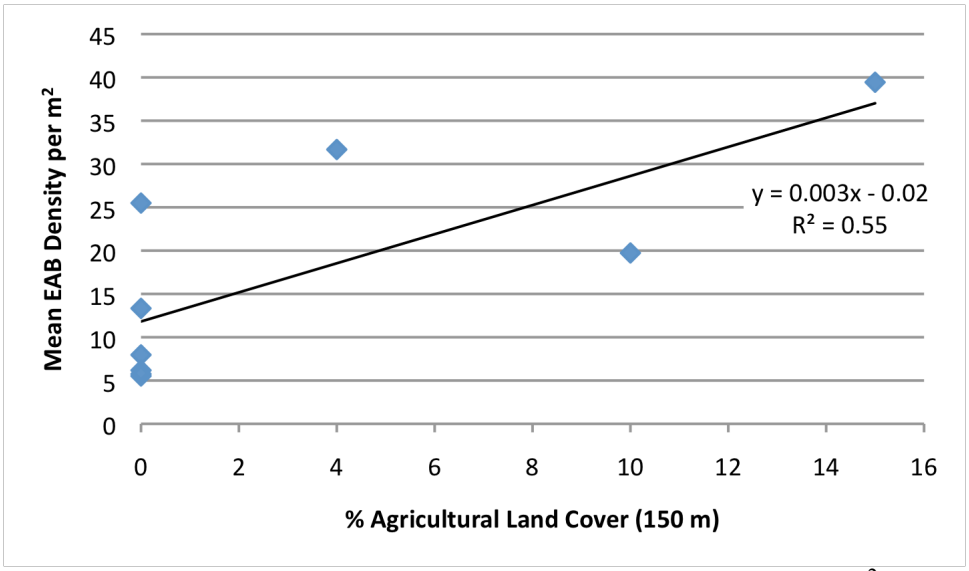
the pest population greatly. Based on findings here along with the findings by MacQuarrie and Sharbach (2015), I hypothesize that before biological control by hymenopteran parasitoids can attribute significant population control for emerald ash borer in these urban systems, bottom up control by natural tree resistance or other emerald ash borer population suppression by pesticides, needs to be established, and these two methods combined may provide a natural balance for future ash trees. In that case, resistant, non-grafted ash cultivars, in areas with minimal impervious surface maybe the best candidates for parasitoid establishment.

Appendix A

NLCD Land Cover Classification Legend for land cover measurements made at field sites.



Appendix B



Relationship between mean emerald ash borer (EAB) density per m^2 phloem area and percent agricultural land cover at 150 m spatial scale.

Bibliography

Abell, K., Duan, J. J., Bauer, L., Lelito, J. P., VanDriesche, R. G. (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). Biol Cont 63: 320-325.

Abell, K. J., Bauer, L. S., Duan, J. J., Van Driesche, R. (2014) Long-term monitoring of the introduced emerald ash borer (Coleoptera: Buprestidae) egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae), in Michigan, USA and evaluation of a newly developed monitoring technique. Biological Control 79, 36–42.

Abell, K. J., Bauer, L. S., Miller, D. L., Duan, J. J., Van Driesche R. G. (2016) Monitoring the establishment and flight phenology of parasitoids of emerald ash borer (Coleoptera: Buprestidae) in Michigan by using sentinel eggs and larvae. Fla Entomol 99(4):667-672.

Analytical Software. *Statistix 10*. Tallahassee, FL: Analytical Software 2013.

Bauer, L. S., H-P Liu, R. A. Haack, R-T Gao, T. H., Zhao, D. L. Miller, Petrice, T. R. (2005) Update on emerald ash borer natural enemies in Michigan and China, pp. 71–72. In V. Mastro and R. Reardon, eds. Proceedings of the Emerald Ash Borer Research and Technology Meeting, Romulus, MI. USDA FSFHTET-2004-15.

Bauer, L. S., Liu, H. P., Miller, D. L., Gould, J. (2008) Developing a classical biological control program for *Agrilus planipennis* (Coleoptera: Buprestidae), an invasive ash pest in North America Newsl. Michigan Entomol Soc 53:38–39.

Bauer, L. S., Duan, J. J., Gould, J. R., Van Driesche R. (2015) Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Can Entomol 147:300-317.

Belokobylskij, S. A., Yurchenko G. I., Strazanac J. Z., Zaldívar-Riveró, N.A., Mastro, V. (2012) A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius Nees* (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Ann. Entomol. Soc. Am 105:165–178.

Bennett, A, B., Gratton, C. (2012) Local and landscape scale variable impact parasitoid assemblages across and urbanization gradient. Landscape and Urban Planning 104(1):26-33.

Benrey, B., Denno, R. (1997) The show-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987-999.

Burks, J. M., Philpott, S. M. (2017) Local and Landscape Drivers of Parasitoid Abundance, Richness, and Composition in Urban Gardens. Community and Ecosystem Ecology 46(2):201-209.

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

Cipollini, D., Wang, Q., Whitehill, J., Powell, J., Bonello, P., Herms, D. (2011) Distinguishing Defensive Characteristics in the Phloem of Ash Species Resistant and Susceptible to Emerald Ash Borer. J Chem Ecol 37:450-159.

Cipollini, D. (2015) White fringetree as a novel larval host for emerald ash borer. J. Econ Entomol 108:370–375.

Cipollini, D., Rigsby, C. M. (2015) Incidence of infestation and larval success of emerald ash borer (*Agrilus planipennis*) on white fringetree (*Chionanthus virginicus*), Chinese fringetree (*Chionanthus retusus*), and devilwood (*Osmanthus americanus*). Environ Entomol 44:1375–1383.

Cipollini, D., Rigsby, C. M., Peterson, D. L. (2017) Feeding and Development of Emerald Ash Borer (Coleoptera:Buprestidae) on Cultivated Olive, *Olea europea*. J. Econ Entomol 110(4):1935-1937.

Dale, A.G., Frank, S. D. (2014) The effects of urban warming on herbivore abundance and street tree condition. PLoS ONE 9 e102996.

Dale, A. G., Youngsteadt, E., Frank, S. (2016) Forecasting the Effects of Heat and Pests on Urban Trees: Impervious Surface Thresholds and the 'Pace-to-Plant' Technique. Arboriculture and Urban Forestry 42(3):181-191.

Dale, A. G., Frank, S. (2017a) Urban warming trumps natural enemy regulation of herbivorous pests. Ecological Applications 24(7):1596-1597.

Dale, A. G., Franks, S. D. (2017b) Warming and drought combine to increase pest fitness on urban trees. PLoS One 12(3).

Davidson, W., Rieske, L. K. (2016) Establishment of classical biological control targeting emerald ash borer is facilitated by use of insecticides, with little effect on native arthropod communities. Biol Control 101:78-86.

Duan, J. J., Bauer, L. S., Ulyshen, M. D., Gould, J., Van Driesche, R. G. (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. J., Bauer, L. S., Abell, K. J., van Driesche, R. (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.

Duan, J. J., Fuester, R.W., Wildonger, J., Taylor, P.B., Barth, S., Spichiger, S. E. (2013a) Parasitoids attacking the emerald ash borer (Coleoptera: Buprestidae) in western Pennsylvania. Florida Entomologist 92:588–592.

Duan, J. J., Watt, T., Taylor, P., Larson, K., Lelito, J. P. (2013b) Effects of ambient temperature on egg and larval development of the invasive emerald ash borer (Coleoptera: Buprestidae): implications for laboratory rearing. J Econ Entomol106: 2101–2108.

Duan, J. J., Bauer, L. S., Abell, K., Lelito, J. P., van Driesche, R. (2013c) Establishment and Abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: Potential for Success in Classical Biocontrol of the Invasive Emerald Ash Borer (Coleoptera: Buprestidae). J Econom Entomol 106(3):1145-1154.

Duan, J. J., Abell, K. J., Bauer, L. S., Gould, J., Van Driesche, R. (2014) Natural enemies implicated in the regulation of an invasive pest: a life table analysis of the population dynamics of the emerald ash borer. Agr For Entomol 16: 406-416.

Duan, J. J., Bauer, L. S., Abell, K. J., Ulyshen, M. D., Van Driesche, R. G. (2015) Population dynamics of an invasive forest insect and associated natural enemies in the aftermath of invasion: implications for biological control. J Appl Ecol 52:1246–1254.

Duan, J. J., Bauer, L. S., Van Dreiesche, R. G. (2017) Emerald ash borer biocontrol in ash saplings: The potential for early stage recovery of North American ash trees. Forest Ecol Manag 394:64-72.

Environmental Systems Research Institute (ESRI). (2012). *ArcGIS v.10.4*. Redlands, CA.

Flower, C. E., Knight, K. S., Rebbeck, J., Gonzalez-Meler, M. A. (2013) The relationship between the emerald ash borer (*Agrilus planipennis*) and ash (*Fraxinus spp.*) tree decline: using visual canopy condition assessments and leaf isotope measurements to assess pest damage. Forest Ecology and Management 303:143–147.

Flower, C. E, Long, L. C., Knight, K. S., Rebbeck, J., Brown, J. S., Gonzales-Meler, M. A., Whelan, C. J. (2014) Native bark-foraging birds preferentially forage in infected ash (*Fraxinus spp.*) and prove effective predators of the invasive emerald ash borer (*Agrilus planipennis* Fairmaire). Forest Ecology and Management. 313:300-306.

Gish, M. De Moraes, C. M., Mescher, M. C. (2015) Herbivore-induced plant volatiles in natural and agricultural ecosystems: open questions and future prospects. Current Opinion in Insect Science. 9:1-6.

Grosman, A. H., van Breemen, M., Holtz A, Pallini, A., Rugama, A. M., Pengel, H., Venzon, M., Zanuncio, J. C., Sabelis, M. W., Janssen, A. (2005) Searching behaviour of an omnivorous predator for novel and native host plants of its herbivores: a study on arthropod colonization of eucalyptus in Brazil. Entomol Exp Appl 116:135–142.

Haack, R. A., Jendek, E., Liu, H-P, Marchant, K. R., Petrice, T. R., Poland, T. M., Ye, H. (2002) The emerald ash borer: a new exotic pest in North America. Newsletter Michigan Entomol Soc 47: 1–5.

Heard, S. B., Stireman, J. O., Nason, J. D., Cox., G. H., Kolacz, C. R., Brown, J. M. (2006) On the elusiveness of enemy free space: spatial, temporal, and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods. Oecologia. 150:421-434.

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

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

Hironaka, Y., Kioke, F. (2013) Guild Structure in the Food Wed of Grassland Arthropod Communities Along and Urban-Rural Landscape Gradient. Ecoscience 20(2):148-160.

Jeffries, M. J., Lawton, J. H. (1984) Enemy free space and the structure of ecological communities. – Biol. J. Linn.Soc. 23: 269–286.

Jennings, D. E., Gould, J. R., Vanderberg, J. D., Duan, J. D., Shrewsbury, P. M. (2013) Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (Agrilus planipennis). PLoS ONE 8(12): e83491.

Jennings, D. E., Duan, J. J., Larson, K. M., Lelito, J. P., Shrewsbury, P. M. (2014) Evaluating a New Method for Monitoring the Field Establishment and Parasitism of *Oobius agrili* (Hymenoptera:Encyrtidae), an Egg Parasitoid of Emerald Ash Borer (Coleoptera: Buprestidae). Fla Entomol 97(3):1263-1265.

Jennings, D. E., Duan, J. J., Bauer, L. S., Schmude, J. M., Wetherington, M. T., Shrewsbury, P. M. (2016a) Temporal dynamics of woodpecker predation on emerald ash borer (*Agrilus planipennis*) in the northeastern USA. Agriculture and Forest Entomology 18(2):174-181.

Jennings, D. E., Duan, J. J., Bean, D., Gould, J. R., Rice, K. A., Shrewsbury, P. M. (2016b) Monitoring the establishment and abundance of introduced parasitoid of emerald ash borer larvae in Maryland, USA. Biol Contr 101:138-144.

Jennings, D. E., Duan, J. J., Bean, D., Rice, K., Williams, G. L., Bell, S. K., Shurtleff, A. S., Shrewsbury, P. M. (2017) Effects of the emerald ash borer invasion on the community composition of arthropods associated with ash tree boles in Maryland, USA. Agriculture and Forest Entomology. 19(2):122-129.

Jones, E. L., Leather, S. R., (2012) Invertebrates in urban areas: A review. European Journal of Entomology 109(4):463-478.

Kenis, M., Auger-Rozenberg, M. A., Roques A., Timms L., Pere, C., Cock, M., Settele, J., Augustin, S., Lopez-Vaamonde, C. (2009) Ecological effects of invasive alien insects. Biol Invasions 11:21–45.

Kovacs, K. F., R. G. Haight, D. G. McCullough, R. J. Mercader, N. W. Siegert, and A. M. Liebhold. (2010) Cost of potential emerald ash borer damage in US communities, 2009–2019. Ecol. Econ 69: 569–578.

Kula, R. R., Knight, K. S., Rebbeck, J., Bauer, L. S., Cappeart, D. L. and Gandhi, K. J. K. (2010) *Leluthia astigma* (Ashmead) (Hymenoptera:Braconidae: Doryctinae) as a parasitoid of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae: Agrilinae), with an assessment of host associations for Nearctic species of *Leluthia cameron*. Proceedings of the Entomological Society of Washington 112:246–257.

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

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

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

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10:689–710.

Macquarrie, C. J. K., Scharbach, R. (2015) Influence of Mortality Factors and Host Resistance on the Population Dynamics of Emerald Ash Borer (Coleoptera: Buprestidae) in Urban Forests. Environ Entomol 44:160-173.

MacFarlane, D. W., S. P. Meyer. (2005) Characteristics and distribution of potential ash tree hosts for emerald ash borer. For. Ecol. Manage. 213: 15–24.

McCullough, D.G., Siegert, N.W., Bedford, J., (2012) Slowing ash mortality: a potential strategy to SLAM emerald ash borer in outlier sites. International Journal of Pest Management 58:9-23.

McIntyre N. E. (2000) Ecology of urban arthropods: a review and a call to action. Ann. Entomol. Soc. Am. 93:825–35.

McKinney, M. L. (2002) Urbanization, Biodiversity, and Conservation. BioScience 52:883-890.

Meineke, E. K., Dunn, R. R. Frank, S. D. (2014) Early pest development and loss of biological control are associated with urban warming. Biology Letters. 10(11).

Moulton, C. A., Adams, L. W. (1991) Effects of urbanization on foraging strategy of Woodpeckers. In Wildlife conservation in metropolitan environments, NIUW Symp. Ser. 2. pp 67–73. Edited by L. W. Adams and D. L. Leedy. Natl. Inst. for Urban Wildl., Columbia, Maryland. 264.

Moran, V.C. and Southwood, T.R.E. (1982) The guild composition of arthropod communities in trees. Journal of Animal Ecology, 51:289–306.

Parisio, M. S., Gould, J. R., Vanderberg, J. D., Bauer, L. S., Fierke, M. K. (2017) Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biol Control 106:45-53.

Peterson, D. L., Cipollini D. (2017) Distribution, Predictors, and Impacts of Emerald Ash Borer (*Agrilus planipennis*) (Coleoptera: Buprestidae) Infestation of White Fringetree (*Chionanthus virginicus*). Environ Entomol 46:50-57.

Philpott, S. M., Cotton, J., Bichier, P., Friedrich, R. L., Moorhead, L. C., Uno, S., Vladez, M. (2014) Local and landscape divers of arthropod abundance, richness, and trophic composition in urban habitats. Urban Ecosyst 17:513-532.

Pimentel, D., McNair S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T., Tsomondo, T. (2001) Economic and environmental threats of alien plant, animal, and microbe invasion. Agric. Ecosyst. Environ 84, pp. 1–20.

Poland, T. M., McCullough, D. G. (2006) Emerald Ash Borer: Infestation of the Urban Forest and the Threat to North America's Ash Resource. J Forest 104(3):118-124.

Poland, T. M., Chen Y., Koch, J., Pureswaran, D. (2015) Review of the emerald ash borer (Coleoptera: Buprestidae), life history, mating behaviors, host plant selection, and host resistance. Canadian Entomologist. 147(3):252-262.

Poland, T. M., Ciaramitaro, T. M., McCullough, D. G. (2016) Laboratory Evaluation of the Toxicity of Systemic Insecticides to Emerald Ash Borer Larvae. Forest Entomology. 105:705-716.

R Core Team (2016) *R*: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Austria [WWW document]. URL http://www.r-project.org [accessed on 11 November 2017].

Raupp, M. J., Shrewsbury, P. M., Herms, D. A.. (2010) Ecology of herbivorous arthropods in urban landscapes. Annu. Rev. Entomol. 55: 19–38.

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

Rutledge, C. E., Arango-Velez, A. (2017) Larval Survival and Growth of Emerald Ash Borer (Coleoptera:Buprestidae) on White Ash and White Fringetree Saplings Under Well-Watered and Water-Deficit Conditions. Enviro Entomol. 46:243-250.

Ryder, T. B., Reitsma, R., Evans, B., Marra, P. P. (2010) Quantifying avian nest survival along an urbanization gradient using citizen- and scientist-generated data. Ecol. Appl. 20: 419–426.

SAS Institute Inc. (2017) *JMP Pro 13.01.* SAS Institute Inc, Gary, NC.

SAS Institute Inc. (2017) *Studio 3.6 University Edition*. SAS Institute Inc, Gary, NC.

Schiers J, De Bruyn L (2002) Temporal variabilty of top-down forces and their role in host choice evolution of phytophagous arthropods. Oikos 97:139–144.

Shrewsbury, P. M., Raupp, M. J. (2000) Evaluation of components of vegetational texture for predicting azalea lacebug, *Stephanitis pyrioides* (Heteroptera: Tingidae), abundance in managed landscapes. Environmental Entomology 29:919–926.

Shrewsbury, P. M., Raupp, M. J. (2006) Do top-down or bottom-up forces determine *Stephanitis pyrioides* abundance in urban landscapes? Ecological Applications 16:262–272.

Siegert, N. W., McCullough, D. G., Liebhold, A. M., Telewski, F. W. (2014) Dendrochronological reconstruction of the epicenter and early spread of emerald ash borer in North America. Diversity and Distributions. 20:847-858.

Smith, A. (2006) Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River watershed in southeast Michigan. MS thesis, Ohio State University.

Thiemann, D., Lopez, V., Ray, A. M., Cipollini, D. (2016) The History of Attack and Success of Emerald Ash Borer (Coleoptera: Buprestidae) on White Fringetree in Southwestern Ohio. Enviro Entomol. 45:961-966.

Uesudi, A. (2015) The slow-growth high-mortality hypothesis: direct experimental support in a leafmining fly. Ecological Entomology. 40:221-228.

Ulyshen, M. D., Duan, J. J., Bauer, L. S., Fraser, I. (2010a) Suitability and Accessibility of Immature *Agrilus planipennis* (Coleoptera:Buprestidae) Stages to Tetrastichus planipennisi (Hymenoptera: Eulophidae). J Econ Entomol. 103(4):1080-1085.

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

Villari, C., D. A. Herms, J.G.A. Whitehill, D. Cipollini, and P. Bonello. (2015) Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer, a model for wood-boring insects that kill angiosperms. New Phytol. 209: 63–79.

Wallander, E., Albert, V. A. (2000) Phylogeny and classification of Oleaceae based on rps16 and trnL-F sequence data. Am. J. Bot. 87:213–231.

Wang, X. Y., Wei, K., Yang, Z. Q., Jennings, D. E., Duan, J. J. (2016) Effects of biotic and abiotic factors on phenotypic partitioning of wing morphology and development in *Sclerodermus pupariae* (Hymenoptera: Bethylidae). Scientific Reports 6:26408.

Yang, Z. Q., Wang, X, Y, Yao, Y. X., Could, J. R., Cao, L. M. (2012) A New Species of *Sclerodermus* (Hymenoptera: Bethylidae) Parasitizing *Agrilus planipennis* (Coleoptera: Buprestidae) From China, With a Key to Chinese Species in the Genus. Annals of The Entomological Society of America. 105:619-627.

Youngsteadt, E., Dale, A. G., Terando, A. J., Dunn, R. R., Frank S. D. (2015) Do cities simulate climate change? A comparison of herbivore response to urban and global warming. Global Change Biology. 21(1):97-105.