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Preference, performance, and selection of historic and novel hosts by emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae)

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PREFERENCE, PERFORMANCE, AND SELECTION OF HISTORIC AND NOVEL HOSTS BY EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE)

A dissertation submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

by

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I HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER MY SUPERVISION BY Donnie L Peterson ENTITLED Preference, performance, and selection of historic and novel hosts by emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy

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Abstract

Peterson, Donnie L. Ph D., Environmental Sciences PhD Program, Wright State University, 2019. Preference, performance, and selection of historic and novel hosts by emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae).

North American and European ash trees are highly susceptible to emerald ash borer (EAB, *Agrilus planipennis*). This buprestid kills hosts via larva feeding on vascular tissue which eventually kills the host plant. Two new hosts have recently been found to support larval development of EAB. White fringetrees (*Chionanthus virginicus*) were found attacked by EAB in 2014 and since then have been found to be attacked throughout other parts of the United States, while olive (*Olea europaea*) has only experimentally been found to support larvae to adulthood. Chemical profiles of these two plants were collected and analyzed to determine how their volatile emissions vary among susceptible and resistant ash trees. Additionally, larvae and adult beetles were tested for their performance on these novel plants. For white fringetree, wild populations were monitored to determine the impact of EAB during the attack wave. These studies find that white fringetree foliage supports adults, but when phloem is healthy it causes high larval morality in contrast to girdled or previously attacked by EAB where larvae survived by the end of assays. In the field, EAB began to use white fringetree quickly, within a couple of years after initial exposure. Female choice suggests white fringetree and susceptible ash are similarly preferred likely due to the similar volatile emissions. These chemicals likely caused host range expansion of EAB to this novel host. In contrast to ash, the impact of EAB on white fringetree is minimal. This plant mostly loses a branch or two from larval girdling, which in ornamentally planted fringetrees can be aesthetically displeasing. On olive, EAB is likely to be even less damaging because larvae take longer

to develop than in ash and larvae die quickly in young, photosynthesizing stems. Adults did not perform well because oleuropein may cause them to compensatory feed and causing malnutrition. In North American forests, if EAB continues to destroy ash species at such high rates, EAB may be driven to use white fringetree more often. With continued use of this host, EAB is likely to adapt to better utilize white fringetree which could eventually lead to host switching.

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Abstract:

Novel hosts lacking a coevolutionary history with herbivore pests can support improved larval performance over historic hosts; e.g., emerald ash borer (EAB, *Agrilus planipennis*) on North American ash (*Fraxinus* spp.) trees. Whether trees are novel or ancestral, stress on plants increases EAB preference and performance. White fringetree (*Chionanthus virginicus*) and olive (*Olea europaea*) are closely related non-ash hosts that support development of EAB to adulthood but their relative suitability as hosts and the impact of stress on larval success has not been well studied. In a series of experiments, survival and growth of EAB larvae on these novel hosts were examined along with the impact of stress. In the first experiment, larvae grew more slowly in cut stems of olive than in green ash (*F. pennsylvanica*) with several adults that successfully emerged from larger stems. In two experiments on young potted olive with photosynthesizing bark, larvae died within a week, but mechanical girdling increased the rate of gallery establishment. The final two experiments on field grown fringetrees found increased larval survivorship and growth in previously EAB attacked and mechanically girdled plants than in healthy stems or stems treated with the defense hormone, methyl jasmonate. Our data demonstrate that these non-ash hosts are less suitable for EAB than preferred ash hosts, but previous EAB attack or girdling led to better survival and growth demonstrating the importance of stress for larval success. In potted olive, high mortality could be due to higher loads of toxic compounds or the presence of chlorophyllous tissue.

Keywords: Host range expansion, stressed host, emerald ash borer, white fringetree, olive

Introduction:

Many woodboring insects perform better and prefer stressed over healthy plants (Koricheva et al. 1998, Evans et al. 2007, Jactel et al. 2012). The exploitation of these weakened hosts can lead to faster development and more damage to plants (Jactel et al. 2012). Buprestids (jewel beetles), are a group of woodboring insects that have evolved to locate and exploit stressed hosts over healthy individuals. The genus *Agrilus* is the most studied because several species have become destructive, costly pests on novel, non-coevolved hosts, even if they are healthy. Examples include bronze birch borer (*A. anxius*) on novel birch hosts (*Betula* spp.) from Eurasia (Muilenburg and Herms 2012), goldspotted oak borer (*A. auroguttatus*) on novel oak hosts in California (*Quercus* spp.; Coleman and Seybold 2008), and the most well studied Buprestid, emerald ash borer (EAB, *A. planipennis*) on novel North American and European ash hosts (*Fraxinus* spp.). These beetles perform better on novel hosts due to two mechanisms. One of these is due to “enemy free space”, a concept whereby an organism escapes its natural enemies by using novel hosts or by moving out of the geographical ranges of natural enemies. In the case of EAB, the accidental introduction into North American provided enemy free space from specialist parasitoids from east Asia (Liu et al. 2003, Duan et al. 2010). The second mechanism is a concept called “defense free space” (Gandhi and Herms 2010), whereby novel hosts are susceptible to exotic herbivores due to the lack of specialized plant defenses that would have evolved if they had shared a coevolutionary history. This idea can be observed in the susceptibility of North American ash trees compared to the resistant ancestral host, Manchurian ash (*F. mandschurica,* (Liu et al. 2003, Rebek et al. 2008, Herms 2014) which appears to have defenses targeted for EAB (Whitehill et al. 2012, Rigsby et al. 2015, 2016, Villari et al. 2016). Combined defense free space and enemy free space can lead to widespread invasiveness of herbivores on novel hosts. When these herbivores reach a new geographic range, they may encounter previously unknown hosts that belong to different genera or families. The question then is how does herbivore performance vary on these novel hosts relative to their ancestral host and what factors affect their relative suitability?

Emerald ash borer has become the most costly North American forest pest (Aukema et al. 2011), killing hundreds of millions of ash trees in the United States and Canada. Previously, EAB was thought to only use ash trees; however, recent research has revealed that this beetle can successfully use two other species in two genera in the Olive family (Oleaceae) as larval hosts. One of these species is white fringetree (*Chionanthus virginicus*) which is closely related to *Fraxinus* (Wallander and Albert 2000). Since the initial discovery of this tree as a host for EAB (Cipollini 2015), larvae have been shown to successfully develop and emerge from white fringetree under a variety of conditions across many sites in the field (Cipollini 2015, Cipollini and Rigsby 2015, Thiemann et al. 2016, Peterson and Cipollini 2017). While not directly compared, we can infer from literature that attack rates by EAB are generally lower on ornamental white fringetrees (Peterson and Cipollini 2017), than in comparably sized ash trees (Rebek et al. 2008, Herms 2014). In addition to variation in attack rates, EAB larvae generally grow more poorly and impact white fringetree less severely than susceptible ash species (Cipollini and Rigsby 2015, Rutledge and Arango-Velez 2017, Hoban et al. 2018, Olson and Rieske 2018). The other non-ash species shown to support EAB is cultivated olive (*Olea europaea*). This plant species is also a close relative of ash (Wallander and Albert 2000) and cut stems can support EAB development from egg to adulthood (Cipollini et al.

2017). Although never directly compared to ash, larval development appears to be slower on olive than on preferred ash hosts (Cipollini et al. 2017). Larvae, for example, fed for over 69 days in cut stems of olive before reaching mature 4th instars (Cipollini et al. 2017), compared to 37-40 days in three susceptible ash species in similar studies (Cipollini and Rigsby 2015, Peterson et al. 2015). Further research needs to be conducted on both white fringetree and olive to determine the relative suitability of these hosts for EAB.

Many plants actively defend themselves with the induction of secondary metabolites leading to reduced performance or mortality of herbivores. These inducible defenses are likely hampered in cut stems of woody plants because the vascular system is separated from the rest of the tree, and hindered in trees with vascular injuries from EAB larvae or other stresses. Many studies have used cut stems to assess larval performance (Cipollini and Rigsby 2015, Peterson et al. 2015, Cipollini et al. 2017, Hoban et al. 2018), which will have constitutive defenses but lack an intact system to transport necessary resources for an induced defense, if resources are transported via long distance. While EAB can routinely feed and reach adulthood on live intact trees in the field (Cipollini 2015, Cipollini and Rigsby 2015, Peterson and Cipollini 2017), survival and growth rates have been observed to be lower on intact stems than on cut stems, as observed in preferred ash species (Peterson et al. 2015, Tanis and McCullough 2015). White fringetrees with intact stems kill larvae at a higher rate (71%) than white ash (*F. americana*; 25%; Rutledge and Arango-Velez 2017). In olive, larval survivorship in cut stems appears to be low (Cipollini et al. 2017), but the relative performance of EAB in this host has not been compared with ash and the examination of larval performance in an intact olive tree has been limited to a single tree (Cipollini and Peterson 2018).

A variety of biotic and abiotic factors can affect the performance of EAB on its hosts (Tluczek et al. 2011, Chakraborty et al. 2014, Whitehill et al. 2014, Rutledge and Arango-Velez 2017, Rigsby et al. 2019). Larval performance increases in ash trees when they are mechanically girdled or attacked previously by EAB (Tluczek et al. 2011, Rigsby et al. 2019). In contrast, larval performance can be hampered from a treatment of methyl jasmonate (MeJA). This compound is involved in the induction of secondary metabolites in response to pathogen or insect attack (Erbilgin et al. 2006, Ballaré 2011). In the EAB system, MeJA increases trypsin inhibitor activity and concentrations of verbascoside and lignin in ash trees which is associated with a decrease in larval survival and growth (Whitehill et al. 2014). In terms of performance on novel hosts, the fitness of EAB is expected to increase in stressed hosts and decrease in MeJA treated plants, as observed in ash trees. Rutledge and Arango-Velez (2017) investigated the effect of drought stress on the susceptibility of white ash and white fringetree to EAB and postulated that survivorship would be higher in stressed plants. This was indeed the case on white ash, but drought stress unexpectedly led to lower larval survival of EAB in white fringetrees. The authors suggest that either the availability of nitrogen or carbon compounds were reduced by drought, lower water availability led to larval desiccation, or fringetrees directed resources to defense rather than growth (Rutledge and Arango-Velez 2017). Regardless of the mechanism, these results demonstrate that larval performance of EAB in at least this novel host is less predictable than expected.

To address gaps in our knowledge of the suitability of non-ash hosts for EAB, we conducted a series of field and laboratory experiments. In the first experiment, the survival and growth of EAB were evaluated in cut stems of mature olive and green ash, which have never been compared. We postulated that survival and growth will be poorer in the novel non-ash hosts. The other experiments used live, intact plants. In two experiments with field-grown white fringetree, we examined the effect of previous attack by EAB, girdling, and MeJA application on growth and survival. Larvae were hypothesized to survive better and grow larger on previously attacked (stressed) and mechanically girdled (stressed) white fringetrees compared to untreated controls. The larvae in MeJA-treated trees were hypothesized to have higher mortality rates and grow slower than those in control plants. In the last experiments, we examined the influence of girdling on the growth and survival of EAB in young potted olive trees. We hypothesized that larvae would grow larger and survive at higher rates in girdled and stressed stems.

Methodology:

Emerald ash borer egg source and stem inoculation.

For all experiments, emerald ash borer eggs used in infestation were obtained from the USDA-APHIS PPQ Biological Control Rearing Facility in Brighton, MI from adults reared from ash logs and fed ash foliage. The eggs were laid by adult females on coffee filter paper three to six days before being sent overnight to Wright State University. Eggs were stored in a growth chamber (25ºC 16:8 Light:Dark cycle) until they reached ~12-14 days old, or about two to three days before eclosure of neonates. Eggs were attached to cut stems or live trees spaced at least 10 cm apart on branches chosen for infestation, as in Cipollini and Rigsby (2015) and Peterson et al. (2015). Eggs were placed on areas of smooth bark and we avoided areas of dead plant tissue or heavy moss/lichen coverage.

Experiment 1. Larval performance in cut stems of olive vs ash.

Emerald ash borer eggs laid between 26-30 November 2016 were sent overnight to Wright State University on 30 November 2016 and stored in a growth chamber (25ºC 16:8 Light:Dark cycle). On 1 December 2016, stems of two cultivars of olive, Barouni (n=17) and Manzanilla (n=9), were cut and shipped from Salinas, CA from older, thick barked trees used in commercial olive production. These logs were cut to lengths between 45-60 cm and ranged in diameter of 7-20 cm. Green ash stems (n=3) from Wright State University were cut on 2 December 2016 to lengths of 30 cm. Olive and ash stems had EAB eggs attached on 6 December 2016 and stored in a growth chamber (25ºC 16:8 Light:Dark cycle) in plastic containers with their lower ends immersed in 5-7 cm of distilled water to maintain moisture, as in Cipollini and Rigsby (2015) and Peterson et al. (2015). Upon inspection, the majority of eggs hatched by 12 December. At 37 days (18 January 2017), 44 days (25 January), and 51 days (1 February) after egg hatch, one ash, three Barouni, and three Manzanilla stems were debarked to reveal EAB larvae and to measured gallery width and survivorship as described in Peterson et al. (2015) and Cipollini et al. (2017). Due to a lower availability of Manzanilla stems, none of these stems were debarked on day 51.

A subset of three Barouni and three Manzanilla stems were kept to determine if larvae could successfully reach adulthood through an artificial overwintering period, as in Cipollini et al. (2017). We recorded the diameter in the middle of each stem (cm). These stems were first exposed to 10ºC in an incubator starting on 14 March and then transitioned to 4ºC on 13 April, 10ºC on 12 May, and then returned to ~23ºC on 1 June. Afterward, stems were placed into 190-liter rearing barrels covered with a metal mesh lid (Thiemann et al. 2016). These barrels were monitored for three months for adult beetle emergence and then debarked to determine fates of any remaining larvae. Emerged adults were kept in growth chamber (25º C, 16:8 L:D) in clear plastic containers (72 oz.) and provided fresh green ash foliage every three-four days. Stems that were overwintered varied in size with Manzanilla stems being significantly larger (15.3±1.7 cm, t= 3.18; df= 3; p=0.026) than Barouni stems (7.1±1.0 cm).

Experiment 2. Larval performance on previously attacked vs healthy white fringetrees in the field.

On 22 of June 2016, white fringetrees (n=16) with branch diameters between 2.5-5.0 cm were selected to be infested with EAB eggs at Spring Grove Cemetery and Arboretum in Cincinnati, Ohio. White fringetrees were split into two treatments: those that were previously attacked by EAB (n=8) and uninfested, healthy trees (n=8). The trees were previously recorded by Cipollini and Rigsby (2015) and Peterson and Cipollini (2017) as previously attacked or healthy based on signs and symptoms of EAB infestation (E.g. D-shaped exit holes, serpentine galleries). The eight selected healthy trees displayed healthy crowns, with little to no canopy thinning or epicormic sprouts, both symptoms of stress. Eight eggs were placed on each stem. Emerald ash borer larvae fed for the remainder of summer 2016. Stems were harvested on 4 November 2016 with care to cut 30 centimeters above and below the terminal placement of EAB eggs and transported to Wright State University. Bark was removed to reveal larval survivorship and galleries using wood chisels. A digital caliper was used to measure the terminal gallery widths of larvae, which is strongly correlated with larval prothoracic size (Fig. 1.4).

Experiment 3: Larval performance on girdled, MeJA, and healthy white fringetrees in the field.

Ornamental white fringetrees at Spring Grove Cemetery and Arboretum in Cincinnati, Ohio were used to examine the performance of EAB larvae on healthy and artificially stressed plants in the summer of 2018. In total we selected and applied each of the treatments to nine healthy white fringetrees with healthy crowns and no signs of EAB. We imposed three treatments to separate stems on each tree consisting of mechanical girdling (n=9), application of methyl jasmonate (MeJA, n=9), and a control, no treatment (n=9) on 12 June. Girdling was conducted by removing the bark from a 10 cm long section of stem around the entire circumference of the stem 1.0 m above the ground. Methyl jasmonate was applied as a 1M solution of MeJA in sterile water with 0.1 % Tween 20 to induce plant defense (Whitehill et al. 2014), to an area of bark ~60 cm long between 0.7 and 1.3 m above ground. The third treatment were control stems, no treatment. One week after treatment on 19 June, six EAB eggs were secured to each stem, as previously described. For the girdled stems, two eggs were placed above the girdle while the other four were placed below. Three weeks after egg placement, stems had a second application of MeJA. Larvae fed for the summer, and on 28 September, stems were harvested, transported back to Wright State University, debarked, and larval survivorship and gallery widths were recorded as previously described. Treatment of MeJA on one replicate of fringetree did not dry before a heavy rain shower and was washed off, so we excluded that stem from this study.

Experiment 4: Larval performance on young live girdled and healthy olive trees.

Potted olive trees (~3-5 cm at 1.3 m), cv. Arbequina (n=15, Willis Orchard Company, Cartersville, GA, USA), were received in February 2018 at Wright State University and stored in a greenhouse until 2 May 2018 at which point they were placed outside. On 15 June, seven olive trees were girdled to stress the plants while the other eight served as controls. Girdling was imposed by removing an 8-10 cm length of bark around the entire circumference of the stem at about 1.3m above the soil. On 20 June, EAB eggs were received from the EAB rearing facility and 10 were attached on olive trees on 22 June as previously described. Girdled trees had four eggs placed above the girdle and the other six eggs were placed below. Olive trees were watered three days a week. On 28 September, olive trees were debarked and larval gallery width and survivorship were assessed. Galleries that were formed by EAB were small so we did not debark the entire stem of the plants in order to use the trees for experiment 5. Gallery establishment rate was also assessed, the number of galleries formed.

Experiment 5: Larval performance on young live, previously infested olives.

In 2019, we used sixteen olive trees that were used in 2018 for larval experiments. These olive trees were previously infested with EAB and only a small bark area, few square centimeters, was removed for each beetle (six to eight areas per tree). This mechanical stress provided a chance to further test stress in a number of olive trees, ten of which were previously healthy, control trees with no treatment besides attachment of EAB eggs in 2018. The other six trees were from girdled plants from experiment 4, which were treated with a girdle 8-10 cm in length in 2018. Egg source and attachment to these trees was done consistent with the previous described methods and debarked in the same manner. On 17th June, EAB eggs were attached to olive trees and on 26th July stems were debarked to assess larval survivorship, gallery widths, and number of galleries formed.

Data analysis:

Data from the field and laboratory were analyzed in SAS Studio. A generalized linear model (PROC GLM) was used to examine variation in prothoracic width, gallery width, and larval mass for experiment 1, with the fixed effects of plant species or cultivar, harvest times, and their interaction. Tukey’s posthoc test were used to determine differences among species. ANOVAs (PROC GLM) were used for experiments 2 through 4 to compare the average gallery width among treatments. Chi squared tests (Fisher) were used to determine differences of survivorship for the four experiments and gallery establishment rate for experiment 4. When data was non normal (Experiment 3), we used a Wilcoxon signed-rank test (PROC NPAR1WAY) to determine if larval performance varied among treatment groups. T-tests (PROC TTEST) were used to determine differences in gallery widths that were above and below girdles in experiment 3, 4, 5.

Results:

Experiment 1. Larval performance in cut stems of olive vs ash.

Overall, gallery width varied among treatments (Fig. 1.1; F= 27.73; df= 2; p= <0.001) and time (F= 6.86; df= 1; p= 0.013), but their interaction was not significant (F= 0.56; df = 2; p=0.576). Larvae in the two olive cultivars were the same size. Survival of larvae was significantly different between species (χ=6.41; df= 2; p= 0.002). Larvae survived at a significantly greater rate in green ash (100%) than in Barouni olive (65.9%) (χ= 6.44; df= 1; p= 0.007). Survival in Manzanilla olive (72.9%) did not vary from that in either green ash (χ= 4.33; df= 1; p= 0.07) or Barouni olive (χ= 0.18; df= 1; 0.263). After the artificial overwintering period, three male EAB emerged from Manzanilla stems (20% of hatched eggs) with no EAB reaching adulthood from Barouni stems.

Experiment 2. Larval performance on previously attacked vs healthy white fringetrees in the field.

Emerald ash borer larvae in previously attacked white fringetrees were 1.3 times larger than healthy trees (Fig. 1.2A; t= 2.29; df= 71; p= 0.025). Similarly, larvae produced galleries that were twice as long in previously attacked trees than in healthy control trees (Fig. 1.2B; t= 2.27; df= 71; p= 0.027). Significantly more EAB larvae were found alive (14.6%) in previously attacked white fringetree than healthy white fringetrees, in which no larvae were found alive (χ= 5.08; df= 1; p= 0.032). Out of the six surviving larvae in previously infested trees, three reached the prepupal stage indication those beetles will emerge as adults the following spring.

Experiment 3: Larval performance on girdled, MeJA, and healthy white fringetrees in the field.

Larvae had 1.46 times wider galleries in girdled stems than in MeJA-treated stems (Fig. 1.3; F= 3.97; df= 2, 61; p=0.0238). Survivorship to the end of the experiment was low in all treatments. Survivorship was significantly different (χ= 6.98; df= 2; p= 0.034) with those in girdled stems having the highest (16%) and no survival seen in MeJA and control trees. Within the girdled treatment, no larvae survived above the girdle and they bored narrower galleries; 1.02±0.20 mm in contrast to those below the girdle, 1.79±0.20 mm (t = 2.08; df= 20; p= 0.050).

Experiment 4: Larval performance on young live girdled and healthy olive trees.

Larval gallery widths in girdled olive trees, 0.81±0.04 mm, were on average the same size as those in control stems, 0.79±0.05 (t= 0.09; df= 21; p=0.767). No larvae were found surviving at the time of debarking of trees; however, the rate of gallery establishment was significantly higher on girdled olives (26.7%) compared to control olives (12.5%; χ= 3.93; df= 1; p= 0.0473). Gallery widths of larvae above the girdle were 0.83±0.04 mm the same as those below the girdle, 0.78±0.06 (t= 1.77; df=13; p= 0.563).

Experiment 5: Larval performance on young live, previously infested olives.
Larval galleries in the olive experiment were the same size in the wounded trees, 0.88±0.12mm and in the wounded, but previously girdled trees, 0.80±0.08mm (t= 0.32; df= 30; p=0.571). Gallery establishment was the same between the girdled, wounded olive (56.5%) and wounded olive (67.6%, χ= 0.745; df= 1; p= 0.421). However, gallery establishment significantly increased in 2019 (63.3%) compared to 2018 (19.2%; χ= 34.837; df= 1; p= <0.001).

Discussion:

In a series of experiments in the field and laboratory, we examined the survival and growth of EAB larvae on two novel hosts and examined the impact of stress on their performance. We examined larval performance on two common olive cultivars from California and compared it to the highly susceptible green ash. Emerald ash borer larvae grew larger in cut stems of green ash than in cut stems of the two olive cultivars, as expected, but this study provides the first direct evidence that olive is a less suitable host for EAB. The cause of reduced survival and growth of larvae in olive is unknown but could be due to nutritional deficiencies, antifeedants, or toxins present in the phloem. Oleuropein is commonly produced in members of the Oleaceae family and is antinutritive by crosslinking with proteins decreasing their nutritive value (Konno et al. 1999). Olive can have 10-25 times higher concentrations of oleuropein (~50 mg/g, Tóth et al. 2015) than North American ash species (1.8-5 mg/g, Whitehill et al. 2012) which could be the cause of reduced growth of EAB in our study. Similarly, variation in oleuropein concentration and/or nutritional qualities may have also contributed to the lower survival of EAB in the Barouni olive cultivar compared to the Manzanilla cultivar. Although survival in the two olive cultivars were similar, adult emergence only occurred in Manzanilla stems indicating this cultivar may be more susceptible than Barouni. This possibility needs to be further explored, but could be confounded with bark thickness. In a previous study, we also observed adults emerging from cut stems of cv. Manzanillia (Cipollini et al. 2017) and in both instances of the successful emergence, the olive stems were greater than 11.9 cm (diameter). At this size, olive stems typically develop thicker, rough bark. In contrast, the Barouni stems that we used were smaller with smooth, thin bark. While speculative, phloem of Barouni stems could have desiccated more quickly in our experimental conditions leading to higher mortality of larvae. In addition or alternatively, younger tissues could have caused the larval mortality since the bark of younger olive stems typically contain higher quantities of secondary metabolites, such as oleuropein, compared with older tissues (Tóth et al. 2015).

Adequate inducible defenses are most likely lacking in cut stems, if resources are allocated from long distance sources, compared to intact photosynthesizing and transpiring plants. To better understand larval performance in olive, we infested actively growing young potted olive trees. All larvae died in healthy olive trees (cv. Arbequina) which is surprising since a fourth instar larvae has been recovered previously in this cultivar (Cipollini and Peterson 2018). Similarly, no larvae survived in girdled olive which was unexpected since stress generally has led to better performance of EAB in ash hosts (e.g. Tluczek et al. 2011, Rigsby et al. 2019). We attached eggs to these olive trees the following summer to determine if the mechanical wounding from debarking in 2018 would increase larval performance. Again, larvae died quickly with no development observed beyond the first instar. These data suggest that young olive trees are likely unsuitable hosts for EAB, at least those stems with smaller diameters (<4 cm). Smaller stems are younger and may have a higher amount of oleuropein (Tóth et al. 2015) or other secondary metabolites that are toxic to EAB larvae. Larvae may also be affected by the age of stems due to the presence of photosynthetic/chlorophyllous tissue in the bark of young olive and ash (Pfanz et al. 2002, Filippou et al. 2007). Photosynthetic capacity is generally higher in younger stems due to higher light transmission through bark than in older stems (Pfanz et al. 2002, Filippou et al. 2007). Larvae may not survive on younger stems due to their high content of chlorophyll or other metabolites, or higher levels of reactive oxygen species associated with the photosynthetic process (Davletova et al. 2005), which makes these tissues more "leaf-like" and would be unfamiliar as a substrate for EAB larvae. This observation could also be one reason why younger ash trees (<2.54 cm) are either not attacked or are more resistant (Herms and McCullough 2014). In contrast, larger and older olive stems, such as those used in our cut stem assay and those ash trees that EAB attacks in the forest might be more susceptible to EAB because these stems have thicker bark that reduces light penetration and have lower levels of chlorophyll.

One effect of stress that we did observe is that larvae established galleries at higher rates in mechanically girdled olive trees compared to healthy plants. When those trees had EAB eggs attached a second time, gallery establishment rate drastically increased suggesting that girdling and mechanical damage increases larval success. Larvae fed down to the phloem layer and then died which suggests that there is something toxic in the phloem of olive plants. Again, these toxins could be chlorophyll, high light penetration, and/or higher concentrations of oleuropein in young stems. Thus, larger stemmed live olive trees, which we postulate to be more susceptible than young, thin-barked stems or young trees, should be investigated to determine whether larval success increases and EAB develop to adults as observed in our cut stem assay.

In live white fringetrees, we observed that larvae were uni- and semi-voltine. Some larvae reached a 4th instar (J-shaped) after one summer and fall of development while others were earlier instars that were going to overwinter in the phloem. Larvae survived and grew only in those plants that were stressed. These stresses appear to have weakened or hampered plant defenses leading to increased larval success similar to what occurs with EAB and stressed Manchurian ash in China and United States (Liu et al. 2003, Chakraborty et al. 2014). The type of stress appears to matter for larval success because drought decreases survival of EAB in white fringetree (Rutledge and Arango-Velez 2017). Therefore, when considering larval likelihood of success in the field, white fringetree could be either a reservoir or population sink for EAB. If white fringetree is healthy or drought stressed, this plant kills or slows development of EAB. In contrast, when plants are girdled in some manner or stressed from previous attack, white fringetree is more susceptible to EAB and larvae can reach adulthood. Emerald ash borer appears to be a secondary pest on white fringetree since its limited success is on stressed plants, and healthy plants appear to resist this plant similar to the historic host in Manchurian ash in East Asia (Liu et al. 2003). This is further supported by the low, observable attack rates in ornamental sites across the lower Midwest and Pennsylvania (Peterson and Cipollini 2017). Egg placement on girdled trees was important in our study because no larva survived above the wound likely due to stem desiccation. Research with gravid females could reveal their preference for egg deposition in relation to the position of wounds on a tree.

Methyl jasmonate treatment applied to trees induces the accumulation of some defense-related metabolites and decreases the success of larvae in ash hosts (Tluczek et al. 2011, Whitehill et al. 2014), but its effects on white fringetree have not yet been studied. We found that MeJA similarly reduced larval size and survival compared to plants that were girdled. We speculate that changes in defense expression or nutritional quality in white fringetree were induced by MeJA and led to the reduce larval performance; however, this has not been studied yet and is worth of future investigation.

Overall, our findings suggest that EAB can perform better on novel hosts with certain types of stress including previous attack by larvae and mechanical girdling. In contrast, healthy plants and those treated with MeJA generally killed EAB. The relative lack of success in thin barked stems of young olive trees suggests they are generally toxic in some way to EAB, but that susceptibility increases in olive as stems age and bark thickens. Larger olive trees and different cultivars need to be tested to better understand their susceptibility to EAB to determine to what extent this pest could threaten this economically important plant.

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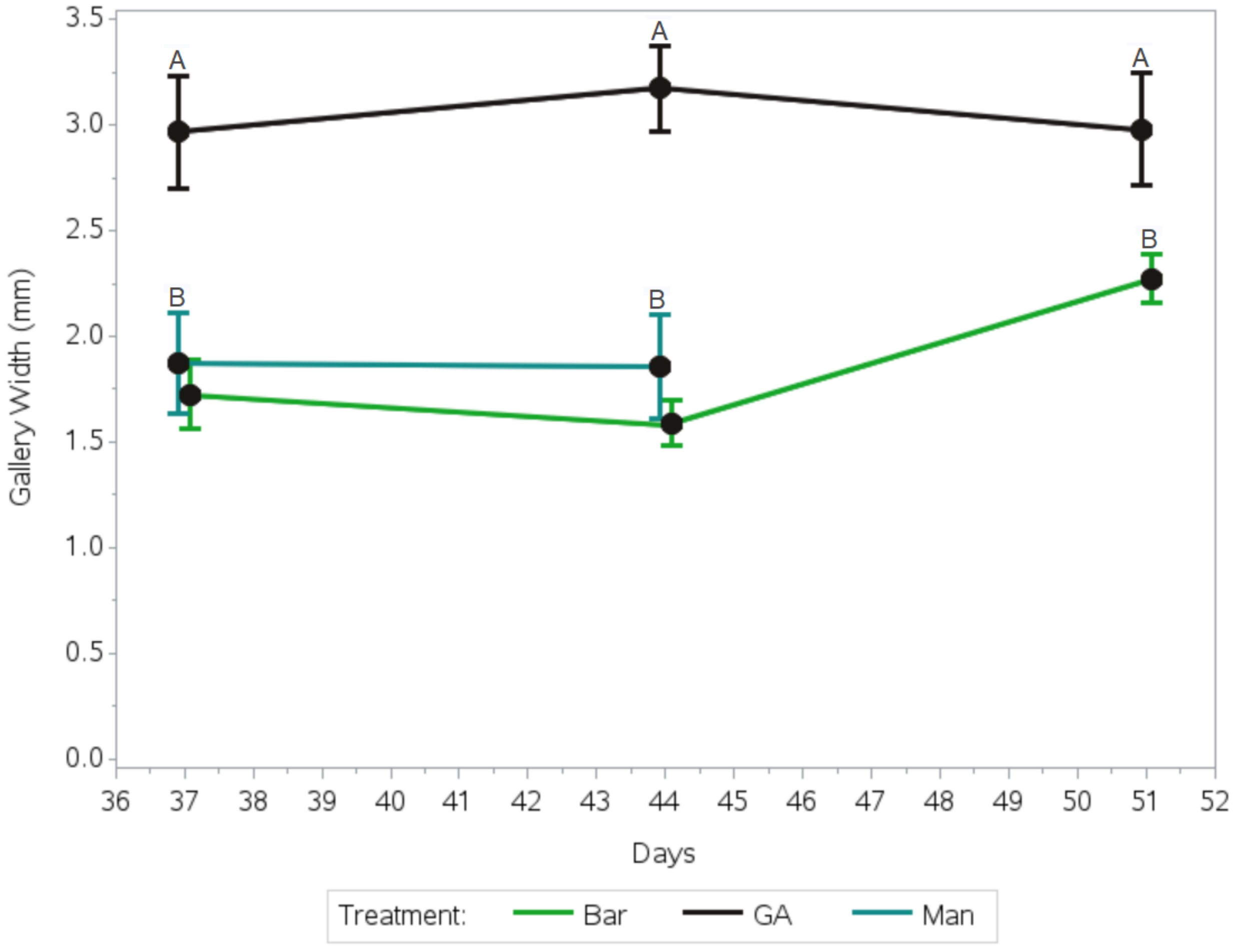

Figure 1.1. Average gallery width (mm±SE) of emerald ash borer (EAB, *Agrilus planipennis*) larvae over time in cut stems of green ash (GA, *Fraxinus pennsylvanica*) and two cultivars of olive (*Olea europaea*); Manzanilla (Man) and Barouni (Bar) in a growth chamber for 51 days after egg hatch at Wright State University, Dayton, Ohio.

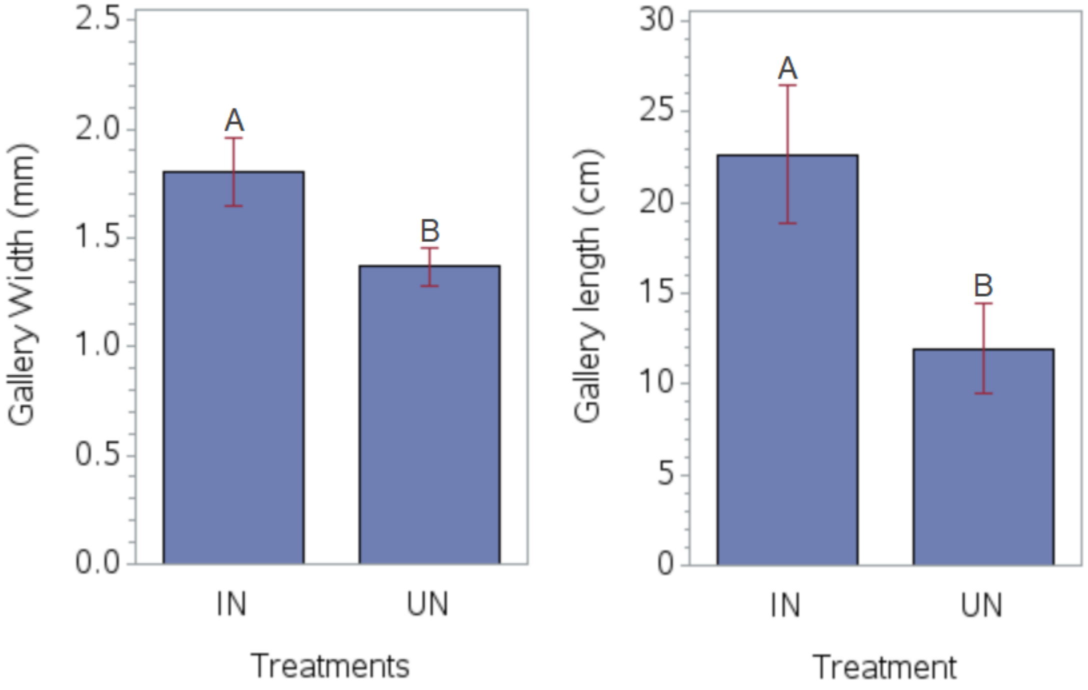

Figure 1.2. A) Average gallery width (mm±SE) and B) gallery length (cm±SE) of emerald ash borer (EAB, *Agrilus planipennis*) larvae that fed on previously attacked by EAB (IN) and healthy (UN) white fringetree (*Chionanthus virginicus*) in summer 2016 at Spring Grove Arboretum and Cemetery, Cincinnati, Ohio.

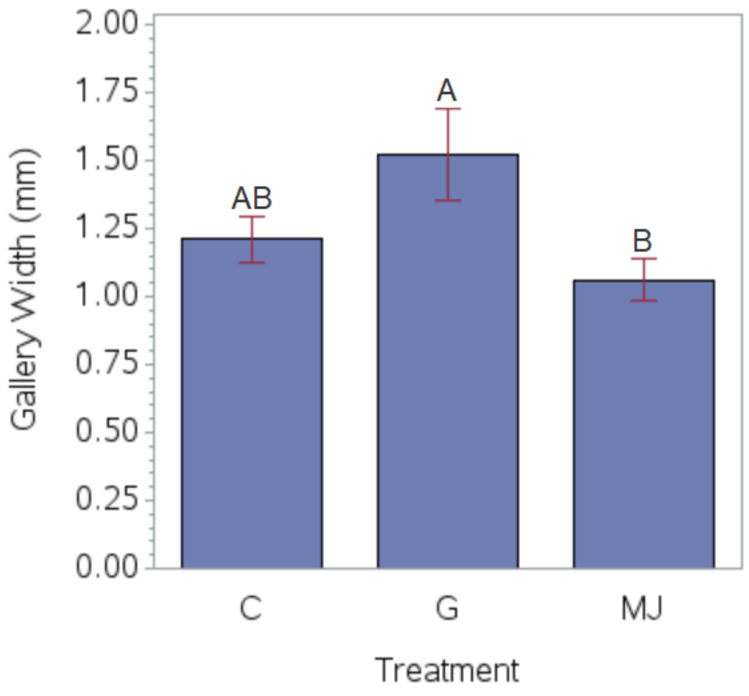

Figure 1.3. Average gallery width (mm±SE) of emerald ash borer (EAB, *Agrilus planipennis*) larvae that fed white fringetree (*Chionanthus virginicus*) treated with a girdle (G), an application of methyl jasmonate (MJ), or untreated, control (C) in summer 2018 at Spring Grove Arboretum and Cemetery, Cincinnati, Ohio.

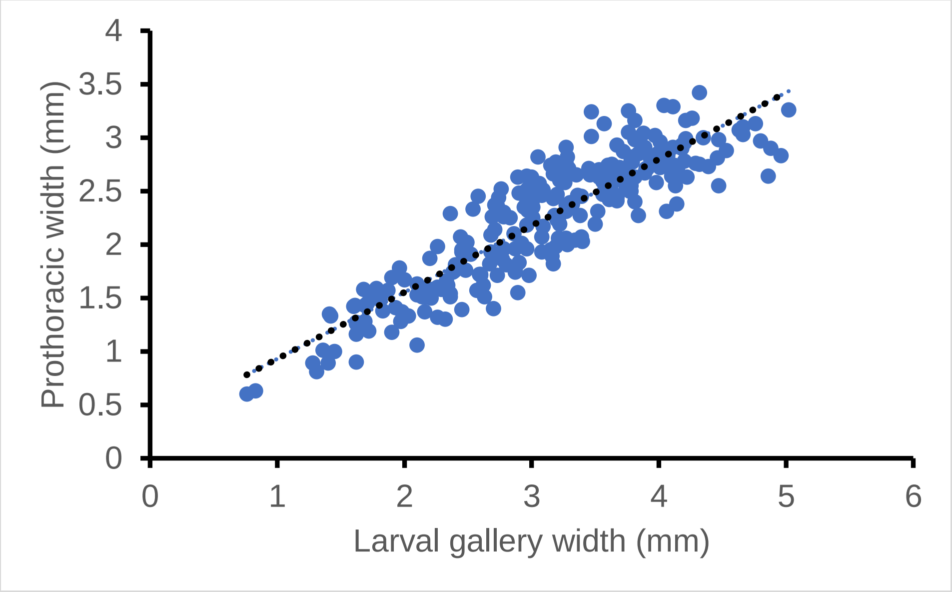

Figure 1.4. Correlation of prothoracic width (mm) and gallery width (mm) of emerald ash borer larvae (*Agrilus planipennis*) based on performance in cut and live stem assays in ash (*Fraxinus* spp.) from Peterson et al. 2015 and DLP unpublished data.

Chapter 2: Fitness and fecundity of adult emerald ash borer on two novel hosts and their influence on herbivore success.

Abstract:

Insect herbivores are more likely to successfully use a novel host if the plant is closely related to the ancestral host and the insect is polyphagous. Emerald ash borer (EAB), *Agrilus planipennis*, is a specialist wood borer of ash (*Fraxinus* spp.) and one of the most destructive forest pests in North American forests. Recent studies have found that larvae can develop in stems of two ash relatives; white fringetree (*Chionanthus virginicus*) and the more distantly related cultivated olive (*Olea europaea*). For EAB adults, the ability to consume, successfully mate, and lay viable eggs on foliage of these hosts is unknown. To examine this, we conducted two no-choice assays with adult EAB on foliage of fringetree and olive which were paired with positive controls of green ash (*F. pennsylvanica*) and tropical ash (*F. uhdei*), respectively. We also examined larval performance in a reciprocal study with cut stems of fringetree and green ash to determine if adult diet impacted progeny. Longevity and consumption rates of adults were similar among fringetree and green ash treatments along with female fecundity. In contrast, adults died more quickly, consumed more over time, and females laid fewer eggs when consuming olive compared to those on tropical ash. Adult diet did not impact progeny. Larvae on white fringetree stems reached the same size and those on green ash were also similar in size. Stem type did impact larval performance with those on green ash growing larger than those on white fringetree. These data suggest that fringetree is a suitable adult host for EAB with no apparent fitness loss. In contrast, the more distantly related olive appears to be a poor host for adult EAB.

Keywords: Emerald ash borer, host range, white fringetree, olive, consumption

Introduction.

In general, exotic phytophagous insects that establish a population on novel hosts are expected to have decreased fitness due to general and specialized defenses that have evolved to defend against native herbivores (Bertheau et al. 2010). Host breadth and the relatedness of the historical and novel host have been found to be the most important factors for impacting the fitness of these insects. Polyphagous insects that consume a broad range of hosts are more likely to survive and perform well on novel hosts. In contrast, oligophagous and monophagous insects that consume few to only one host plant are less likely to survive and perform well on novel hosts (Bertheau et al. 2010). The exceptions of this trend are insects that become destructive pests. These insects perform better on novel plants likely due to not having adequate defenses from a lack of a coevolutionary history, “defense free space” (e.g. Villari et al. 2016, Showalter et al. 2018), and/or the herbivores escape their natural enemies, “enemy free space”(e.g Gandhi and Herms 2010).

The potential for further exotic introductions has increased over the decades due to increased globalization and world trade. Many of these exotic insects are not of concern because they either fail to establish or cause minor damage to plants. But, those exotics that do establish and cause serious damage to plants can become important invasive pests. These pests cost billions of dollars to manage each year in North American forests (Aukema et al. 2011). In the last two decades, wood boring insects have increasingly become the most expensive forest pests (Aukema et al. 2011) with two such examples being Asian long-horned beetle (*Anoplophora glabripennis*) and emerald ash borer, EAB, (*Agrilus planipennis*: Buprestidae). Buprestids, specifically those in the genus *Agrilus*, have demonstrated a particularly ability to become invasive on evolutionarily naïve congeneric hosts. Examples include the previously mentioned EAB on ash trees in North American and European forests, bronze birch borer (*A. anxius*; Muilenburg and Herms 2012) on Eurasian birch trees (*Betula* spp.), and gold spotted borer (*A. coxalis*) on Californian coast live oaks (*Quercus* spp.; Coleman and Seybold 2008); all of them appear to prefer and readily attack healthy trees (Coleman and Seybold 2008, Muilenburg and Herms 2012, Herms and McCullough 2014), when the historical strategy is to attack stressed, weakened hosts.

Emerald ash borer was accidently introduced into North American in the late 1980s-early 1990s (Siegert et al. 2014). In the native range of EAB, historical hosts are only attacked when stressed, but in North America on novel ash hosts, EAB readily attacks and kills healthy trees in a few years. *Fraxinus* has been the only known genus to support EAB, but in 2014, a close relative of ash trees, white fringetree (*Chionanthus virginicus*) was observed to support development to adulthood (Wallander and Albert 2000, Cipollini 2015, Cipollini and Rigsby 2015). A second novel host, cultivated olive (*Olea europaea*) was also found to support larval development (Cipollini et al. 2017; Peterson and Cipollini in review), although this plant is more distantly related than fringetree to the ancestral hosts (Wallander and Albert 2000). So far, white fringetree appears to be a less suitable host for larvae compared to North American ash (Cipollini and Rigsby 2015, Peterson and Cipollini in review), although performance is better than on its historic host, Manchurian ash (*F. mandshurica*; unpublished data, Don Cipollini). Olive, in contrast, appears to be an even less suitable host for larvae since they have only been reared from cut stems (Cipollini et al. 2017) and die quickly on young potted trees that have photosynthesizing stems (Peterson and Cipollini, in review).

Feeding by adult EAB on host trees has received less attention than that of larvae likely because foliage damage caused by adults is less detrimental to the host than consumption of vascular tissue by larvae. The life cycle of adults begins in the spring with emergence from hosts (Brown-Rytlewski and Wilson 2005). Adults must consume foliage for two weeks before they begin to mate and lay eggs. Previous feeding assays among ash hosts have found that beetles vary in the amount of foliage consumed in no-choice assays (Haack and Petrice 2005, Tanis and McCullough 2015) and when given a choice, beetles generally prefer green ash (*F. pennsylvanica*), white ash (*F. americana*), and black ash (*F. nigra*; Pureswaran and Poland 2009) that are susceptible to larvae. This is in contrast to the more resistant hosts (Rebek et al. 2008, Tanis and McCullough 2012): blue ash (*F. quadrangulata*) and Manchurian ash, which are less preferred and consumed in lower amounts by adults (Haack and Petrice 2005, Pureswaran and Poland 2009, Tanis and McCullough 2015; T. Petrice per. communication). Shortly after the discovery of EAB in North America, adult feeding assays were conducted on ash relatives including white fringetree. In 48-hour studies, adult beetles were found to consume white fringetree foliage, but in lower amounts than more susceptible North American ash trees (Haack and Petrice 2005). Since this research, no further work on adult feeding was performed using non-ash hosts.

Feeding preference and performance of EAB adults on host foliage are likely mediated by secondary metabolites and other nutritional factors (Pureswaran and Poland 2009, Chen and Poland 2010). Phenolics are a dominant group of secondary metabolites in ash and other members of Oleaceae, with secoiridoids and coumarins being commonly found in ash tree foliage (Kostova and Iossifova 2007). White fringetree foliage similarly contains many of these compounds with ash foliage, as well as flavonoids, quercetin triglycosides, and triterpenoids (Pourrat et al. 1954, Harborne and Green 1980, Gülçin et al. 2009). This suggests that performance and preference for this host will be similar to ash hosts. In contrast, olive is more distantly related than white fringetree to *Fraxinus* and contains high concentrations of a oleuropein, a toxic iridoid compound (Soler-Rivas et al. 2000, Tóth et al. 2015), and likely adults would perform poorly on olive due to the antinutritive properties of this compound (Konno et al. 1999). Foliage of olive is evergreen, xerophytic, and have a high degree of toughness which likely would be more difficult for beetles to consume since leaves of their ancestral hosts are deciduous and thin.

Our goal for this study is to determine whether white fringetree and olive leaves are suitable food sources for EAB adults, such that either of these species could serve as a reservoir for EAB in the absence of ash. Our first objective was to determine consumption and longevity of adult beetles on these novel hosts compared to susceptible ash species. We hypothesized that adult beetle consumption rates of foliage and survival would be similar on fringetree and ash trees because their leaf chemistry and leaf thickness are similar. In contrast, olive leaves are thick and have an abundance of oleuropein compared to ash foliage, so we postulated that consumption rates and survival of adults would be lower than on ash trees. The second objective was quantifying female fecundity, hypothesizing that females that consume ash and white fringetree will lay a similar number of eggs and have the same rates of fertilized and hatched eggs. Adults on olive are expected to lay fewer eggs and have lower rates of fertilized and hatched eggs compared to ash. The final objective was to determine if parent diet impacts the performance of progeny on alternate hosts. We postulated that larvae emerging from eggs of parents with a diet of green ash will grow to a similar size than those larvae from parents that fed on white fringetree. Furthermore, we predicted that larvae in green ash stems will grow larger than those in white fringetree as observed in Cipollini and Rigsby (2015).

Material and Methods:

Research location and foliage sources.

Bioassays were conducted at USDA APHIS PPQ Biological Control Production Facility in Brighton, MI. We conducted two no-choice assays, the first was run from 1st October – 3rd November 2017 with green ash (GA, *Fraxinus pennsylvanica*) and white fringetree (WF, *Chionanthus virginicus*) foliage and the second assay was run from 8th April- 15th May 2019 with tropical ash (TA, *F. uhdei*) and olive (OL, *Olea europaea*) foliage. Foliage for the first assay came from green ash trees in the Wright State University Woods (Dayton, OH), an ornamental white fringetree near Wright Brothers Memorial (Dayton, OH), and for the second assay from potted olive trees cvs. Lucca and Arbequina bought from Willis Orchard Company (Cartersville, Georgia). Olives were grown outside at Wright State University during the summer and stored in a greenhouse during the winter to avoid mortality from freezing. Tropical ash foliage was overnighted from USDA collaborators in California and Texas twice per week during the second assay. Sun exposed foliage was collected biweekly and sent to Brighton Rearing Facility where it was washed and either used immediately for assays or stored in a plastic bag in a refrigerator (4º C) to delay desiccation for a few days. Only mature, sun-exposed leaves of each host were used in assays because adult beetles prefer these over young, shaded foliage (Chen and Poland 2009a, 2009b). Olive leaves were provided for adults with both mature and developing, immature leaves, which reflects what would be available to beetles in the field.

Adult feeding and longevity bioassays.

Two adult males and two females were placed into 32 oz. plastic cups with foliage of one of the hosts placed in plastic water vials. Three-four leaflets of tropical and green ash and three-four leaves of white fringetree were placed in water picks. For olive, 15-25 leaves still attached to a stem were cut from an olive tree and inserted into the water pick. Plastic lids with filter paper covered holes prevented the escape of beetles and provided ventilation. Beetles were kept in growth chambers at 27° C with a 16:8 light:day cycle. After 14 days, coffee filter papers replaced the plastic lids and were secured to the cups with rubber bands. These coffee filters serve as a substrate for oviposition. Beetles were transferred to clean plastic cups with new foliage and coffee filters/lids twice per week: Monday-Thursday or Tuesday-Friday. Removed coffee filters were checked for eggs which were then monitored for fertilization (fertilized eggs characteristically change color from white to brown over the course of a few days, while unfertilized eggs do not brown). Fertile eggs were also examined for their hatch rate. Foliage on which beetles had fed was scanned and the area consumed was quantified with Fiji Software (ImageJ). Area consumed was divided by the number of beetles in the containers, number of days they fed, and the average specific mass of the leaves (mg/cm^2) in order to obtain a daily consumption rate (mg/day) for each beetle. To estimate leaf mass of each species , we weighed 10, one cm^2 areas collected from five trees/species. Finally, mortality of adult beetles was recorded daily and those that died were removed immediately to prevent potential spread of any pathogens. The first assay was stopped when green ash foliage of good quality, clean of pathogen or previous herbivore damage, could not be obtained.

Due to laboratory limitations and logistical constraints, consumption data was not collected consistently and did not begin until day eight for the first assay. The second assay was stopped once all beetles feeding on olive died.

Influence of adult diet on larval performance in white fringetree and green ash. From the first assay, a subset of eggs (n=264) from white fringetree and green ash were used to reciprocally infest cut green ash and white fringetree stems, as described in Peterson et al. (2015). In brief, stems of both plant species were cut on 26 October 2017 to ~40 cm lengths (6-10 cm in diameter), soaked in bleach to kill pathogens, washed with DI water, dried, and then placed into plastic containers with green floral foam. Distilled water was added as needed. White fringetree stems were obtained from planted trees at Spring Grove Cemetery and Arboretum in Cincinnati, Ohio and green ash stems were obtained from wild trees in the Wright State University Woods. Eggs were overnighted to Wright State University from the USDA APHIS PPQ Biological Control Production Facility in Brighton, MI. On 27 October, six eggs were attached to each of the 22 green ash stems and 22 white fringetree stems. Eleven stems of both plant species were infested with fertile eggs laid by females that consumed green ash leaves, while the other set of stems were infested with eggs laid by beetles that consumed white fringetree foliage. Majority of neonates eclosed by 3 November and larvae fed on stems until 29 November. Stems were then debarked to measure survival and the widths of galleries formed (mm) by larvae.

Statistical analysis.

Data were analyzed using SAS Studio (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Kaplan Meier survival analyzes (PROC LIFETEST) were used to compare the survivorship of emerald ash borer different hosts for both assays. PROC GLMMIX was used to run a generalized linear mixed model to determine the differences of the amount of foliage consumed by beetles (mg/day/beetle) for both assays and the egg laying rate (eggs/day/female) for the first assay only by plant species, time, and their interaction. For the second assay, females with a diet of olive laid few eggs for a short duration, so we compared the average number of eggs laid per female using a T-test (PROC TTEST) The PROC FREQ was used to run a Fisher's chi-squared test to compare the fertilization rate and hatch rate of eggs for the feeding assays and hatch rates and gallery establishment rates in the larval performance assay. PROC GLM was used to determine differences in the mean gallery width (two-way ANOVA), proxy for larval size for the larval performance assay, among larval stem host and adult foliage host.

Results:

In the first experiment comparing adult feeding on green ash versus white fringetree, adults survived the same length of time on green ash and white fringetree leaves (Fig. 2.1; $\chi = 0.066$; df = 1; $p = 0.797$). Consumption rate significantly changed over time for all beetles (Fig. 2.2; $F = 18.51$; df = 4, 106; $p = <0.001$), but there were no differences in feeding by adults on the two plant species ($F = 0.07$; df = 1, 106; $p = 0.895$) and there was no interaction of time and plant species ($F = 1.50$; df = 4, 106; $p = 0.208$). For white fringetree, adults successfully mated (Fig 2.3A) and laid fertile eggs (Fig. 2.3B; 77.5%) at the same rate as those consuming green ash foliage (78.2%; $\chi = 0.085$; df = 1; $p = 0.785$). Similarly, hatch rate of eggs was the same for beetles that consumed green ash (57.1%) and white fringetree (54.5%; $\chi = 0.413$; df = 1; $p = 0.566$). The egg laying rate (eggs/day/female) was the same for females in both treatment groups (Fig. 2.4; $F = 2.10$; df = 1,18; $p = 0.165$). Egg laying rate varied over time for both groups (F = 2.78; df = 8, 69; p = 0.010) with an increasing over time until day 23, decreasing until day 27, and a second increase until the end of the experiment, day 33. There was no interaction between species and time on egg laying rate (F = 1.06; df = 7, 69; p = 0.401).

The second assay compared adult feeding on tropical ash versus olive, and adults fed readily on olive foliage (Fig. 2.3C), but did not survive as long as those on tropical ash foliage (Fig. 2.5; χ = 113.122; df = 1; p = <0.001). Even though mortality was higher for adults on olive, some beetles lived long enough to mate (Fig. 2.3C). Consumption rate was significantly different among olive and tropical ash (Fig. 2.6; F = 12.91; df = 1, 15; p = 0.003), over time (F = 8.78; df = 9,103; p = <0.001), and the interaction of plant species and time (F = 5.28; df= 9,38; p = <0.001). Females on average laid significantly fewer eggs than those that consumed olive having 0.29±0.03 eggs/female/day (n=3, Fig. 2.3D) compared to tropical ash fed females which laid 7.05±0.58 eggs/female/day (n= 2,854; t = 11.69; df = 69; p = <0.001). There was a trend in fecundity of eggs with tropical ash fed females producing more fertile eggs (87.5% vs 66.7% in olive) and more hatched (74.2% vs 50% in olive). One neonate emerged from an olive egg which appeared healthy (Fig. 2.3E) and vigorous.

Hatch rates and gallery establishment rates were the same among all four treatments (Table 1) Larval performance was significantly different for larval stem host (Fig. 2.7; F = 562.01; df = 3, 114; p = <0.001) with those larvae developing on white fringetree stems boring gallery widths half the width of those in green ash stems. Adult diet did not significantly impact progeny, same size galleries (F= 0.58; df= 3, 114; p= 0.883).

Discussion.

We conducted a set of experiments in the laboratory to examine the longevity and fecundity of EAB adults feeding on foliage of white fringetree, which is closely related to the ancestral hosts, and olive, which is more distantly related (Wallander and Albert 2000). We also examined the impact of adult diet on subsequent larval performance in these novel hosts. Before this study, little was known about the ability of EAB adults use olive and white fringetree other than that they would consume foliage of white fringetree in short term studies (Haack and Petrice 2005) and that females will select both hosts for egg laying (Cipollini 2015, Peterson and Cipollini 2017, Cipollini and Peterson 2018).

Adults survive an average of 21 days on ash trees in the field (Wang et al. 2010), but can range to over 40 days (Herms and McCullough 2014). Consistent with these results, 50% of beetles lived at least 28 days on green ash and white fringetree. Although we ended the first experiment before all beetles had died, adults had similar longevity on each of these plant species with ~40% reaching an age of 32 days. For the first time, adult beetles were observed to live long enough to feed, mate, and lay eggs when provided only white fringetree foliage. Over the course of the experiment, adult beetles displayed the same feeding pattern on fringetree and green ash with an increase of consumption over time peaking near 18 days, then falling. Although not quantified in our study, the nutritional quality of white fringetree apparently meets the needs of EAB, and, consistent with the phytochemical similarities of ash and fringetree foliage (Pourrat et al. 1954, Harborne and Green 1980, Gülçin et al. 2009). Therefore, adult EAB are clearly capable of tolerating the secondary compounds present in the foliage of this species. For fecundity, females had similar laying rates, fertilization rates, and hatch rates of eggs after feeding on green ash and white fringetree. These data suggest that a diet of white fringetree does not reduce the fecundity of females. Consumption rates peaked at day 18 which appears to be correlated with the largest peak in egg laying by females for both treatments a few days later. Together these data suggest that EAB females increase their feeding to obtain the additional nutrients to mature their eggs which is similar to the pattern of feeding and oviposition observed in pine weevil (*Hylobius abietis*; Bylund et al. 2004). We have found that EAB will readily consume and performs well on white fringetree foliage in a no-choice assay; however, future research needs to determine whether adults choose to feed on this novel host in the field. Adults are likely to visit fringetree at the same rate as ash in the field because in olfactometer assays with foliage volatiles of both plant species, mated females tend to orient toward volatiles emitted by white fringetree and green ash at the same rate (Peterson et al. in review). Furthermore, in a choice feeding assay, adults ate similar amounts of fringetree and green ash foliage (T. Petrice pers. communication). These data suggest that preference of these hosts is similar and adults are likely to select fringetrees at the same rate as green ash trees in the field. Overall, if adults choose to use white fringetree, beetles have no apparent fitness or fecundity loss which shows that white fringetree is a suitable host for adults.

In contrast to white fringetree, olive appears to be a poor host for adult EAB similar to a previous report (Cipollini and Peterson 2018). Adults died quickly on olive with >50% mortality by day 10 while only 5% died on tropical ash over the same period. Ten days is roughly the length of time needed for females to complete maturation feeding (Wang et al. 2010). By day 18, 90% of adults consuming olive were dead which is a few days after average egg deposition occurs in the field (Wang et al. 2010). Feeding dynamics were different among beetles on olive compared to those on tropical ash. Near the beginning of the assay, beetles consumed less olive foliage than tropical ash, but increased their consumption, eventually surpassing the rate of beetles with a diet of tropical ash. The majority (75%) of mortality occurred between days 6 and 14, which is when some of the highest amounts of olive foliage was consumed. The high mortality after a period of increased feeding indicates that beetles are compensatory feeding, a response of insects to increase consumption to compensate for poor quality hosts (Kondoh and Williams 2001). On olive, this adult feeding behavior could be due to the foliage containing compounds that are antinutritive and/or toxic and leaf toughness (Cipollini and Peterson 2018). Oleuropein, a phenolic compound, occurs throughout Oleaceae and is linked with herbivore resistance in olive and relatives (Konno et al. 1999, Spadafora et al. 2008). Levels of this compound can be ~24-85 times higher in olive (~85.2 mg/g; Tóth et al. 2015) compared to susceptible North American ash species (0-3.6 mg/g; Chen et al. 2011). When EAB feed on olive foliage, they activate oleuropein leading to foliage being less nutritious due to the crosslinking of protein (Konno et al. 1999). Thus, EAB feeding on olive may experience a protein deficiency which they try to compensate for, which eventually leads to mortality. Quantities of oleuropein in white fringetree foliage are unknown and should be investigated. However in the phloem, levels of this compound are more similar (~30 mg/g, DC unpublished data) to olive, 0.6 times (~50 mg/g, Tóth et al. 2015) than in susceptible ash trees (1.8-5 mg/g, Whitehill et al. 2012). Thus, the implication of oleuropein lethality for EAB adults should be treated with caution because adults performed well on white fringetree and foliage may contain similar levels of this compound if phloem chemistry is similar. Since the majority of adults died quickly from feeding on olive, very few females survived to an age to successfully mate and lay eggs. Of those females, only three eggs were laid in stark contrast to the nearly 3000 eggs laid by females that consumed tropical ash. While speculative, the reduced fecundity could be due to higher concentrations of toxic compounds in olive foliage. For example, extracts of olive foliage applied to sorghum led to a decrease in the amount of eggs laid and fertility of eggs of migratory locust (*Locusta migratoria*). The olive foliage treatment caused a significant decrease in egg quality (Abdellaoui et al. 2018). While low, adults with a diet of olive foliage survived and laid eggs indicating that EAB can persist on olive in field where populations are much higher than the quantity used in our experiment. Olive is grown in dense monocultures providing an abundant supply of food which EAB could adapt to use more efficiently via natural selection as suggested by Cipollini and Peterson (2018). This scenario is premised on larval success of reaching adulthood on older olive tissues (Peterson and Cipollini in review). Overall, olive is a poor host for adults because we observed decreased longevity and fecundity. This was not surprising, because larvae similarly perform worse on cut stems of olive compared to ash (Chapter 1) and young, live olive stems, even if stressed, kill larvae (Chapter 1).

We initially looked at consumption rate of foliage by area consumed rather than by mass. The area measurement suggested that EAB consumed more ash foliage over both white fringetree and olive foliage which could have led to a conclusion that compounds in olive are antifeedant, chemicals that cause organism to consume less. Olive foliage is much thicker than ash foliage and has more mass per unit of area. When our data was reanalyzed with mass of foliage consumed, we found that olive foliage appears to be antinutritive, as previous mentioned. Future studies should take this under consideration when comparing adult feeding rates on foliage.

Olive is mostly unsuitable for adult EAB, yet oviposition has been observed on this plant in the field (Cipollini and Peterson 2018). Oviposition preference has yet to be determined, but those neonates placed on olive are likely to die suggesting this host may be a population sink, at least on young, photosynthesizing stems (Chapter 1). White fringetree in contrast is more suitable since larvae reach adulthood on living plants in ornamental and wild populations (Cipollini 2015, Cipollini and Rigsby 2015, Thiemann et al. 2016, Peterson and Cipollini in prep). As observed in this study, adults appear to experience no fitness loss when provided only white fringetree foliage. However, neonates and larvae could be impacted by the paternal diet. An example of this has been observed in progeny of adult blue willow beetle (*Phratora vulgatissima*) that fed on different willow (*Salix* spp.) varieties. The pupae had significantly lower fitness with prolonged pupation time and decreased pupal weight when reared on the cultivar, *S. dasyclados*, when the parents fed on other hosts (Peacock et al. 2004). In our study, larval performance was unaffected by parental diet. When performance was compared among the same plant species, progeny of EAB females with a diet of white fringetree grew to the same size as progeny of females with a diet of green ash. When larval performance was compared between fringetree and green ash, they bore significantly wider galleries in green ash, consistent with previous studies with fringetree and susceptible ash trees (Cipollini and Rigsby 2015, Cipollini et al. 2017, Peterson and Cipollini, in review). These data indicate that female preference for different hosts is more important for progeny success than adult diet. It is possible that time to pupate or fitness of the progeny is reduced by parental diet and is worth further investigation.

In conclusion, white fringetree is a suitable host for adult EAB to use. While olive is a poor host for EAB, some beetles should be able to use this host and performance could improve through time, if larvae can reach adulthood on living olive stems. Females survived long enough to mate and lay fertile eggs, but those on olive produce very few eggs. No apparent legacy effects were passed onto progeny from parents consuming white fringetree. This may be similar for parents that consumed olive because the neonate that emerged was vigorous. The preference in both feeding and oviposition for white fringetree needs to be determined to understand host use in the field. Our study provides some support that relatedness of the historical and novel host can reduce insect fitness (Bertheau et al. 2010) with EAB fitness being reduced on more distant relatives (Olive) in contrast to the more closely related host, white fringetree (Wallander and Albert 2000, Besnard et al. 2009, Yuan et al. 2010, Hong-Wa and Besnard 2013). To expand on this relationship, other closely related genera of Oleaceae should be investigated to determine if they can support EAB. For example, *Osmanthus americanus* is closely related to *Chionanthus* and *Fraxinus* and has been found to support 2nd-3rd instars (Cipollini and Rigsby 2015, DLP unpublished data).

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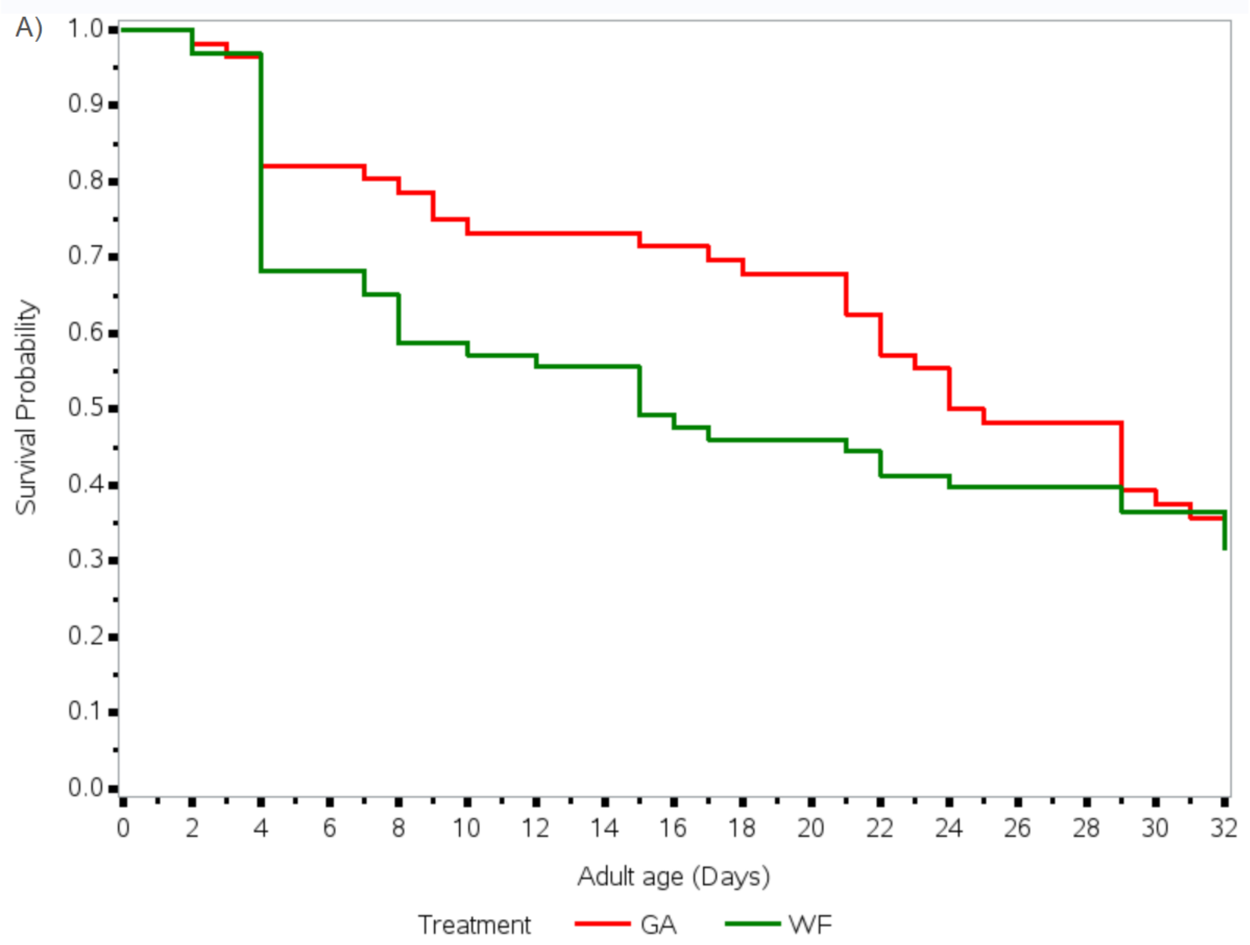

Figure 2.1. Kaplan Meier survival analyzes of emerald ash borer (*Agrilus planipennis*) on white fringetree (WF, *Chionanthus virginicus*) and green ash (GA, *Fraxinus pennsylvanica*) foliage over 32 days.

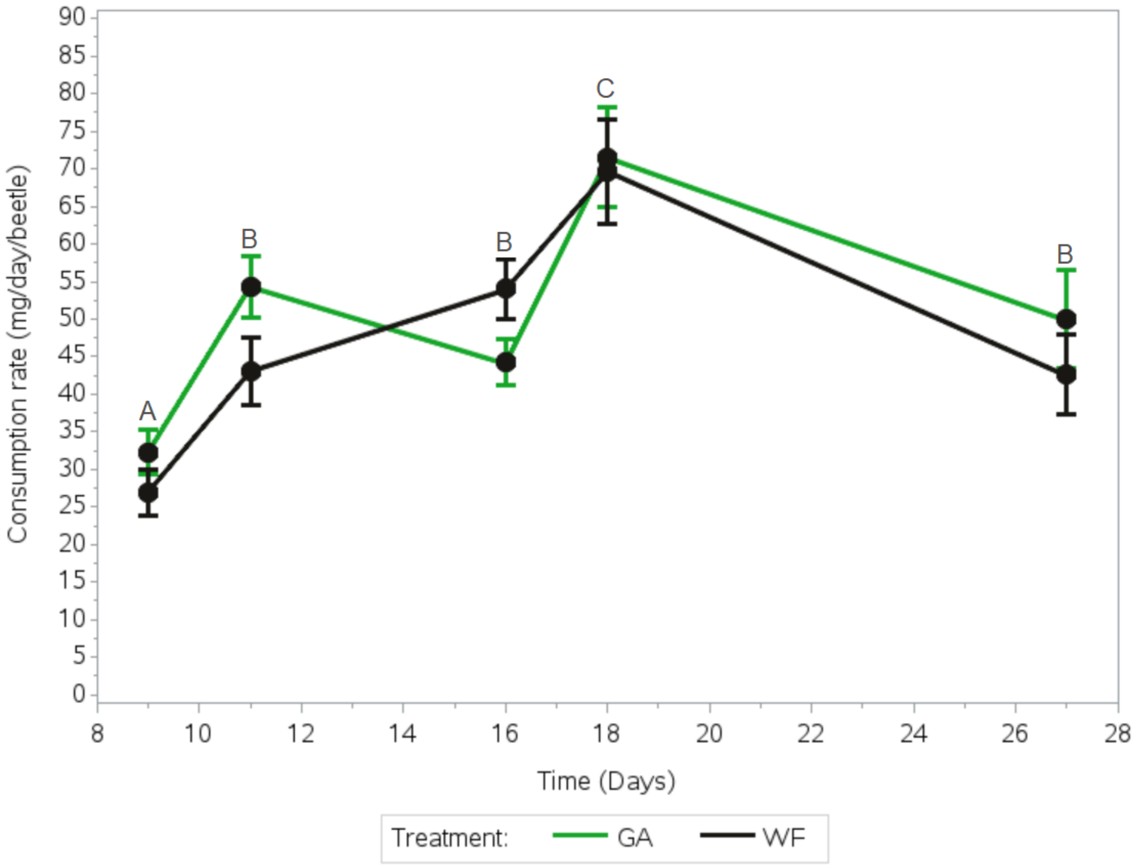

Figure 2.2. Consumption rate (mg/day/beetle±SE) of white fringetree (WF, *Chionanthus virginicus*) and green ash (GA, *Fraxinus pennsylvanica*) by emerald ash borer adults (*Agrilus planipennis*).

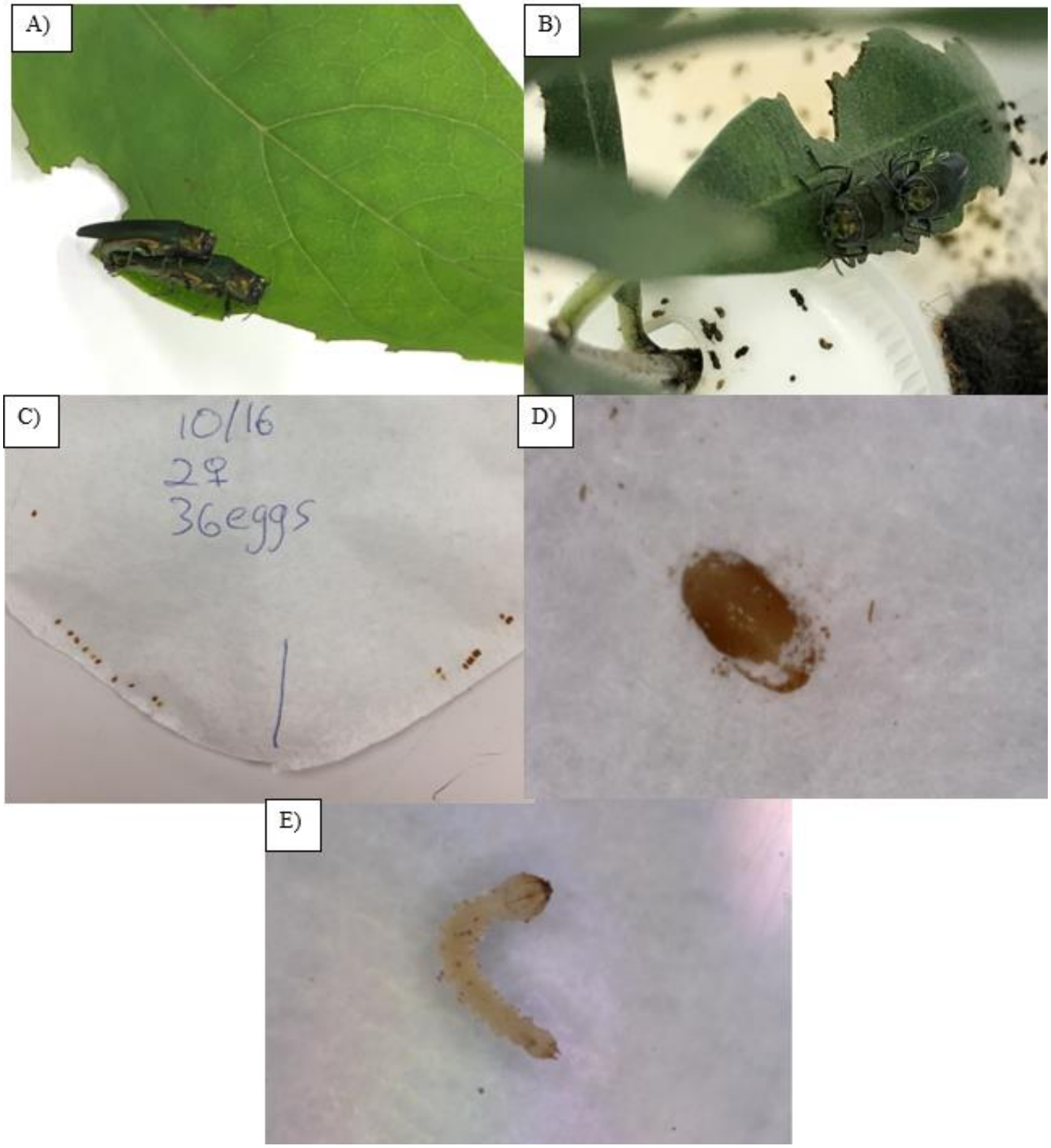

Figure 2.3. A male and female emerald ash borer (EAB, *Agrilus planipennis* mating on A) white fringetree (*Chionanthus virginicus*) and B) olive foliage (*Olea europaea*). C) Eggs laid by female EAB that consumed only white fringetree leaves. D) Single EAB egg laid by female that fed on olive foliage. E) Neonate that successfully emerged from an EAB egg laid by a female beetle that only consumed olive leaves.

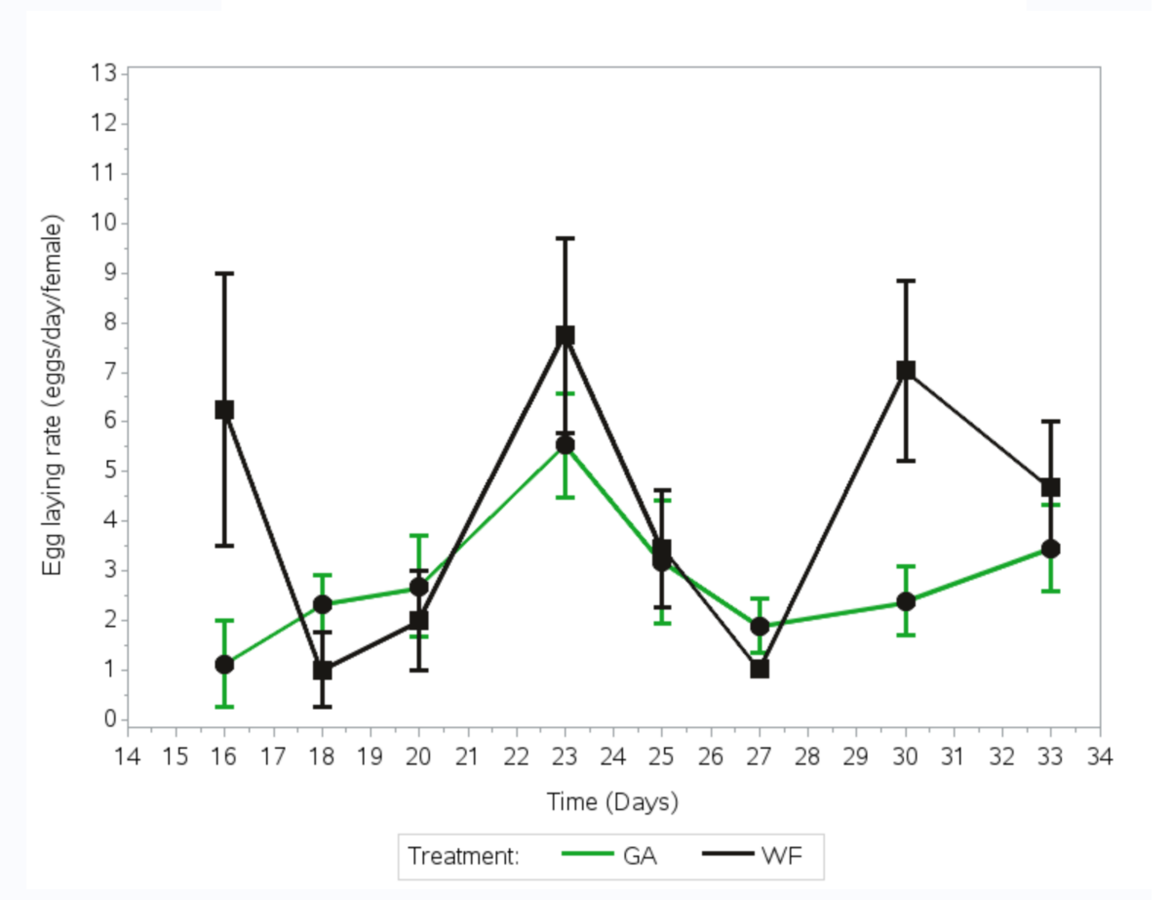

Figure 2.4. Egg laying rate (eggs/day/beetle±SE) over time of female emerald ash borers (*Agrilus planipennis*) that consumed green ash (GA, *Fraxinus pennsylvanica*) and white fringetree (WF, *Chionanthus virginicus*).

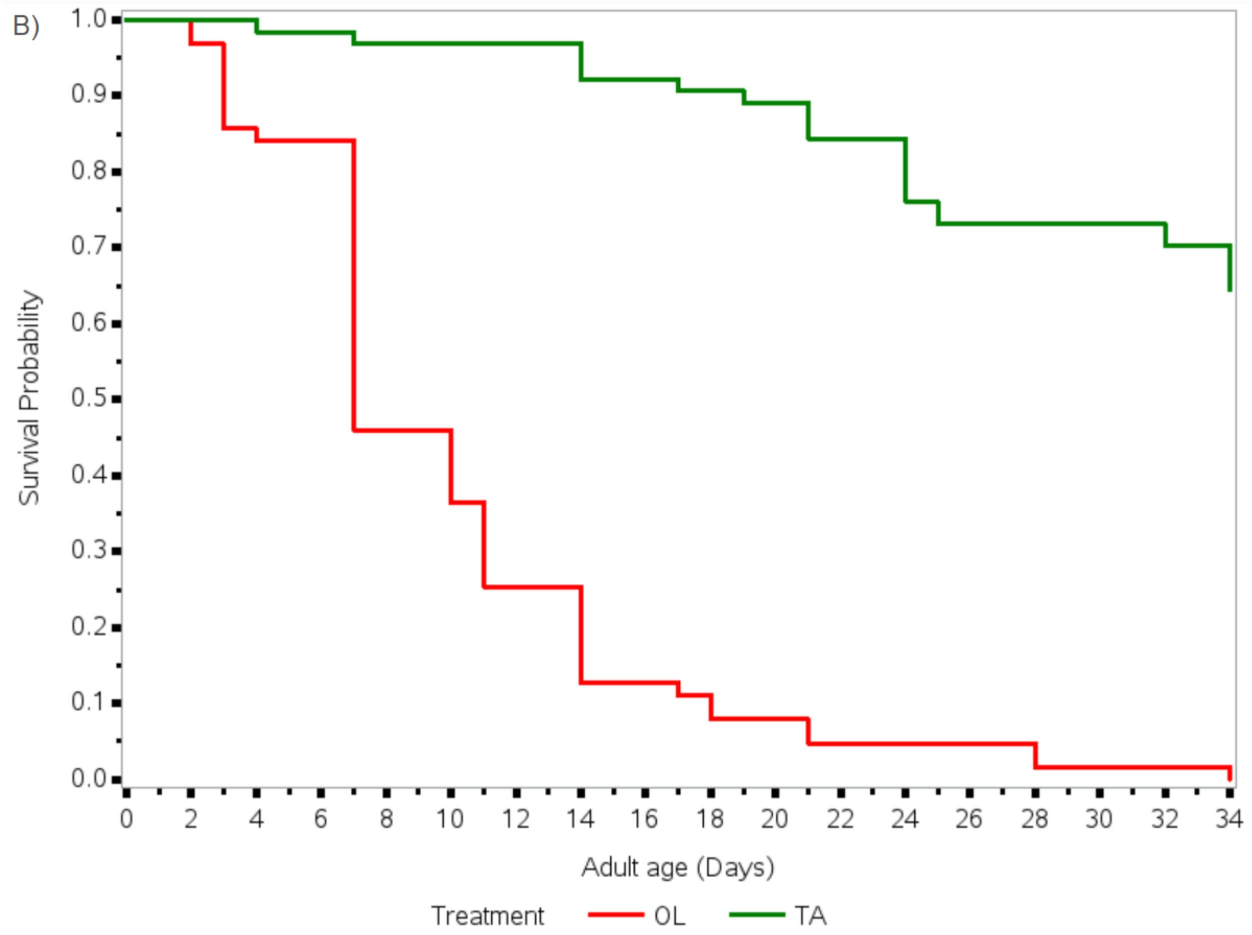

Figure 2.5 Kaplan Meier survival analyzes of emerald ash borer (*Agrilus planipennis*) on olive (OL, *Olea europaea*) and tropical ash (TA, *F. uhdei*) foliage over 34 days.

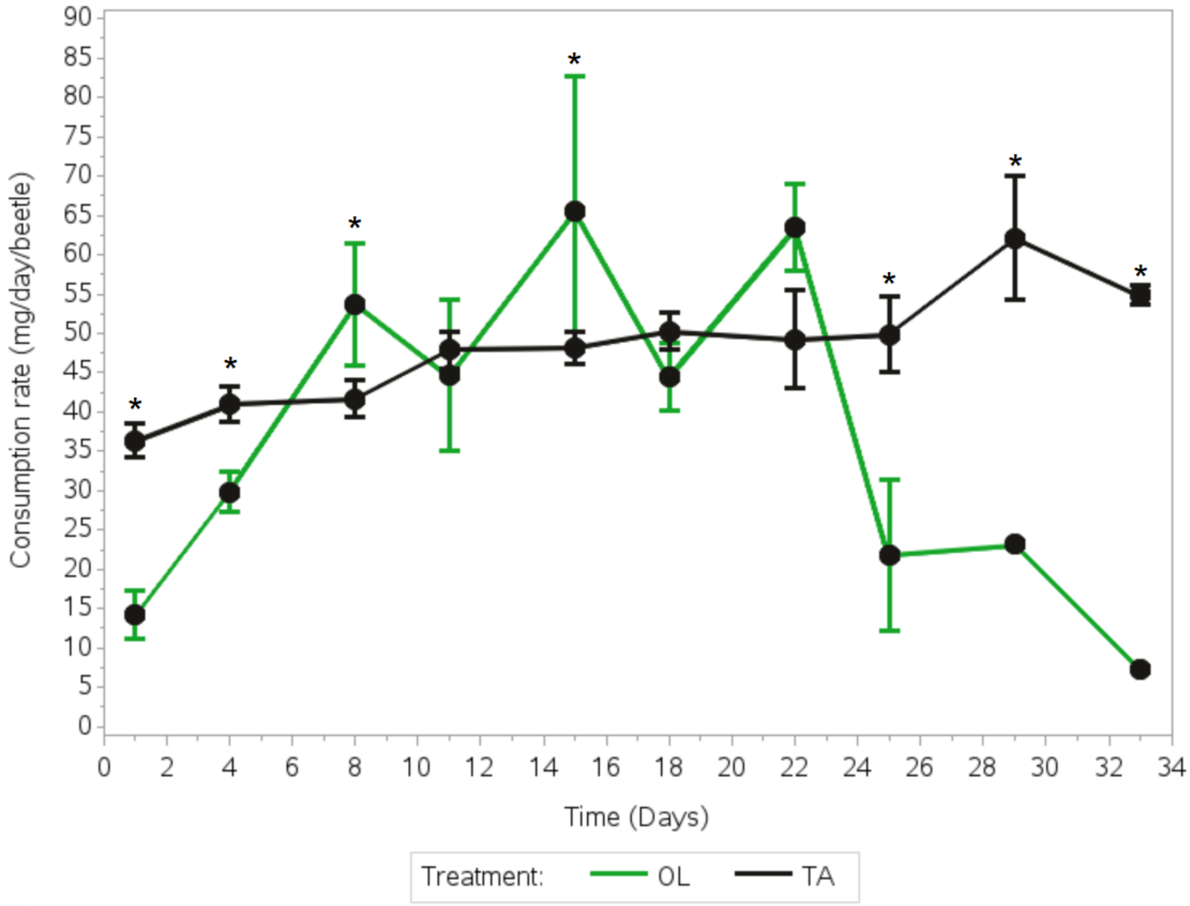

Figure 2.6. Consumption rate (mg/day/beetle±SE) of tropical ash (TA, *Fraxinus uhdei*) and cultivated olive (OL, *Olea europaea*) by emerald ash borer adults (*Agrilus planipennis*).

*

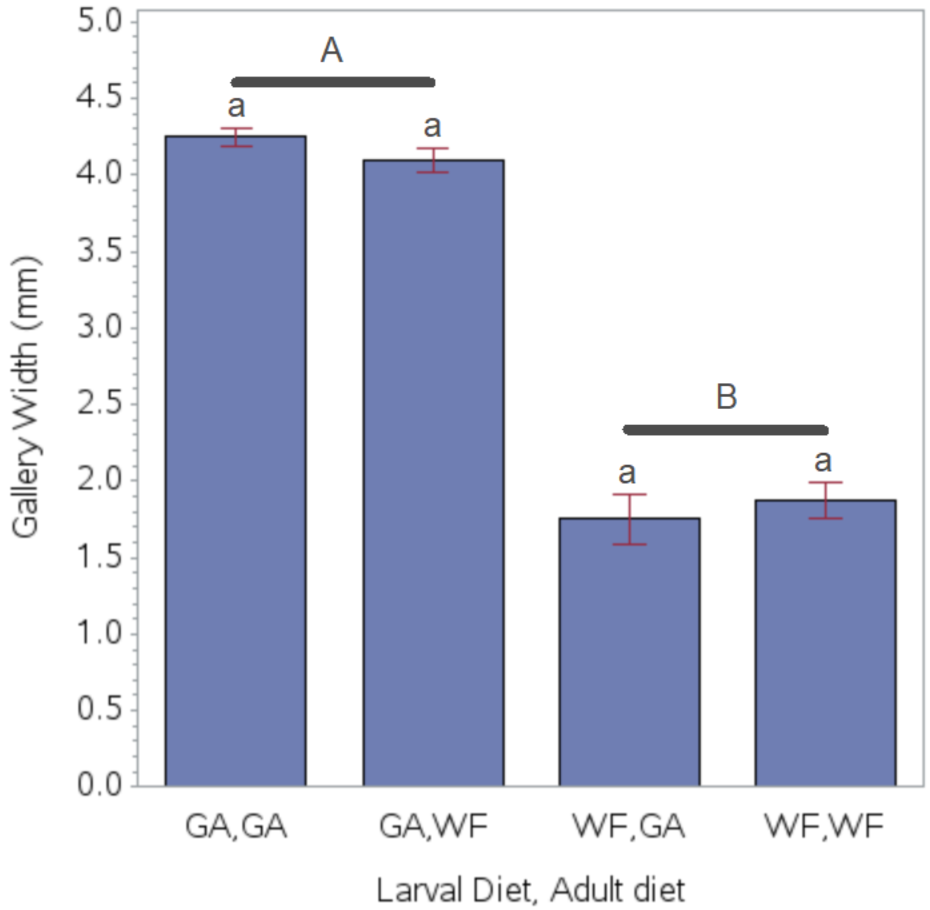

Figure 2.7. Larval performance (Gallery width, mm) of emerald ash borer (*Agrilus planipennis*), a reciprocal test of the influence of mother and larval diet of green ash (GA, *Fraxinus pennsylvanica*) and white fringetree (WF, *Chionanthus virginicus*).

Table 2.1. Hatch rates and gallery establishment rates of emerald ash borer larvae (*Agrilus planipennis*), a reciprocal study of influence of mother and larval diet of green ash (*Fraxinus pennsylvanica*) and white fringetree (*Chionanthus virginicus*).

Treatment (Larval diet, Adult diet)	Hatch rate	Gallery establishment rate
GA, GA	74.2%	89.8%
GA, WF	69.7%	76.1%
WF, GA	63.3%	71.4%
WF, WF	60.0%	71.8%
Statistics	$\chi = 3.56$; df = 3; $p = 0.314$	$\chi = 5.97$; df = 3; $p = 0.089$

Chapter 3: Ecological fitting: chemical profiles of plant hosts provide insights on selection cues and preferences for a major Buprestid pest.

Abstract

The specific cues used by emerald ash borer (EAB, *Agrilus planipennis*) to select hosts are largely unknown. Known attractants are likely general and the use of novel host plants provides an opportunity to investigate the commonality of these cues. We examined volatile profiles emitted by five host plants of EAB and their importance for known oviposition preferences.

Canopy volatiles were collected from black ash (*Fraxinus nigra*), Manchurian ash (*F. mandshurica*), blue ash (*F. quadrangulata*), white fringetree (*Chionanthus virginicus*), and olive (*Olea europaea*) and analyzed using GC-MS.

Fifty-nine compounds were at least partly identified including eight green leaf volatiles (GLV), 12 monoterpenes, and 21 sesquiterpenes. Ordination plots show separation of species by full canopy profiles, monoterpenes, sesquiterpenes, and known antennally active compounds, but GLVs were similar. Random Forest (RF) analysis revealed eight compounds that separated plant species with an error rate of ~19%, consisted mostly of sesquiterpenes.

Similarity of GLV profiles suggests they serve as general cues for host selection. Manchurian ash, a resistant host, produced the highest quantities and variety of sesquiterpenes indicating these chemicals are antixenotic. All compounds identified by

RF have been implicated as deterrents or attractants to woodborers in other studies and should be investigated for adult antennal activity and attraction.

Introduction

Introductions of herbivore insects has increased dramatically over the past few decades due to international shipping. Many of these pests survive by establishing on novel hosts, but they need to first find these plants. Herbivorous insects select their hosts with visual and chemical cues, secondary metabolites produced by the plant. Insect herbivores rely on plant chemistry similarity to find new hosts which can lead to host shifts or range expansions (Becerra 1997, Murphy and Feeny 2006, Coley et al. 2018). For example with Bursera (Burseraceae) and Blepharida beetles, molecular phylogenies demonstrate how herbivores (beetles) have followed the terpene profiles of host plants (Bursera) through evolutionary time. Recent insect success for establishing in novel environments and finding these new hosts is likely due to ecological fitting, a process "whereby organisms colonize and persist in novel environments, use novel resources, or form novel associations with other species as a result of the suites of traits that they carry at the time they encounter the novel condition" (Janzen 1985). In the context of plant-insect interactions, herbivores can use ecological fitting to expand their host range, mostly to closely related taxa, using chemical, tactile, and/or visual similarities. Thus, for an insect to find novel hosts via ecological fitting, herbivores need to possess traits that can be used to find hosts from long distances, volatile organic compounds that are similar among the historical and novel hosts.

Host selection is relatively well studied in major forest pests such as long-horned beetles (Cerambycidae: Coleoptera) and bark beetles (Curculionidae: Coleoptera) (Hanks 1999, Allison et al. 2004). Bark beetles that can kill their plant hosts use random landing to find their hosts for those that are pioneer colonizers, while secondary colonizers are drawn in with aggregation pheromones produced by conspecifics. This mass attack of a host is used to quickly overcome plant defenses. Long-horned beetles attack dead, stressed, or healthy trees and range from specialists to generalists (Hanks 1999). These large beetles use a variety of secondary metabolites to select their hosts including floral (Hanks et al. 1990), smoke (Evans et al. 2007), trunk (Allison et al. 2004), and leaf (Allison et al. 2004) volatiles from plants, and some even use bark beetle pheromones (Allison et al. 2004). Monoterpenes and ethanol excite the antennae of and are used as attractants by Cerambycids to select stressed, damaged, or dead hosts (e.g. Schroeder and Weslien 1994, Suckling et al. 2001, Trapp and Croteau 2001, Morewood et al. 2002, Allison et al. 2004, Sweeney et al. 2004).

Other woodborers have received less attention due to their inconspicuous life style and status as secondary pests of stressed or dying trees (Evans et al. 2007). In their native range, these pests typically remain secondary, but can become a primary pest on evolutionarily naïve host plants (Liu et al. 2003, Hu et al. 2009). Buprestidae, jewel beetles or metallic woodboring beetles, is a family of beetles consisting of 15,000 species (Evans et al. 2007). Most of these beetles are tree specialists that select hosts that are stressed from biotic or abiotic damage to the root, stems, or branches (Evans et al. 2007). Due to the movement of trees in the horticultural trade and the use of wooden shipping material, several Buprestids have been moved and exposed to and cause high morality in novel hosts (Haack et al. 2002, Liu et al. 2003, Poland and McCullough 2006, Coleman and Seybold 2008, Muilenburg and Herms 2012). Bronze birch borer (*Agrilus anxius*) is a secondary pest of North American birch trees (*Betula* spp.); however, when exposed to novel Eurasian birch, these beetles readily kill healthy plants within a few years (Nielsen et al. 2011, Muilenburg and Herms 2012). One of the few studies on cues it uses to find hosts proposed that it used rhododendrol as a contact oviposition cue in susceptible Eurasian hosts, but further investigation refuted this hypothesis due to a precursor of rhododendrol being found in high concentrations in a resistant, North American hosts (Santamour Jr 1990, 1999, Santamour and Lundgren 1997). Another Buprestid, goldspotted oak borer (*A. auroguttatus*) attacks declining oak trees (*Quercus* spp.) in the southwestern U.S. and Mexico (Coleman and Seybold 2008). In the 2000s, extensive mortality of novel oak species in California was caused by this beetle (Coleman and Seybold 2008). This pest is attracted to purple traps and males prefer traps baited with Manuka oil, Phoebe oil, or 3-*Z*-hexenol in contrast to no bait (Coleman et al. 2014). The two oils are distillations of plants and consisting of many sesquiterpenes so it is difficult to determine which compounds are important for host selection. These data suggest specific cues are not well understood for this species. 3-*Z*-hexenol is one compound that increases capture rates in goldspotted oak borer and bronze birch borer (Coleman et al. 2014, Silk et al. 2019) but it is produced by many plants suggesting it may only serve as a general attractant.

Emerald ash borer (EAB) is the most damaging and costly woodborer in North America (Aukema et al. 2011) and is the most extensively studied Buprestid. This invasive Asian beetle prefers to oviposit on North American ash species (*Fraxinus* spp.), including green ash (*F. pennsylvanica*), black ash (*F. nigra*), white ash (*F. americana*), and blue ash (*F. quadrangulata*) over its ancestral, resistant host, Manchurian ash (*F. mandshurica*) (Tanis and McCullough 2012, 2015, Herms 2014, Rigsby et al. 2014, 2017). Based on laboratory and field experiments, along with general observations, the order of preference appears to be green ash=black ash>white ash>blue ash>Manchurian ash (Rebek et al. 2008, Tanis and McCullough 2012, 2015, Herms 2014, Rigsby et al. 2014, 2017). Blue ash is unique among the North American ash because it demonstrates some level of deterrence or resistance to EAB with ~30-40% of trees surviving (Tanis and McCullough 2012) in EAB "aftermath" forests, compared to <1% of other North American ash trees (Klooster et al. 2014). Larval performance is delayed in blue ash, but survivorship is the same compared to beetles in green ash (Peterson et al. 2015). These data, in conjunction with few galleries found in blue ash compared to other North American ash (Tanis and McCullough 2015), suggests that female beetles oviposit fewer eggs on this host than other North American species. Thus, blue ash may either possess deterrents to EAB or it lacks attractants that EAB uses for host plant selection.

The known attraction to visual and volatile cues for goldspotted oak borer and bronze birch borer are consistent with emerald ash borer (Crook and Mastro 2010a, Francese et al. 2010, Coleman et al. 2014, Silk et al. 2019). Host selection by this beetle is partially mediated by host volatiles. Adult beetles respond to at least 16 volatiles produced by Manchurian, white ash, and green ash (Rodriguez-Saona et al. 2006, de Groot et al. 2008, Crook et al. 2009), many of which are green leaf volatiles. In general, females respond more strongly to monoterpenes such as linalool, while males respond more strongly to hexenals in electroantennograms (Rodriguez-Saona et al., 2006). Six bark sesquiterpenes: α-cubebene, α -copaene, 7-epi-sesquithujene, trans-β-caryophyllene, α-humulene (α -caryophyllene), and one unidentified compound elicit responses in antennae of adult beetles (Crook et al. 2009). These compounds occur in Manuka and

Phoebe oils (Crook et al. 2009) and when used in conjunction with purple traps, these oils increase captures of EAB. Traps with Phoebe oil catch more than those with Manuka oil apparently due the presence of 7-*epi*-sesquithujene in phoebe oil. Black ash receives much higher egg loads over Manchurian ash and this pattern is in part attributed to higher levels of the 7-*epi*-sesquithujene (Rigsby et al. 2014, 2017). In contrast, when healthy, Manchurian ash receives little to no eggs from female beetles compared to North American species (Rigsby et al. 2014, 2017). Although this tree is attractive in the field when stressed, the low egg load on Manchurian ash indicates that this plant is antixenotic when healthy. This resistance may be from higher emissions of potentially deterrent volatile compounds in Manchurian ash compared to North American ash as postulated by (Pureswaran and Poland 2009), specifically due to chemicals emitted at higher levels such as abietatriene and abieta-8,12-diene.

Since 2014, emerald ash borer has been observed using a novel non-ash host, white fringetree (*Chionanthus virginicus*: Oleaceae), in ornamental and wild populations in North America (Cipollini 2015, Cipollini and Rigsby 2015, Peterson and Cipollini 2017, Cipollini and Peterson 2018). This beetle will oviposit on the closely related cultivated olive (*Olea europaea*) in the field and completes development on cut stems in the laboratory (Cipollini et al. 2017). Infestation rates of white fringetree are lower (~26%,(Peterson and Cipollini 2017) than in similarly sized ash trees (≥75%, (Rebek et al. 2008, Herms 2014) in ornamental settings. Larval survival is similarly lower in healthy trees (<15%, DLP unpublished data) compared to ash trees (>52% in green and blue ash Peterson et al. 2015). Limited field observations have shown that females prefer ash over olive trees for oviposition (Cipollini and Peterson 2018). Regardless of preference or performance, EAB likely uses host plant traits, including volatile cues, to find and select among novel NA hosts, including white fringetree, a form of ecological fitting. Several hosts of EAB emit methyl salicylate and Z-3-hexonal (Rodriguez-Saona et al. 2006, Rigsby et al. 2017) including white fringetree (DC unpublished data) and olive (Flamini et al. 2003, Brahmi et al. 2012), which may facilitate the use of these hosts by EAB. Olive and ash emit several other compounds in common, including hexanols and linalool (Brahmi et al. 2012), that are attractive to adult beetles (Rodriguez-Saona et al., 2006; de Groot et al., 2008). Ecological fitting of EAB to novel NA hosts is unclear, specifically it needs to be determined what compounds are shared that facilitates attraction and which compounds may deter adults, if any, from the historic host when healthy. The volatile organic compounds, chemical profiles, of novel and ancestral hosts need to be explored to better explain host preference and attraction of EAB.

Conducting studies to determine the chemical differences between the ancestral and novel hosts will contribute to understanding better how emerald ash borer, and Buprestids use ecological fitting via volatiles for host selection. The chemical differences with shared attractants will explain why certain plants are preferred over other hosts while unique compounds to the ancestral/resistant host are avoided or less preferred by EAB. In this study, we investigated the volatile profiles of five EAB hosts: Manchurian ash (ancestral), blue ash (novel ash), black ash (novel ash), white fringetree (novel non-ash), and olive (novel non-ash). Canopy emissions were collected with SuperQ filters to determine how volatiles vary among these hosts. We hypothesized several compounds are shared between the ancestral host with novel hosts that facilitates the host range expansion of EAB. Specifically, green leaf volatiles are likely to be very similar since they appear to be general cues while sesquiterpenes vary significantly both qualitatively and quantitatively, as observed in Rigsby et al. (2017). Using Random Forest machine learning, we determined what compounds are the most important for determining differences among plant species. Finally, we performed a preliminary bioassay to give some indication of EAB female choice among the two novel non-ash hosts, green ash (proxy for black ash), and Manchurian ash in order to gain some insights on the relative preference of EAB preference for these hosts.

Methods

Volatile collection and analysis.

Canopy volatiles of five plant species, Manchurian ash (n=5), black ash (n=5), blue ash (n=5), white fringetree (n=5), and olive (n=6), were collected twice, once on 27 and 28 June 2017 and again 31 July and 1 August 2017. Plants were acquired between 2015-2017 including five blue ash trees from the Wright State University Woods (2015); five white fringetrees from Siebenthaler's Garden Center in Beavercreek, OH in (2016); six cultivated olive trees, cultivars ‘Mission’ (n=3) and ‘Arbequina’ (n=3) from Willis Orchard Company, Cartersville, GA, USA (2016); and black ash, cultivar “Fallgold” and Manchurian ash, cultivar ‘Mancana” from Schumacher’s Nursery and Berry Farm Inc., Heron Lake, MN, USA (2017). All trees were potted with diameters one to three cm in diameter at the base of the scion. All trees were similarly size of two to three cm in diameter at the base of the scion with blue ash and Manchurian ash being 1.5-1.8 meters in height, while olive, white fringetree, and black ash were 1.0 meters;

Using methodology as described in Rigsby et al. (2017) and Böröczky et al. (2012), we used portable, tackle boxes (n=8) each having two pumps for volatile collection of canopy emissions. A sheet of Teflon (FEP100 fluoropolymer film; Dupont, Wilmington, DE, USA) was wrapped around a large branch with foliage, rolled up to create a tight seam, and secured with nylon string on the top and bottom ends of the sheet to create a bag. The tackle box uses two pumps to push and pull air through a headspace to collect volatiles. The first pump, pulled air from the background and pushed the air through a charcoal filter and then onward to the headspace of the tree. The second pump pulled air from the headspace of the plant through a SuperQ filter (30 mg SuperQ sorbent; Alltech Associates, Deerfield, IL, USA) which absorbed emitted volatiles. After two hours, we removed the filter, wrapped it in scentless Teflon tape and then aluminum foil to reduce contamination from background volatiles. Filters were sent to the Chemical Ecology Laboratory at Penn State University for analysis. Volatile profiles were collected for two hours/tree using a push rate of 0.75±0.05 L/min and pull rate of 0.5±0.05 L/min. Leaves were gathered and weighed to get an average mass per leaf after each volatile profile collection. Teflon sheets were cleaned with 95% ethanol between samplings to prevent cross contamination. SuperQ filters were processed in the lab and analyzed with GC-MS using a target compound identification method (based on retention index and target ion abundances), as described in Rigsby et al. (2017). Canopy emissions are expressed in units of pg/hr/g leaf for analysis and presentation.

Olfactometer setup and host volatile profile preference.

Gravid EAB were used in a Y-tube olfactometer (24mm, Volatile Collection Systems Company, LLC, Gainesville, FL, USA) to conduct a two-choice bioassay. Bioassays were run at Brighton EAB rearing facility in Brighton, MI (9-13 July 2018) and the Beneficial Insects Introduction Research Unit in Newark, Delaware (12-14 September 2018). Bioassays were set-up with Y-tube olfactometers with two external air inserts, a carbon filter, air humidifier, two air flow meters, and two glass elbow flasks interconnected with Teflon tubing. Air was pumped through an activated carbon filter and then bubbled through distilled water to humidify. Air was then split into elbow Erlenmeyer flasks with plant foliage or blank (no leaves) at 0.5 L/min (O'Neal et al. 2004, Saïd et al. 2006). Plant volatiles or blank air was pushed out of the flasks through the external air inserts that connect to the Y-tube olfactometer. White foam boards were used to surround the olfactometer to block visual clues that adult beetles may use for host selection. Bioassays were conducted at 23-25 Cº (ambient room temperature).

We conducted bioassays for 1800 second (30 minutes) with introducing gravid females individually at the base of the olfactometer and exposing them to two air treatments. The treatments presented to female beetles were either blank air (BL, control) or volatiles emitted by various plant species: olive (OL), white fringetree (WF), Manchurian ash (MA), or green ash (GA). In total, there were six treatments: OL vs BL (n=12), WF vs BL (n=8), GA vs BL (n=10), OL vs GA (n=11), WF vs GA (n=12), WF vs MA (n=8), and OL vs MA (n=8). Due to travel time constraints, the treatments of MA vs GA and MA vs BL were not completed. Host foliage was randomly assigned for each bioassay. We filmed all bioassays using a Panasonic V180K Full HD 1080p. After each trial, the Y-tube olfactometer and external air inserts were washed and cleaned with ethanol and then dried with Kimwipes to remove residual odors from previous bioassays. Flasks were cleaned when treatments were switched as previously described. To eliminate pseudoreplication, individual beetles were used once. We used the videos to record the total amount of time(s) beetles were in the left and right arms of Y-tube. The location of beetles was recorded at 30 second intervals due to low beetle movement and to efficiently analyze the videos. We then multiplied the quantity for each area by 30 to get total time spent during the 30 minute bioassay.

Statistical analysis.

Volatile data were analyzed using SAS (“SAS Studio” 2017) and R (R Core Team 2018). Canopy volatile profiles were visualized using nonmetric multidimensional scaling (NMDS) with the Euclidean dissimilarity index using the ‘vegan’ package in R. Euclidean dissimilarity was used because it provided the highest index available in the ‘vegan’ package and estimated the lowest stress statistic, <0.2 for ordinations. A Permutational Multivariate Analysis of Variance, PERMANOVA (Adonis in R), was used to determine quantitative differences in plant volatiles among species and a post-hoc test (Pairwise Adonis in R) was used to determine differences between species. Randomforest and VarSelRF, packages in R, were used to determine the most important volatiles predicting species differences, as well as oviposition and feeding preferences based on the deduced preferences BA>Blue>WF≥MA>OL (Rebek et al. 2008, Pureswaran and Poland 2009, Herms 2014, Rigsby et al. 2014, Tanis and McCullough 2015). Using varSelRF, models were selected to have the smallest out of the bag error using 200 bootstrap replicates and ntrees set to 1000 (Jaeger et al. 2016). Mean decrease in accuracy was presented in very low values in R that were unscaled (i.e. values <0.01). We present R output values in a scaled value to better display importance of variables. PROC ANOVA (SAS) was used to determine differences in the emission of volatiles by tree species. Olfactometer bioassays were only used when beetles made a choice, such as spending any amount of observable time in a treatment area. Due to this, sample sizes were small and non-normal; therefore, we used Wilcoxon signed-rank T-test (PROC UNIVARIATE) to determine if time spent in treatment areas differed.

Results

Identification and category assignment of compounds.

A total of 59 compounds (Table 3.1) were emitted from the five host species, including nine green leaf volatiles (GLV), 12 monoterpenes, and 21 sesquiterpenes. Total canopy and monoterpene emissions did not differ significantly among species (Figure 3.1a and 3.1e, respectively). More GLV were emitted from Manchurian ash than olive (Fig. 3.1c) likely due to a high emission of (*Z*)-3-hexenyl acetate. Manchurian ash emitted significantly higher levels of antennally active compounds (Fig. 3.1b) compared to blue ash and of sesquiterpenes compared to blue and black ash (Fig. 3.1d). The remaining compounds emitted were a mixture of aromatics, homoterpenes, phenylpropanoids, esters, and an alkane. Fourteen compounds were emitted by plants that are antennally active for adult beetles (Rodriguez-Saona et al., 2006; de Groot et al., 2008; Crook et al., 2009) including (Z)-3-hexen-1-ol, n-hexanol,(Z)-3-hexenyl acetate, hexyl acetate, (E)-b-ocimene, linalool, nonanal, 4,8-dimethyl-1,3,7-nonatriene (DMNT), a-cubebene, a-copaene, b-caryophyllene, a-humulene, eremophilene, and (E,E)-a-farnesene.

Volatile profile differences between plant species.

NMDS (Fig. 3.2a-e) visualized volatile profiles while PERMANOVA was used to statistically assess differences in these profiles (Table 3.2 and SI Table 3.1). PERMANOVA with sampling date indicated that there were no significant differences in profiles between sampling date within species and that both olive cultivars had the same overall profiles. Therefore, we combined data from the two sample dates and the two olive cultivars to one species, olive (OL). Volatile profiles (Fig. 3.2a) were significantly dissimilar among species overall with the ash species: black ash (BA), Manchurian ash (MA), and blue ash (BLUE) having mostly similar profiles. The volatile profile of olive was dissimilar from all other species. Black and blue ash profiles were significantly dissimilar from white fringetree (WF) while Manchurian ash was similar to white fringetree. When we examined differences of volatile profiles in chemical classes, green leaf volatiles (GLV, Fig. 3.2b) were found to be similar among species. Monoterpene profiles (Fig. 3.2c) were emitted by species dissimilarly with white fringetree being the most dissimilar (Fig. 3.2c, Table 3.2) from the other four plant species likely due to the unique express of b-pinene, a-terpinene, and limonene. The ash species were similar among each other, while olive was only similar to black ash and Manchurian ash and dissimilar to blue ash. Species emitted dissimilar profiles of sesquiterpenes (Fig. 3.2d) with only blue and black ash having similar profiles. The emission of antennally active compound (AA, Fig. 3.2e) by the ash species and white fringetree were similar. The profile for olive was significantly dissimilar from all other species except for Manchurian ash.

Individual volatile differences between species.

Out of 59 volatiles consistently detected (emitted in ≥50% of replicates) in the five plant species, 15 were emitted at significantly different rates (Table 3.3). α-pinene and sabinene were emitted at higher rates in white fringetree than other hosts and Manchurian and blue ash did not produce any sabinene. Blue ash and Manchurian ash emitted the highest amount of mesitylene. Manchurian ash emitted the most (Z)-3-hexenyl acetate. Limonene was only produced in olive and white fringetree with white fringetree emitting the highest amount. Olive and white fringetree produced the most benzyl alcohol out of all five species. Both (Z)-b- and (E)-b-ocimene were abundant in black ash; although olive produced a similar amount of (E)-b-ocimene. Blue ash and olive emitted at higher rates while DMNT was produced mostly by Manchurian ash. White fringetree produced the most (Z)-3-hexenyl isobutyrate and (Z)-3-hexenyl isovalerate with neither Manchurian or blue ash emitting isobutyrate. B-caryophyllene and y-cadinene were emitted highly in Manchurian ash than in white fringetree and olive.

Random forest.

Random forest was used to determine the most important volatiles for distinguishing plant species and the inferred EAB host preference rankings. The model with the lowest out of bag error rate (19.23%) included eight volatiles that had the highest mean decrease in accuracy (Table 3.1). Three of these compounds were unique to two species such as *(R)-(+)*-limonene (present in WF and OL), a-trans-bergamotene (present in WF and OL), and abietatriene (present in BA and MA). Sabinene, methyl benzoate, b-caryophyllene and y-cadinene were emitted at significantly different levels demonstrating species differences. The final compound was caryophyllene oxide that was emitted highly in Manchurian ash followed by olive and white fringetree.

Olfactometer bioassays.

The average time spent by female beetles was not statistically different among treatments (Fig. 3.3), with GA vs BL (*t*=-3.0, *p*= 0.719, n=8), GA vs BL (*t*=-3.0, *p*= 0.250, n=3), GA vs OL (*t*=-11.5, *p*= 0.125, n=8), MA vs OL (*t*=-1.0, *p*= 0.875, n=4), MA vs WF (*t*=3.0, *p*= 0.375, n=4), WF vs BL (*t*=-1.5, *p*= 0.500, n=2), and WF vs GA (*t*=-3.0, *p*= 0.688, n=7). There was a tendency for beetles to spend more time near foliage volatiles of green ash over blank air, white fringetree over blank air, and green ash over olive.

Discussion:

For over a decade, emerald ash borer has been a major pest of ash trees in Europe and North America; yet, the specific cues it uses to select their hosts are poorly understood. A recent host range expansion has provided an opportunity to better understand the chemical differences among resistant ash, susceptible ash, and novel non-ash hosts by evaluating volatile emissions. Common green leaf volatiles (GLVs), such as Z-3-hexanol, increase trap captures (de Groot et al. 2008), but many plants produce these compounds. Gold-spotted oak borer (*A. auroguttatus*), a close relative of EAB that attacks oak trees (*Quercus* spp.), is similarly attracted to GLVs (Coleman et al. 2014) while bronze birch borer (*A. anxius*) demonstrates the same trend (Silk et al. 2019). The common emission of GLVs in our study with the previously mentioned studies suggest these compounds are not specific for host selection of EAB and instead serve as general cues. Our random forest analysis further supports this concept because Z-3-hexanol, which increases trap capture of EAB (de Groot et al. 2008, Crook and Mastro 2010b), was the least important volatile in differentiating species. Green leaf volatile similarities of white fringetree and olive to the ash trees suggests these compounds contribute to the ecological fitting to novel hosts. Interestingly, one GLV, (Z)-3-hexenyl acetate, which is an antennally-active compound, was emitted the most by Manchurian ash (Rodriguez-Saona et al., 2006). Based on the avoidance of healthy Manchurian ash by EAB when they are presented with other ash trees (e.g.(Rigsby et al. 2014, 2017), these data would suggest that (Z)-3-hexenyl acetate is a deterrent to females, but it may be a general attractant, as we postulate for GLVs, and is not significant for host preference since emissions were highest from a less preferred host. White fringetree emitted higher quantities of (Z)-3-hexenyl isobutyrate which was produced by the most attractive host, black ash, also. These data indicate that if this compound is antennally active, it would be one of many shared compounds that EAB has used to expand its host range to white fringetree.

In contrast to GLVs, monoterpenes varied significantly among species particularly in white fringetree due to the unique or higher emission of *a*-, *b*-pinene, sabinene and *a*-terpinene. Two monoterpenes known to excite antennae of EAB are linalool and Z-*b*-ocimene (Rodriguez-Saona et al., 2006). When plants are stressed, emissions of Z-*b*-ocimene and linalool increase in trees such as silver birch (*B. pendula*) and Manchurian ash (Rodriguez-Saona et al., 2006; Vuorinen et al., 2007; Gossner et al., 2014). Compounds that increase with plant stress are consistent with what should be used by EAB to locate susceptible hosts. In our study, the highest emission of Z-*b*-ocimene was in black ash, the most preferred plant species (Rigsby et al. 2014), indicating this volatile may be an important attractant for EAB. Several compounds were found to be unique or emitted at a higher level in white fringetree: *b*-pinene, limonene, *a*-terpinene, and sabinene. Terpinene, although not revealed to be an important variable in our random forest analysis, may be important for adult EAB plant selection since it excites the antennae of the congener two-spotted oak borer (*A. biguttatus*) from Europe (Vuts et al. 2016) and is worthy of future investigation. Two of these compounds, limonene and sabinene were important variables in our random forest analysis for separation of plant species. The biological activity of these compounds is unknown for EAB, but in the woodborer mountain pine beetle, limonene causes insects to feed less (Chiu et al. 2017, Erbilgin et al. 2017). Emerald ash borer larvae grow slower in white fringetree and olive compared to in green ash (Cipollini and Rigsby 2015, Cipollini et al. 2017) and limonene, as an antifeedant, would explain the delayed growth. Traps baited with limonene generally do not catch Buprestids (Chénier and Philogene 1989) indicating a potential deterrence to beetles such as EAB and its relatives. Lower attack rates in the white fringetrees (Peterson and Cipollini 2017) compared to similar sized North American ash trees (Rebek et al. 2008, Herms 2014) could therefore be explained due to higher emissions of limonene. On the other hand, sabinene in a blend attracts some wood borers such as redbay ambroisa beetle (*Xyleborus glabratus*, Curculionidae; (Martini et al. 2015) and juniper bark borer (*Semanotus bifasciatus*, Cerambycidae; Jingfang, 1989). Sabinene was an important volatile for the separation of plant species in our study with emissions detected from olive, black ash, and white fringetree but not in blue and Manchurian ash. Manchurian ash has previously been found to produce sabinene, but black ash produced ~6.5 times more (Rigsby et al. 2017) demonstrating a similar pattern to our data. The lack of emission of sabinene in less preferred hosts and higher production in some preferred hosts indicate that this compound may serve as an attractant for host plant selection. Furthermore, white fringetree and olive emitted sabinene and this compound may be one more component of EAB attraction to the two novel non-ash hosts. Future studies should test the antennal response and attraction of EAB to sabinene.

Sesquiterpenes showed the largest difference between species and may explain preferences for adult EAB. Comparing Rigsby et al. (2017) with our data, several parallels are drawn. Black and Manchurian ash emitted sesquiterpenes differentially with many of them occurring at higher levels in the historic host (Rigsby et al. 2017). We suspect sesquiterpenes delineate the differences between the novel ash and the ancestral ash hosts of emerald ash borer and this is further supported by sesquiterpenes being the majority of the eight important variables (from Random Forest) for plant identification. These data help us better understanding the host selection/preference of EAB particularly in regard to antixenosis. A previous hypothesis from Pureswaran and Poland (2009) suggested that EAB prefer green ash due to low volatile emissions, while Manchurian ash is less attractive due to high quantities of volatile emissions. Our data supports this hypothesis; specifically, Manchurian ash and the two novel hosts, both hypothesized to be less attractive than NA ash, emitted more sesquiterpenes than black and blue ash (i.e. antixenosis) which both produced very little. Of the twenty sesquiterpenes detected, only (E,E)-a-farnesene was emitted by black and blue ash. In contrast, Manchurian ash, white fringetree, and olive emitted at least 11 or more of these compounds. Six sesquiterpenes excite the antennae of EAB including *a*-copaene, *cis*-caryophyllene, *b*-caryophyllene, caryophyllene oxide, *a*-humulene, (Z,E)-*a*-farnesene, and (E,E)-*a*-farnesene (Rodriguez-Saona et al., 2006; Crook et al., 2008). Manuka and Phoebe oil emit these antennally active compounds, except for the farnesenes, and increase captures of EAB when present in traps (Crook et al. 2008). Those data suggest that sesquiterpenes are attractants for EAB. We did not detect these compounds in black and blue ash; surprisingly, since preferred hosts would likely emit higher levels of attractants in contrast to less preferred hosts. Black ash has previously been found to emit these compounds, except *b*-caryophyllene (Rigsby et al. 2017). The differences in our study compared to Rigsby et al. (2017) may be from their plants being stressed due to EAB infestation that caused a higher degree of dieback in black ash compared to Manchurian ash. In our study, trees were not infested with EAB and plants were located several hundred meters away from the nearest infested ash trees, EAB populations were very low at the time of sampling (DLP unpublished data), and no dieback was observed. We found that four sesquiterpenes: a-trans-bergamotene (WF and OL), b-caryophyllene (MA, WF, and OL), y-cadinene (MA, WF, and OL), and caryophyllene oxide (MA, WF, and OL), and one diterpene: abietatriene (BA and MA) are important for plant species identification. Adult antennal response to abietatriene, y-cadinene, and a-trans-bergamotene are unknown. These chemicals were emitted uniquely or in higher amounts in Manchurian ash compared to black and blue ash. Thus, we hypothesize that these compounds could deter adult beetles, an idea previously postulated by Rigsby et al. (2017) because Manchurian ash in their study emitted these compounds uniquely or at higher levels compared to black ash. Manchurian ash, white fringetree, and olive emitted two other sesquiterpenes; (E)-nerolidol and germacrene D, uniquely compared to blue and black ash. These compounds have some antixenotic effect for insects (Doskotch et al. 1980) and may influence EAB behavior, but this remains to be tested.

For EAB to find and select white fringetree and olive, beetles are likely using similar volatiles that are shared with ash hosts. We found that the host trees shared GLVs and antennally active compounds. Plants commonly emit GLVs, so similarity of these compounds is not surprising. White fringetree emitted similar antennally active compounds as the other ash trees and only olive emitted a different profile. These data indicate that EAB should prefer white fringetree and ash species over olive, although we have seen oviposition on olive by EAB in the field (Cipollini and Peterson 2018). Thus, white fringetree appears to be selected as a host by EAB due to ecological fitting via chemical similarities. The five plant species share several compounds and these chemicals will contribute to better understanding the host selection cues used by EAB and other Buprestids.

Gravid females demonstrated somex trends in their preferences for hosts among green ash, Manchurian ash, white fringetree, and olive. Females that made a choice spent more time with volatiles from foliage of white fringetree and green ash over blank air. Yet, when EAB were exposed to volatiles of both host trees, beetles spent equal time with green ash and white fringetree. These female choices support a previous study that found that EAB attacks white fringetrees and North American ash trees at about the same time (Thiemann et al. 2016). We also found that female beetles choose foliage of green ash over olive indicating preference for the more susceptible ash host, as we have suggested before (Cipollini and Peterson 2018). Combined, these data indicate that females likely prefer green ash and white fringetree over olive. These trends, along with previous observations, suggest that the overall preference of hosts is the following: green ash=black ash=white fringetree>white ash>blue ash>Manchurian ash=olive. These results are speculative and should be treated with caution due to the assays not providing significant differences due to low replication.

Conclusions:

To conclude, this study has contributed to furthering our understanding of the similarities and differences in volatile profiles of five plant species in the Olive family that are known to host EAB. Random forest analysis revealed eight compounds that were particularly important for separating plant species. These compounds may explain host preferences by EAB providing a guideline for future studies. Plants varied in their emissions of sesquiterpenes with Manchurian ash producing the most. The high quantity and variety of sesquiterpenes in Manchurian ash may serve as deterrents or at least lower EAB preference.

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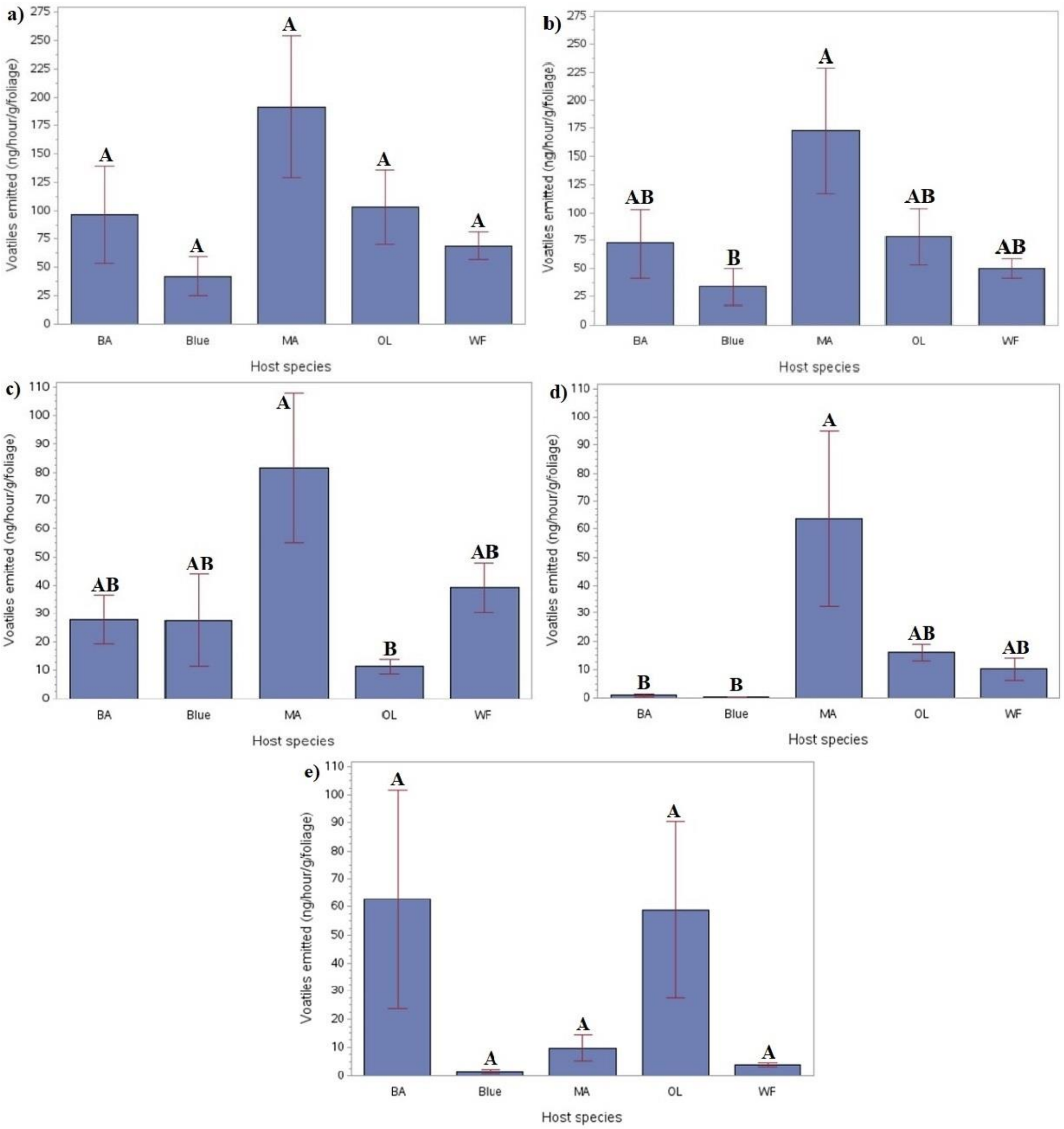

Figure 3.1.Volatiles emissions (pg/hr/g/foliage±1SE) of five plant hosts of emerald ash borer collected in summer 2017. a) Overall plant profiles, b) antennally active compounds c) Green leaf volatile (GLV) profiles, d) sesquiterpene profiles, and e) Monoterpene profiles.

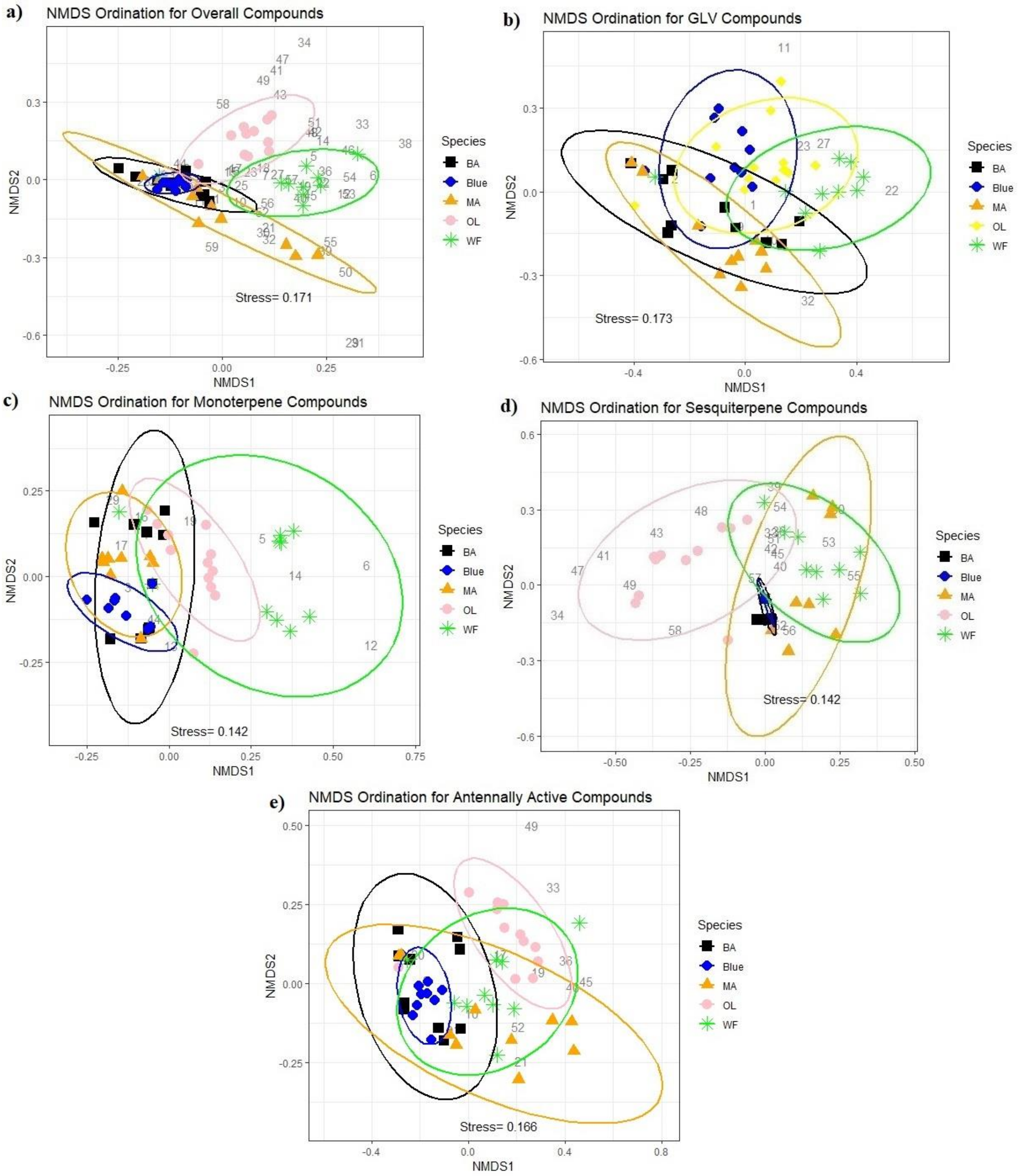

Figure 3.2. Ordination (nonmetric multidimensional scaling) plots of volatiles profiles of five plant hosts of emerald ash borer collected in summer 2017. a) Overall plant profiles, b) Green leaf volatile (GLV) profiles, c) monoterpene profiles, d) sesquiterpene profiles, and e) antennally active compounds.

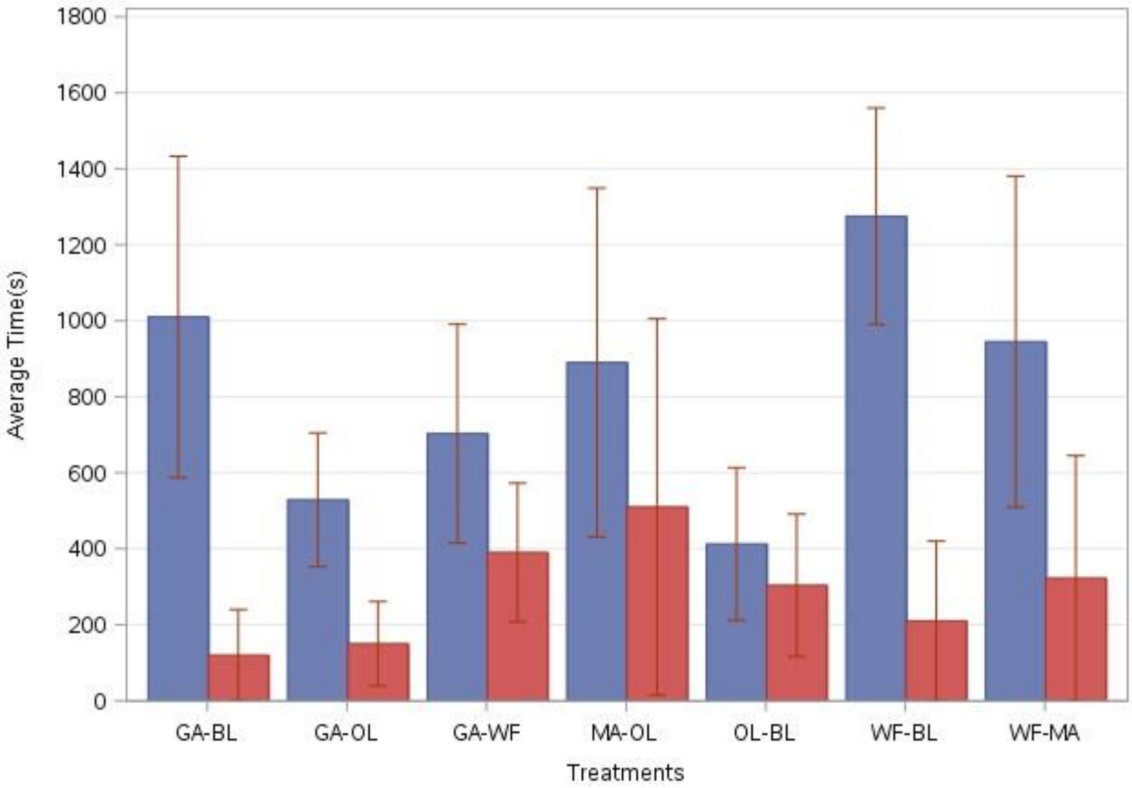

Figure 3.3. Average time spent (±1SE) of gravid emerald ash borer females (*Agrilus planipennis*) in arms of Y-tube olfactometer with foliage emissions of olive (OL, *Olea europaea*), white fringetree (WF, *Chionanthus virginica*), green ash (GA, *Fraxinus pennsylvanica*), Manchurian ash (MA, *F. mandshurica*) or blank air (BL).

Table 3.1.Tenatative identification, chemical category, identification method, and rank order of importance (Mean decrease in accuracy, MDA, Random Forest) of volatiles emitted by five hosts of emerald ash borer (*Agrilus planipennis*); black ash (BA, *Fraxinus nigra*), Manchurian ash (MA, *F. mandshurica*), blue ash (Blue, *F. quadrangulata*), white fringetree (WF, *Chionanthus virginicus*), and olive (OL, *Olea europaea*).

Peak	RT (min)	RI	Target Ion	RF	Tentative ID	Category	Identification	Rank (MDA)*
1	4.528	854	67	5.23	(*Z*)-3-hexen-1-ol	GLV	Standard, Library	59 (-1.93)
2	4.761	864.6	56	4.03	n-hexanol	GLV	Standard, Library	56 (-0.05)
3	6.472	934.4	93	3.66	(1S)-(-)-α-pinene	monoterpene	Standard, Library	24 (5.74)
4	7.235	956.1	106	5.68	benzaldehyde	aromatic ald.	Library	30 (4.31)
5	7.661	973.2	93	3.48	sabinene	monoterpene	Standard, Library	**2 (12.33)**
6	7.773	977	93	3.64	(1S)-(-)-β-pinene	monoterpene	Standard, Library	9 (10.56)
7	8.196	991	93	4.08	myrcene	monoterpene	Standard, Library	16 (7.91)
8	8.311	994.8	105	3.17	mesitylene	alkylbenzene	NA	12 (9.78)
9	8.759	1007	67	3.87	(*Z*)-3-hexenyl acetate	GLV	Standard, Library	21 (6.83)
10	8.963	1013.8	43	3.68	hexyl acetate	GLV	Standard, Library	38 (3.18)
11	9.069	1016.3	43	3.61	(*E*)-2-hexenyl acetate	GLV	Standard, Library	35 (3.87)
12	9.134	1016.8	121	5.08	α-terpinene	monoterpene	Standard, Library	48 (1.97)
13	9.404	1024.4	119	2.40	*p*-cymene	monoterpene	Standard, Library	51 (0.71)
14	9.547	1028.1	68	6.28	(*R*)-(+)-limonene	monoterpene	Standard, Library	**7 (10.92)**
15	9.683	1031.7	79	5.28	benzyl alcohol	aromatic alcohol	Standard, Library	50 (0.91)
16	9.881	1036.6	93	4.31	(*Z*)-β-ocimene	monoterpene	Standard, Library	10 (10.09)
17	10.296	1046.9	93	4.94	(*E*)-β-ocimene	monoterpene	Standard, Library	17 (7.88)
18	12.108	1095	105	3.23	methyl benzoate	aromatic ester	Library	**5 (11.38)**
19	12.296	1100	93	8.70	linalool	monoterpene	Standard, Library	29 (4.34)
20	12.484	1104.5	57	8.65	n-nonanal	alkyl aldehyde	Standard, Library	25 (5.68)
21	13.002	1116.7	69	2.99	nonatriene (DMNT)	homoterpene	Standard, Library	11 (9.95)
22	14.142	1143.4	67	4.34	(*Z*)-3-hexenyl isobutyrate	GLV ester	Standard, Library	15 (8.55)
23	15.966	1186.6	67	4.31	(*Z*)-3-hexenyl butyrate	GLV ester	Standard, Library	28 (4.52)

Peak	RT (min)	RI	Target Ion	RF	Tentative ID	Category	Identification	Rank (MDA)*
24	16.294	1194.4	110	9.12	RI 1194.4	NA	NA	53 (0.55)
25	16.288	1195	120	3.47	methyl salicylate	phenylpropanoid	Standard, Library	36 (3.66)
26	16.525	1200	57	4.53	dodecane	alkane	Standard, Library	54 (0.10)
27	17.949	1233	82	4.49	(Z)-3-hexenyl isovalerate	GLV ester	Library	27 (5.04)
28	19.229	1262.5	133	2.95	*p*-ethylacetophenone	aromatic ketone	Library	59 (-1.96)
29	19.593	1270.9	69	4.47	geranial	monoterpene	Standard, Library	42 (2.41)
30	19.565	1271.4	120	2.67	ethyl salicylate	phenylpropanoid	Standard, Library	32 (4.02)
31	20.493	1292	117	2.41	indole	aromatic	Standard, Library	39 (2.95)
32	21.909	1325	82	4.80	(*Z*)-3-hexenyl tiglate	GLV ester	Standard, Library	22 (6.53)
33	22.968	1350.3	161	7.13	*α*-cubebene	sesquiterpene	Standard, Library	44 (2.17)
34	23.613	1366	161	11.85	*α*-ylangene	sesquiterpene	Library	55 (<0.01)
35	23.680	1367.4	81	6.78	RI 1367.4	NA	NA	31 (4.22)
36	24.073	1378.3	161	7.84	*α*-copaene	sesquiterpene	Standard, Library	14 (8.56)
37	24.967	1397.8	81	6.86	RI 1397.8	NA	NA	33 (3.99)
38	25.263	1404.9	178	4.91	methyl eugenol	phenylpropanoid	Library	52 (0.58)
39	25.378	1407.2	93	14.13	*cis*-caryophyllene	sesquiterpene	Library	41 (2.56)
40	25.886	1420	93	14.22	*β*-caryophyllene	sesquiterpene	Standard, Library	**1 (13.74)**
41	26.274	1429.8	161	4.84	*β*-copaene	sesquiterpene	Library	26 (5.39)
42	26.544	1436.6	119	6.46	*α*-*trans*-bergamotene	sesquiterpene	Library	**3 (11.68)**
43	26.844	1444	69	7.74	sesquisabinene B	sesquiterpene	Library	46 (2.10)
44	27.233	1453.5	151	23.01	geranyl acetone	monoterpene	Library	40 (2.73)
45	27.259	1453.9	93	4.71	*α*-humulene	sesquiterpene	Standard, Library	18 (7.60)
46	27.403	1458.1	69	5.66	(*E*)-*β*-farnesene	sesquiterpene	Standard, Library	57 (-0.67)
47	28.205	1477.4	161	6.97	*γ*-muurolene	sesquiterpene	Library	43 (2.20)
48	28.380	1481.6	161	6.45	germacrene D	sesquiterpene	Standard, Library	20 (6.84)
49	28.633	1488.1	161	15.84	eremophilene	sesquiterpene	Library	19 (6.92)
50	28.955	1495.8	93	9.90	(Z,*E*)-*α*-farnesene	sesquiterpene	Standard, Library	45 (2.13)
51	29.154	1501.1	105	6.49	*α*-muurolene	sesquiterpene	Library	48 (1.73)

Peak	RT (min)	RI	Target Ion	RF	Tentative ID	Category	Identification	Rank (MDA)*
52	29.475	1510.9	93	7.79	(*E*,*E*)-*α*-farnesene	sesquiterpene	Standard, Library	37 (3.65)
53	29.694	1517.2	161	6.07	*γ*-cadinene	sesquiterpene	Library	**6 (11.02)**
54	30.067	1528.4	161	7.79	*δ*-cadinene	sesquiterpene	Library	23 (5.88)
55	31.526	1573.5	69	8.04	(*E*)-nerolidol	sesquiterpene	Standard, Library	13 (9.75)
56	31.715	1579.2	105	5.07	(*Z*)-3-hexenyl benzoate	ester	Standard, Library	34 (3.92)
57	32.038	1589.2	79	19.29	caryophyllene oxide	sesquiterpene	Library	**4 (11.52)**
58	33.832	1695.5	108	16.70	RI 1696.5	sesquiterpene	NA	49 (1.41)
59	36.813	2092.5	255	7.39	abietatriene	diterpenoid	Library	**8 (10.87)**

*Mean decrease in accuracy (MDA) from Random Forest analysis, higher values are more important for the accuracy of the model. The eight most important are bolded, determined by model selection (VarSelRF, R), while the ten compounds of least importance are underlined, each contributing to <1% of model accuracy.

Table 3.2. Overall statistical differences of PERMANOVAs of chemical groups emitted by five hosts of emerald ash borer (*Agrilus planipennis*); black ash (BA, *Fraxinus nigra*), Manchurian ash (MA, *F. mandshurica*), blue ash (Blue, *F. quadrangulata*), white fringetree (WF, *Chionanthus virginicus*), and olive (OL, *Olea europaea*).

Volatiles	BA	Blue	MA	WF	OL
Overall	A	A	AB	B	C
GLVs	A	A	A	A	A
Monoterpenes	AB	A	AB	C	B
Sesquiterpenes	A	A	B	C	D
AA	A	A	AB	A	B

Table 3.3. Canopy emissions (mean±1SE ng/hour/g of foliage) among five hosts of emerald ash borer (*Agrilus planipennis*); black ash (*Fraxinus nigra*), Manchurian ash (*F. mandshurica*), blue ash (*F. quadrangulata*), white fringetree (*Chionanthus virginicus*), and olive (*Olea europaea*).

Putative ID	Peak	Category	Black ash	Manchurian ash	Blue ash	White fringetree	Olive	F-value	P-value
(Z)-3-hexen-1-ol[†]	1	GLV	2.29±0.87	5.92±1.9	7.77±6.34	4.3±1.37	3.19±1.96	0.45	0.773
n-hexanol[†¥]	2	GLV	0.21±0.06	0.57±0.2	0.36±0.15	0.63±0.21	0.49±0.13	1.01	0.414
(1S)-(-)-alpha-pinene	3	monoterpene	0.04±0.01b	0.11±0.03b	0.1±0.02b	0.31±0.07a	0.05±0.01b	8.13	<0.001
Benzaldehyde	4	aromatic ald.	0.47±0.17	0.79±0.16	0.75±0.13	0.67±0.16	0.98±0.28	0.86	0.497
sabinene	5	monoterpene	0.06±0.04b	ND	ND	0.50±0.15a	0.07±0.01b	6.45	0.006
(1S)-(-)-beta-pinene	6	monoterpene	ND	ND	ND	0.13±0.03	ND		
myrcene	7	monoterpene	1.6±0.8	0.29±0.06	ND	0.68±0.22	0.97±0.60	0.99	0.407
mesitylene	8	alkylbenzene	0.05±0.01c	0.13±0.02ab	0.16±0.02a	0.05±0.01c	0.08±0.02bc	8.53	<0.001
(Z)-3-hexenyl.acetate[†¥]	9	GLV	24.45±7.02b	71.39±21.93a	18.89±8.8b	31.41±6.33b	6.28±1.29b	4.60	0.003
hexyl.acetate[†¥]	10	GLV	1.58±0.54	3.16±1.6	0.43±0.15	1.58±0.45	0.71±0.23	1.12	0.362
(E)-2-hexenyl acetate	11	GLV	ND	ND	0.12±0.07	0.35±0.18	ND	1.14	0.318
a-terpinene	12	monoterpene	ND	ND	ND	0.02±0.004	ND		
p-cymene	13	monoterpene	0.01±0.01	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.01	0.48	0.752
(R)-(+)-Limonene	14	monoterpene	ND	ND	ND	0.38±0.11	0.11±0.02	5.17	0.036

Benzyl alcohol	15	aromatic alcohol	0.25±0.1c	0.23±0.04c	0.16±0.04c	1.19±0.42a	0.99±0.45ab	2.50	0.049
Putative ID	Peak	Category	Black ash	Manchurian ash	Blue ash	White fringetree	Olive	F-value	P-value
(Z)-b-ocimene	16	monoterpene	37.69±17.92a	0.7±0.32b	ND	0.1±0.03b	10.14±6.55b	4.50	0.009
(E)-b-ocimene†	17	monoterpene	85.48±45.73a	8.29±3.8b	2.16±1.48b	1.28±0.35b	47.23±24.35ab	2.50	0.049
Methyl benzoate	18	aromatic ester	0.01±0.003b	ND	0.10±0.03b	1.05±0.48a	0.08±0.04b	3.30	0.032
linalool†	19	monoterpene	0.14±0.08	0.55±0.23	ND	0.39±0.11	0.39±0.12	1.61	0.213
nonanal†	20	alkyl aldehyde	1.06±0.21b	2.55±0.69b	5.03±1.68ab	2.60±0.75b	9.12±2.32a	4.80	0.003
4,8-dimethyl-1,3,7-nonatriene.(DMNT)†	21	homoterpene	0.19±0.09b	27.08±12.2a	0.73±0.32b	3.47±1.69b	1.04±0.35b	3.04	0.029
(Z)-3-hexenyl isobutyrate	22	GLV ester	0.03±0.01b	ND	ND	0.40±0.15a	0.03±0.01b	3.87	0.037
(Z)-3-hexenyl butyrate	23	GLV ester	0.24±0.16	0.15±0.07	0.2±0.13	0.66±0.31	0.65±0.29	1.14	0.350
RI.1194.4	24	NA	0.22±0.11	0.21±0.06	0.33±0.12	0.10±0.04	0.24±0.08	0.46	0.767
Methyl salicylate	25	phenylpropanoid	0.27±0.07	1.26±0.64	1.08±0.43	2.76±1.31	1.63±0.74	0.90	0.473
Dodecane	26	alkane	0.64±0.14	1.15±0.27	1.43±0.44	0.58±0.11	0.54±0.07	2.49	0.056
(Z)-3-hexenyl.isovalerate	27	GLV ester	0.06±0.02b	0.11±0.06b	0.1±0.04b	1.34±0.46a	0.39±0.21b	3.59	0.014

Putative ID	Peak	Category	Black ash	Manchurian ash	Blue ash	White fringetree	Olive	F-value	P-value
p-ethylacetophenone	28	aromatic ketone	0.72±0.18	1.77±0.52	1.73±0.74	0.81±0.25	0.47±0.0	1.97	0.114
Geranial	29	monoterpene	ND	0.08±0.04	ND	ND	ND		
Ethyl salicylate	30	phenylpropanoid	ND	0.28±0.22	ND	0.25±0.15	0.003±0.001	0.61	0.561
Indole	31	aromatic	ND	0.35±0.16	ND	ND	ND		
(Z)-3-hexenyl tiglate	32	GLV ester	0.1±0.04	0.38±0.14	ND	0.15±0.06	0.02±0.01	1.57	0.224
a-cubebene$^{\lambda}$	33	sesquiterpene	ND	ND	ND	0.36±0.18	0.03±0.01	3.43	0.097
a-yanglene	34	sesquiterpene	ND	ND	ND	ND	0.05±0.02		
RI.1367.4	35	NA	0.37±0.21	0.31±0.09	0.56±0.12	0.18±0.03	0.45±0.18	0.87	0.486
a-copaene$^{\lambda}$	36	sesquiterpene	ND	1.38±1.19	ND	0.69±0.4	0.59±0.22	0.75	0.566
RI.1397.8	37	NA	0.75±0.39	0.63±0.2	1.23±0.23	0.47±0.09	1.04±0.37	1.03	0.404
Methyl eugenol	38	phenylpropanoid	ND	ND	ND	0.12±0.06	ND		
cis-caryophyllene	39	sesquiterpene	ND	1.36±0.66	ND	ND	0.09±0.03	3.58	0.091
b-caryophyllene$^{\lambda}$	40	sesquiterpene	ND	38.22±17.78a	ND	2.14±1.35b	7.53±1.59b	3.48	0.044
b-copaene	41	sesquiterpene	ND	ND	ND	ND	0.10±0.03		
a-trans-bergamotene	42	sesquiterpene	ND	ND	ND	0.26±0.13	0.17±0.03	0.46	0.507
Sesquisabinene	43	sesquiterpene	ND	ND	ND	ND	0.09±0.03		

Putative ID	Peak	Category	Black ash	Manchurian ash	Blue ash	White fringetree	Olive	F-value	P-value
Geranyl acetone	44	monoterpene	0.18±0.12	ND	0.37±0.2	0.28±0.09	0.11±0.04	1.01	0.401
a-humulene[λ]	45	sesquiterpene	ND	4.51±2.1	ND	1.81±1.46	0.86±0.19	1.79	0.188
(E)-b-farnesene[†]	46	sesquiterpene	ND	ND	ND	0.13±0.09	0.03±0.01	0.54	0.489
Gamma-cadinene, y-muurolene	47	sesquiterpene	ND	ND	ND	ND	0.06±0.02		
Germacrene D	48	sesquiterpene	ND	1.78±1.33	ND	0.89±0.65	1.96±0.52	0.43	0.654
Eremophilene	49	sesquiterpene	ND	ND	ND	ND	0.39±0.10		
(Z,E)-a-farnesene[†]	50	sesquiterpene	ND	3.22±1.67	ND	0.14±0.06	0.10±0.02	2.10	0.173
a-muurolene	51	sesquiterpene	ND	ND	ND	0.06±0.02	0.11±0.04	0.89	0.371
(E,E)-a-farnesene[†]	52	sesquiterpene	1.47±0.7	9.37±4.47	0.27±0.14	0.91±0.24	1.31±0.34	2.43	0.065
γ-cadinene	53	sesquiterpene	ND	1.1±0.5a	ND	0.75±0.2ab	0.08±0.02b	5.30	0.012
d-cadinene	54	sesquiterpene	ND	1.88±1.34	ND	0.89±0.58	0.19±0.04	1.58	0.226
(E)-nerolidol	55	sesquiterpene	ND	0.38±0.19	ND	0.54±0.26	ND	0.22	0.641
(Z)-3-hexenyl benzoate	56	ester	0.19±0.07	0.66±0.2	0.12±0.04	2.23±1.04	0.17±0.07	2.40	0.067
Caryophyllene oxide	57	sesquiterpene	ND	4.65±2.1	ND	0.44±0.35	1.95±0.44	1.79	0.188
RI.1696.5[€]	58	sesquiterpene	ND	ND	ND	ND	1.36±1.10		
Abietatriene	59	diterpenoid	0.02±0.01	0.15±0.05	ND	ND	ND	3.51	0.08

[†¥λ] Volatiles known to elicit antennal reactions of adult emerald ash borer. [€]Unknown and co-eluted peaks. ND= No detection

Chapter 4: Attack dynamics and impacts of emerald ash borer on wild white fringetree populations.

Abstract:

North American forests have been heavily impacted from the loss of ash trees (*Fraxinus* spp.) due to attack by emerald ash borer (EAB, *Agrilus planipennis*). In 2014, ornamental white fringetrees were found attacked by EAB in southwestern Ohio. Since then, dozens of ornamental trees have been found infested across several states in the lower Midwest, mostly outside of the native range of this tree. Previous surveys at ornamental sites have also been conducted retrospectively, so we aimed to examine wild populations of white fringetree before and throughout the attack wave to determine what factors may predict attack on these trees. We monitored populations of white fringetrees and white ash (*F. americana*) at three sites in southeastern Ohio from 2015-2019. We assessed trees for their size, attack rates, epicormic branching, and canopy dieback, and trapped beetles to track the presence and density of EAB populations throughout the years. We found no evidence of EAB in the initial year of monitoring in 2015 in any of our sites on either ash or white fringetrees. By 2016, EAB had attacked some white ash at two sites and in 2017, the first exit holes and galleries of EAB were confirmed on white fringetrees at our Vinton site. For those fringetrees that eventually were attacked (19-30% at two sites), they were more likely to display epicormic branching, a symptom of stress in plants before being infested by EAB. This data suggests that EAB attacks stressed white fringetrees over healthy plants. At our third site, only small white fringetrees were present and showed no signs of EAB. The white ash also had no EAB signs, but beetles

were captured the final two years in traps in a nearby ash tree indicating that ash and white fringetrees were likely to be attacked soon. During the EAB attack wave, white ash trees were attacked heavily and all mature overstory trees were killed. We caught more beetles in traps hung in ash trees than white fringetrees. White fringetrees were attacked in a manner that reflected beetle densities; when EAB densities were high, attack rates were high and as beetle levels declined so did the infestation of these trees. Other variables measured were not significant for predicting EAB attack besides canopy dieback and epicormic branching with higher degrees of these stress responses being more likely to be attacked. While no trees were killed by attack, those white fringetrees that were infested were stressed suggesting that EAB prefers or is at least more successful on stressed hosts as observed with ancestral hosts.

Introduction:

Invasive herbivores cost billions of dollars per year in economic damage (Pimentel et al. 2005), with forest pests alone causing an estimated 1.7 billion dollars annually (Aukema et al. 2011). Extensive ecological damage such as severe injury or death to trees can have small scale impacts such as local extirpation of specialist herbivorous insects (e.g. Gandhi and Herms 2010a, Wagner and Todd 2016) or ecosystem wide changes such as gap formations and influencing biogeochemical cycling of nutrients (Gandhi and Herms 2010b). The impacts of invasive herbivores could be magnified if they expand their host range to novel hosts, a process that can occur by ecological fitting. This concept is when a herbivore can detect and utilize a novel host due to morphological and biochemical similarities to historic hosts, often due to their close relatedness (Janzen 1985, Agosta 2006).

Buprestids are an emerging group of pests that are known historically as secondary pests, those that attack and kill stressed hosts. However, over the last several decades, these beetles have increasingly been observed causing major damage to evolutionarily naïve hosts. Bronze birch borer (*Agrilus anxius*), for example, started killing healthy Eurasian birch trees (*Betula* spp.; Muilenburg and Herms 2012) when these plants were introduced to North America. Similarly, gold spotted oak borer (*A. coxalis*) was discovered in the 2000s attacking and killing healthy oak trees in southern California (Coleman and Seybold 2008). Emerald ash borer (EAB), *Agrilus planipennis* is a prime example of the destruction that this beetle family can cause. From eastern Asia, EAB was accidentally introduced to North America where it kills evolutionarily naïve ash hosts (*Fraxinus* spp.: Oleaceae; Cappaert et al. 2005), and has since become one of the most expensive forest pests (Kovacs et al. 2010, Aukema et al. 2011). In Asia, EAB is a secondary pest and only attacks and kills stressed Manchurian (*Fraxinus mandshurica*) and Chinese ash (*F. chinensis*) by girdling the plant via larval feeding on the phloem. In North America, EAB attacks and readily kills >99% of most healthy North American ash trees (Klooster et al. 2014).

In 2014, EAB was found attacking white fringetree (*Chionanthus virginicus*: Oleaceae), the first recorded non-ash host. This host range expansion was surprising because EAB was thought to be a specialist of ash trees, but white fringetree is closely related to ash trees (Wallander and Albert 2000). The extent to which these beetles will damage fringetree is of concern because EAB is so lethal to evolutionarily naïve hosts. The first few studies were conducted on ornamental populations in the lower Midwest, outside the native range of this tree. The average attack rates of fringetrees were 19-26% (Cipollini 2015, Peterson and Cipollini 2017), with infestation rates varying among sites (4-75%). Variation in attack rates is likely due to differences in EAB densities, proximity to ash hosts, and whether or not the plants were stressed (Peterson and Cipollini 2017). Mortality rates are drastically lower (~1%) on white fringetrees than North American ash species of similar sizes in common garden studies (>75%; Rebek et al. 2008, Herms 2014, Tanis and McCullough 2015, Peterson and Cipollini 2017).

Peterson and Cipollini (2017) provide some insights of EAB impact on fringetree, but that research was retrospective, only providing a snapshot of attack during or after the EAB attack wave. More recently, sites from Peterson and Cipollini (2017) were reevaluated and found that attack rates on fringetrees decreased in the years following peak EAB densities (Ellison et al. in review). The authors also found that stressed trees were more likely to be attacked, as did Peterson and Cipollini (2017). The attack rates of EAB during or after peak populations appear to be low in contrast to ash trees, but initial attacks and the impact on wild populations have yet to be revealed. One major question, not answered in previous studies, is when does EAB begin to attack white fringetree in relation to ash trees. A dendrochronological study indicated white fringetrees get infested by EAB within a few years of ash tree infestation (Thiemann et al. 2016), but dynamics that lead to initial EAB attack on white fringetree have not been studied. Another area of interest is whether attack rates and impacts vary in wild white fringetree populations compared to those observed in ornamental sites from previous studies. In ornamental trees, attack rates and impacts may be affected by human management such as the pruning of dead branches, fertilization, and watering. Furthermore, most ornamental trees that we have observed are grown in optimal conditions in open, sunny areas (DLP per. observation) at low densities which reduces competition for sunlight, moisture, and soil nutrients. In contrast, wild fringetrees are unmanaged and typically grow in dense groups competing for light, space, and nutrients in the understory and could make plants more susceptible to attack (e.g. Folgarait et al. 1995). In one wild population growing under these conditions, white fringetrees were attacked at relatively higher rates (~50%; DLP unpublished data) compared to those in ornamental landscapes (13-26%; Peterson and Cipollini 2017, Cipollini and Peterson 2018, Ellison et al. in review) indicating that wild fringetrees may be more impacted by EAB.

The signs of EAB attack in white fringetree are the same as in ash and include D-shaped exit holes, presence of larvae and/or adults, and serpentine larval feeding galleries containing larval frass (Peterson and Cipollini 2017). Symptoms of EAB infestation in ash include crown dieback, epicormic branching, bark splits, and woodpecker holes (Cappaert et al. 2005, Gould et al. 2015). All of these signs and symptoms have been observed on white fringetree (Cipollini 2015, Cipollini and Rigsby 2015, Peterson and Cipollini 2017). Crown dieback and epicormic branching are both plant responses that can be caused by various agents, such as insects, pathogens, or abiotic stress (e.g. drought (Matusick et al. 2012)). The presence of epicormic branching and higher rates of dieback are two variables that are associated with EAB attack in white fringetree (Peterson and Cipollini 2017), but in retrospective studies, it is unclear if these two factors were caused by previous infestation by larvae or other mechanisms. Other variables, such as plant sex and proximity to other white fringetrees, were not predictive of EAB attack (Peterson and Cipollini 2017), but infested white fringetrees were on average closer to ash trees than uninfested trees, indicating that distance to ash hosts may affect the likelihood of EAB attack. Large white fringetrees were also more likely to be infested by EAB than smaller individuals, which could be due to higher volatile emissions than their smaller counterparts and higher amounts of available phloem for larvae as postulated for ash (McCullough and Siegert 2007).

The goal of this research was to determine the dynamics and impacts of EAB attack on wild populations of white fringetrees. Our first objective was to determine the timing of EAB attack on white fringetree relative to white ash trees. We hypothesized that EAB will infest white fringetree within a couple of years of attack on ash trees as observed in dendrochronological studies (Thiemann et al. 2016). A second objective was to determine the incidence of infestation and to examine the influence of factors including epicormic sprouting, crown dieback, tree size, and ash tree adjacency on attack rates (Peterson and Cipollini 2017). We selected three sites that did not have EAB present at the beginning of this study providing the opportunity to determine what factors are important for determining attack on white fringetrees. We postulated that stressed plants, displaying epicormic sprouting and crown dieback, will be more likely to be attacked by EAB. Additionally, fringetrees closer to ash trees and larger white fringetrees were predicted to be attacked over those that are farther way from ash or that are small in size. Another objective was to compare the impact of EAB on attacked fringetrees compared to unattacked plants specifically whether they increase canopy dieback and cause epicormic sprouting. Our last hypothesis was attack rates in white fringetree populations would be higher in wild populations than those observed in ornamental populations in previous studies (Peterson and Cipollini 2017, Ellison et al. in review) due to the higher density of plants and the lack of management.

Materials and Methods:

White fringetree sites:

Three wild populations of white fringetree were monitored beginning in 2015 in southeastern Ohio: Vinton, Jackson, and Shawnee. Vinton Furnace State Experimental Forest is owned by the Ohio Division of Forestry and comanaged by the US Forest Service Northern Research Station. This property consists of 486 ha of upland mixed oak forest with chestnut oaks (*Quercus montana*) on the ridgetops. The white fringetrees (n=33; *Chionanthus virginicus*) at Vinton were understory shrubs ranging from one to three meters in height. Jackson is a private property near Jackson, Ohio. The land was historically used as pasture and has been recolonized by the forest with the overstory consisting of over mature Virginia pine (*Pinus virginiana*) and mixed broadleaf trees on a southeast facing hillside. The white fringetrees (n=31) were previously in shaded conditions, but a series of strong wind storms occurred 2-3 years before 2015 creating canopy gaps exposing the plants to increased sunlight. Shawnee, near Shawnee State Forest, consists of two smaller populations of white fringetrees on private property within two miles of each other with all 13 individuals growing in a shaded understory. Populations of fringetrees at Vinton and Jackson were in an area of 0.2 ha while the trees at Shawnee were more spread out in an area of 1.2 ha. The overstory consisted of red maple (*Acer rubrum*), white ash (*Fraxinus americana*), and oaks (*Quercus* spp.) with a mixture of other broadleaf trees. White ash trees at Shawnee (n=4), Vinton (n=16), and Jackson (n=5) growing among and within 50 m of the white fringetree populations were monitored along with the white fringetrees. No visual signs of EAB were detected at these sites in 2015, but detections had been found within 1,500 m at Vinton and 500 m at Jackson, so beetles were likely at or near these sites, but at low levels.

Field examinations:

White fringetrees and white ash trees were examined for signs and symptoms of EAB infestation each year 2015-2019 in early August. In 2015 we conducted the work in early September. Presence of EAB was determined by searching trees for D-shaped exit holes or serpentine galleries. On the final year of surveying (2019), we removed small areas of bark tissue with wood chisels to reveal feeding larvae or galleries over suspected areas. These areas are bark swellings caused by larval feeding in the phloem of trees and have been used in previous studies to positively detect EAB (Thiemann et al. 2016, Peterson and Cipollini 2017). The limited bark removal caused superficial wounds damaging small areas on trees with caution taken to prevent mortality of branches. This technique can reveal both galleries bored in the current year and galleries created years prior up to 4-5 years (Thiemann et al. 2016), all of which were recorded. Since not all of the bark could be explored in this way, our determination of EAB attack on fringetrees is conservative. Diameter of ash trees were measured at 1.3 m above ground while the largest stem of each white fringetree was measured at 10 cm above the soil line. White fringetrees are measured in this way because they have multiple stems and branches tend to split several times at or near 1.3 m. Crowns of all trees were rated on the amount of dieback that they exhibited using the following rating system, 1 = alive, 0-12% dieback; 2 = ~13-37% dieback, 3 = ~38-62% dieback, 4 = ~63-87% dieback, and 5 =~88-100%, dead, based on the rating system used for EAB infestation in ash trees (Smith 2006, Gould et al. 2015) and white fringetrees (Peterson and Cipollini 2017). For white fringetrees, presence or absence of epicormic sprouts was recorded. For each white fringetree, distance to the nearest ash tree, as described in Peterson and Cipollini (2017) was measured, but all of the fringetrees were within 50 m, so this variable was removed from analyses due to no differences in ash distance from fringetrees.

At each site, we monitored for the presence and abundance of EAB in white ash and white fringetrees with purple prism traps starting in 2016. Traps (Great Lakes IPM, Vestaburg, MI, USA) were baited with Manuka oil (Synergy Semiochemicals Corp., Delta, BC, Canada) and were deployed each year in mid-May, approximately near the beginning of EAB emergence (~230 DD_{10}). A prism trap was placed in an ash tree and one in a white fringetree at each site and collected in early August at the time of tree examinations. The total number of beetles were counted on each trap each year.

Statistical analysis:

Data from the field was analyzed using SAS (SAS Studio, SAS Institute Inc., Cary, NC). Fisher's Exact tests (PROC FREQ) were used to compare trap counts among sites and years across individual sites, attack rates of white ash and white fringetrees among sites, across all sites, and among overstory and understory ash at Vinton. PROC LOGISTIC was used to run two binary regressions. The first regression was used to examine the influence of the presence of epicormic sprouts (yes or no), basal diameter of the largest stem (proxy for plant size), crown dieback, site, and year on the likelihood that white fringetrees would be attacked by EAB (yes or no). A second regression was used to determine what variables were important for eventual EAB attack by using data from 2015, the conditions before EAB arrived at all sites. Stepwise, forward, and backward model selection were used and the model with the lowest Akaike information criterion (AIC) was presented. For basal stem diameter of fringetree, we only used measurements from 2015 due to inconsistency of measuring at 10 cm off the ground among years, and because stems varied little over this time period. T-tests using PROC TTEST were used to compare mean basal stem diameter, epicormic branching, and canopy dieback between attacked and unattacked white fringetrees. Welch's T-tests were used because sample sizes were unequal.

Results:

Trap counts. Trap capture of EAB varied among years and sites (Table 4.1) with the highest number caught in the first year of trapping in 2016 at both the Vinton Furnace and Jackson sites. Captures then declined over time at those sites through 2019. Our Shawnee site caught a couple of beetles the first year, then no captures until 2018 and 2019, which were the highest capture rates for this site; all were caught in ash tree traps (Table 4.1). Among years, the pattern for the beetle captures were similar with more on white ash and fewer on white fringetree at Vinton Forest (χ^2= 2.20; df= 3; p= 0.816), Jackson (χ^2= 2.59; df= 3; p= 0.789), and Shawnee. At all sites, there was a significantly higher proportion of beetles caught on traps placed in ash trees (0.90) compared to traps in white fringetrees (0.10; χ^2= 6.50; df= 2; p= 0.043). When we compared the proportion of beetles in white ash traps among sites, a higher proportion of traps caught EAB at Shawnee (1.00) than at Vinton (0.84; χ^2= 5.51; df= 1; p= 0.027). No beetles were caught on traps placed in the small white fringetrees at Shawnee.

Emerald ash borer attack rates. Attack rates by EAB on white ash were significantly higher than those on white fringetree over time for Vinton (Table 4.1; χ^2= 67.65; df= 1; p= <0.001) and Jackson (χ^2= 23.66; df= 1; p= <0.001). Cumulative rates of attack by the end of 2019 on white ash were significantly higher than on white fringetrees across sites (χ^2= 18.90; df= 1; p= <0.001). Among sites, no white fringetrees were attacked at Shawnee, but EAB attack was the same rate at Vinton and Jackson (χ^2= 0.91; df= 1; p= 0.410). White ash trees were most abundant at our Vinton site, 14 white ash in total were monitored with both larger, overstory plants (n= 9) and smaller understory plants (n=5). Of ash trees in the understory, they were attacked (n=2) at the same rate (40%) as white fringetrees (Table 4.1; χ^2= 1.33; df= 1; p= 0.337) by the end of the experiment. By the end of 2019, all of the overstory white ash were attacked and killed by EAB, a significantly higher rate than the one understory white ash that was killed (χ^2= 10.08; df= 1; p = 0.005). Jackson had four ash trees that were monitored and all were attacked by 2019 with one overstory ash being killed by EAB and a second one displayed a high degree of dieback (50%) caused by EAB damage. Shawnee differed from the other two sites with none of the ash trees being attacked by EAB.

Binary regression. Binary regression with the lowest AIC value was forward selected for both regressions. For the full set of data from 2016-2019, the model included year, white fringetree epicormic branching, and white fringetree dieback. Epicormic branching was significant (Table 4.2) with fringetrees showing this plant response being 6.02 times more likely to be infested with EAB than a plant with no branching. Dieback on white fringetrees was also found to be significant (Table 4.2) with plants being 9.84 times more likely to be infested with EAB for each 25% loss of canopy. The second model included site, canopy dieback of white fringetree, stem size, and epicormic branching (Table 4.3). The only significant variable was epicormic branching with fringetrees being 8.13 times more likely to be infested if they displayed epicormic branches.

Comparisons of variables among white fringetrees and white ash. Overall, attacked white fringetrees displayed a significantly higher percentage (Table 4.3) of epicormic branching compared to unattacked fringetrees. A similar pattern was observed for canopy dieback with average dieback being significantly higher in attacked than in unattacked white fringetree (Table 3). Average diameter between attacked and unattacked trees was the same overall and for each year. Among attacked white fringetrees, those that were found to have exit holes displayed a higher proportion of canopy dieback, 0.46±0.08 (t = 2.22; df= 17; p = 0.040) in contrast with 0.18±0.08 in those that had EAB galleries, but no exit holes. White ash trees were most abundant at Vinton, providing a chance to compare diameters of overstory ash trees which were significantly larger (17.1±2.4 cm) than white fringetrees (4.3±0.3 cm, t = 9.33; df= 40; p = <0.001)

Discussion:

Emerald ash borer (EAB; *Agrilus planipennis*) is a destructive pest on North American ash (*Fraxinus* spp.; Herms and McCullough 2014). When EAB was found attacking white fringetree (*Chionanthus virgincus*) in 2014 (Cipollini 2015), there was concern that a new North American native plant species had the potential of being adversely impacted like susceptible ash trees. Ornamental white fringetrees are attacked at low rates (13%; Ellison et al. in review) years after peak beetle levels compared to during the peak of the attack wave of 26% of trees (Peterson and Cipollini 2017). Together, these studies provided evidence that EAB attack (observed only during and after) the invasion wave has a lower impact on fringetree than susceptible ash trees, but these observations were found at ornamental sites mostly outside of the native range of this plant species. Furthermore, these studies were retrospective, and other questions remained to be answered such as when does EAB begin to attack white fringetrees in relation to ash trees, what tree or site factors predict attack, and how do attack dynamics change through time. To address these questions, we monitored three sites harboring wild populations of white fringetree over time from the beginning to the end of the EAB attack wave .

Attack rates of EAB on white fringetrees are low in contrast to susceptible ash trees of comparable sizes (Rebek et al. 2008, Herms 2014, Cipollini 2015, Peterson and Cipollini 2017). We observed that EAB attacked ash trees at higher rates than nearby white fringetrees at two of our sites. At Vinton, all of the overstory white ash were killed compared to only 20% of the smaller ash trees in the understory. These understory ash trees are of a size similar to white fringetrees and experience the same growing conditions. If we compare the attack rates of EAB only among understory ash and white fringetree, then cumulative infestation rates were not significantly different (40% vs 30% of trees, respectively). These results suggest that EAB is attracted to and attacks white ash and white fringetree at similar degrees, when size and growing conditions are the same. Support for this assertion has been found in olfactometer assays (Peterson et al. in review) of female beetle attraction to volatile emissions of white fringetree and green ash (*F. pennsylvanica*), a close relative of white ash (Wallander 2008). Both ash species are highly preferred by adults for feeding and oviposition (Pureswaran and Poland 2009, Rigsby et al. 2014). In the olfactometer assay, gravid EAB females were found to choose ash and fringetree at the same rate (Peterson et al. in review). We also observed that EAB attacked white fringetree within one or two years after the larger ash trees were already attacked at Vinton and Jackson, respectively, supporting previous dendrochronological research (Thiemann et al. 2016). White fringetrees appear to be attacked quickly upon detection by EAB and not when ash hosts are exhausted.

To better understand the attack dynamics, EAB populations were tracked through time using purple prism traps baited with Manuka oil, an attractive lure for EAB (Crook et al. 2008). Overall, ~90% of EAB were caught on traps in white ash with our site at Vinton having the highest capture of EAB. This finding was likely due to the higher densities of ash trees at this site. Captures were high in 2016 on traps hung in white ash at Vinton and decreased over time suggesting that EAB were indeed present in 2015, the first year of the inventory when attack on trees was not yet apparent. Captures of beetles decreases with an increase in symptoms and mortality rates on white ash, which is expected since as ash trees are killed by beetles, they have fewer hosts and therefore disperse to find living hosts (Siegert et al. 2010). There appears to be a “spillover effect” during peak populations of EAB. When EAB are in high numbers due to abundant ash trees, more eggs are likely laid on fringetrees by female adults. Fall cankerworm (*Alsophila pometaria*) is an example of this "spillover," where outbreaks lead to more caterpillar damage on a more resistant host, cottonwoods (*Populus angustifolia* × *P. fremontii*) due to higher numbers of larvae (White and Whitham 2000). Only 10% of EAB were caught on traps in white fringetree with Vinton having the highest captures, followed by Jackson. White fringetree at Vinton also had decreasing captures of EAB over time corresponding with decreased attack rates. These results indicate that EAB attack on white fringetree corresponds with beetle population densities in the vicinity of the trees and, therefore, fringetree may be more heavily attacked when ash density is high in an area. This pattern was also observed at Jackson, where low ash densities likely led to lower beetle densities. We monitored only four ash trees at this location and only one was killed by the end of 2019. Beetle captures were lower in white ash traps in 2016, rose in 2017, and fell the final two years at Jackson. At both Vinton and Jackson, ash trees were first observed to be attacked by EAB in 2016, but the first observed attack on fringetree was in 2017 at Vinton and in 2018 at Jackson, in accordance with beetle densities at these sites. Our final site, Shawnee, had no captures of beetles on fringetree, and EAB only appeared in abundance in the final two years of the study. If monitoring had continued for one or two years at Shawnee, we believe that EAB would have been found attacking fringetrees and captured on traps, as we observed at our two other sites. However, attack rates would likely be lower because the density of fringetrees is low at this site and the tree species were both small.

White fringetrees that are attacked by EAB are more likely to be stressed (Peterson and Cipollini 2017, Ellison et al. in review). Both the presence of epicormic branching and higher rates of canopy dieback were found to be important factors associated with EAB attack on white fringetree in our first regression, and this was also observed in ornamental populations (Peterson and Cipollini 2017). These plant stress responses could have been caused by EAB larvae. Alternatively, these responses could have been caused by other agents that led to increased attraction and susceptibility to EAB (Liu et al. 2003, McCullough et al. 2009). To address these possibilities, we analyzed tree traits from the year before EAB was present to predict the probability of EAB attack by the end of 2019. We found that the presence of epicormic sprouts at the start of monitoring increased the likelihood that fringetrees would be attacked indicating that EAB prefers attacking stressed white fringetree over healthy hosts which is similarly observed in ash trees (Liu et al. 2003, Cappaert et al. 2005, McCullough et al. 2009). Canopy dieback was not a significant predictor of attack and was likely due to very few white fringetrees had canopy thinning at the start of the experiment. Supporting that EAB are more successful on stressed hosts, Peterson and Cipollini (in review) found that larval survival and performance was highest on mechanically girdled or previously attacked white fringetrees. In our study, we recorded attack of white fringetrees by the presence of larval galleries or adult exit holes. With one exception, no larva reached adulthood on stems in which we found only larval galleries, larvae in these trees were small and died within the stem. The exception was for one larva that had reached a prepupal stage, but was killed by a woodpecker. We found exit holes on several white fringetrees demonstrating that beetles can succeed on some trees as was also demonstrated in ornamental plants (Cipollini 2015, Peterson and Cipollini 2017). Fringetrees with exit holes displayed a higher degree of canopy dieback compared to those that only had larval galleries, indicating that EAB perform better on stressed hosts and that larvae significantly damage the phloem and cause canopy thinning. The fringetrees that were attacked but with no exit holes were healthier suggesting that larvae die in healthy white fringetrees as observed in Peterson and Cipollini (in review). Besides stress factors, larger white fringetrees have been found to be attacked more frequently (Peterson and Cipollini 2017), but there was a lack of support for this in our study which could be due to lower variation of diameter sizes in contrast to Peterson and Cipollini (2017). Yet, size could be important since fringetrees in our study are smaller than overstory ash trees and most beetles were caught in white ash. The preference for larger trees is likely due to the higher quantities of volatiles that are emitted compared to small plants.

In ornamental landscapes, EAB attack corresponded positively with white fringetree density (Peterson and Cipollini 2017). Because of natural seed dispersal patterns, white fringetrees in wild populations tend to grow in more dense groupings than those in ornamental plantings. One large population of naturally growing fringetrees in Pennsylvania had 50% of trees attacked by EAB in 2016 (DLP unpublished data). We hypothesized that wild white fringetree would similarly be attacked at higher rate than those from ornamental sites because populations that were monitored were similar to the PA site. However, the attack rates on white fringetrees at our two sites with EAB appears to be similar to those trees in ornamental sites (Peterson and Cipollini 2017). These data suggest, as mentioned previously, it is more likely that overall EAB populations via high densities of susceptible ash trees increases the attack rates on white fringetrees. At the PA wild population, ash trees were much more abundant than those at our Vinton site and we therefore speculate that EAB populations were much higher at the PA site. As in previous studies (Peterson and Cipollini 2017, Ellison et al. in review), the impact of EAB on white fringetree is low with a lower proportion attacked compared to nearby ash and those trees that were infested displayed epicormic branching and canopy thinning. The epicormic branching was present before EAB arrived and this stress was likely used by adults to select fringetrees. In contrast, canopy thinning is caused by larval feeding since white fringetrees at the beginning of the experiment had mostly full canopies. Beetle capture and attack on white fringetree was highest in a site which had the highest abundance of white ash. As EAB continues to spread farther into the native range of white fringetree, the hypothesis of beetle densities predict attack rates on white fringetree could be tested and additionally it could be determine to whether adults are more attracted to stressed novel hosts as we observed in our study and in ash trees (Liu et al. 2003, McCullough et al. 2009).

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Table 4.1 Study site locations monitored for emerald ash borer (*Agrilus planipennis*) overall and by year attack rates and trap counts by year in wild white fringetrees (*Chionanthus virginicus*) and white ash trees (*Fraxinus americana*) in southern Ohio from 2015-2019.

Site	Year	White ash		White fringetree	
(GPS Coordinates)		Proportion attacked (quantity)	Trap count	Proportion attacked (quantity)	Trap count
Vinton Forest					
39.202408	2015	0.00 (0)	-	0.00 (0)	-
-82.391101	2016	0.36 (5)	27	0.00 (0)	7
	2017	0.64 (9)	15	0.18 (6)	3
	2018	0.79 (11)	6	0.12 (4)	0
	2019	0.86 (12)	3	0.06 (2)	0
	Overall	0.86 (12)	53	0.30 (10)	10
Jackson					
39.085189	2015	0.00 (0)	-	0.00 (0)	-
-82.621573	2016	0.25 (1)	12	0.00 (0)	2
	2017	0.25 (1)	4	0.00 (0)	0
	2018	0.25 (1)	7	0.13 (4)	0
	2019	1.00 (4)	6	0.10 (3)	0
	Overall	1.00 (4)	29	0.19 (6)	2
Shawnee					
38.820956,	2015	0.00	-	0.00	-
-83.169374	2016	0.00	2	0.00	0
38.812384,	2017	0.00	0	0.00	0
-83.136957	2018	0.00	17	0.00	0
	2019	0.00	12	0.00	0
	Overall	0.00	31	0.00	0

Table 4.2**.** Binary regressions of A) 2016-2019 data examining the likelihood of host plant traits in predicting emerald ash borer (EAB, *Agrilus planipennis*) attack on wild white fringetrees (*Chionanthus virginicus*) across three sites in southeastern Ohio, USA and of B) 2015 conditions before EAB arrival at sites and likelihood of host plant traits to predict cumulative EAB attack on white fringetree.

Predictor variable	DF	*P* – value	χ^2 – value
A) First regression			
Crown dieback	1	0.029	4.75
Epicormic branching	1	0.001	10.80
Year	3	0.250	4.08
B) Second regression			
Crown dieback	1	0.240	1.38
Epicormic branching	1	0.022	5.26
Site	2	0.253	2.75
Stem size	1	0.080	3.06

Table 4.3. Characteristics of wild white fringetrees (*Chionanthus virginicus*) either attacked or unattacked by emerald ash borer (*Agrilus planipennis*) from 2016-2019 across three sites in southern Ohio, United States. Numbers represent means ± SE.

Variable	Attacked trees	Unattacked trees	*P*-value
Basal stem diameter (cm)	5.2±0.3	4.5±0.1	0.086
Proportion of canopy dieback	0.36±0.7	0.18±0.01	0.021
Proportion of trees displaying epicormic branching	0.58±0.12	0.23±0.02	0.008

Chapter 5: Summary

Introduction:

A host range expansion of an herbivore begins with an encounter with a novel host. These interactions, for instance, occur with the introduction of a plant or phytophagous insect to a novel range which can be due to international trade (Meyerson and Mooney 2007, Westphal et al. 2008). In order to use these novel hosts, herbivores have the ability of to use a novel host by using already evolved traits due to ecological fitting (Janzen 1985, Agosta 2006, Cipollini and Peterson 2018). Ecological fitting generally occurs when herbivores encounter close relatives of their historic hosts (e.g. Erbilgin et al. 2014), although it can also occur with distantly related plants that are chemically similar (Murphy and Feeny 2006). Due to ecological fitting, phytophagous insects can find and survive on novel hosts, but the historic host is likely the preferred plant to use. However, organisms may be forced to use less preferred hosts which can lead to host switching and eventually a host shift. For example, natural enemies, a biotic mechanism, can kill herbivores at high rates on their historical host, leading to the phytophagous insects to be more successful on novel host and escaping predation as observed in *Chrysomela lapponica* beetles (Zvereva et al. 2010). Similarly, climate change, an abiotic mechanism, can alter precipitation patterns and temperatures affecting host phenology and herbivore synchrony. Potential asynchrony can drive herbivores to use alternative, novel hosts that are present at the time of insect emergence (e.g. Lurgi et al. 2012). Another scenario that may cause host switching by an herbivore could be from the loss of preferred hosts due to extinction or local extirpation. Ash trees (*Fraxinus* spp.) from North America are highly susceptible and preferred hosts (Pureswaran and Poland 2009, Rigsby et al. 2014) by the woodboring beetle, emerald ash borer (EAB, *Agrilus planipennis*). This invasive beetle threatens to cause the extinction, or at least local expiration of ash because larvae kill greater than 99% of most *Fraxinus* in North American forests (Klooster et al. 2014, 2018). If the high mortality of ash trees continues, beetles may be forced to colonize other closely related hosts such as white fringetree (*Chionanthus virginicus*) or horticulturally planted olive (*Olea europaea*), two recently discovered novel hosts of EAB (Cipollini 2015, Cipollini et al. 2017). Emerald ash borer is major pest on novel ash trees, so when these two novel hosts were discovered there was concern about the extent to which beetles could use, survive, and select these non-ash hosts. And furthermore, will these two novel hosts face a similar impact, high mortality as susceptible ash or will they only be secondary hosts of EAB?

EAB impact on white fringetree populations:

Ornamental white fringetrees were the first non-ash hosts discovered attacked by EAB in 2014 (Cipollini 2015). But dendroecological evidence reveals that EAB has been attacking this host for years, which this observation had previously gone undocumented at sites in Ohio and Michigan (Thiemann et al. 2016, Ellison et al. in review). Peterson and Cipollini (2017) evaluated EAB attack on white fringetrees throughout the lower Midwest and found ~26% of the fringetrees were attacked and trees displaying dieback and epicormic branching were more likely to be infested (Peterson and Cipollini 2017). Larger fringetrees appeared more likely to be attacked than smaller individuals in Peterson and Cipollini (2017), but further studies suggest that there is no apparent preference for fringetree size (Ellison et al. in review, Peterson and Cipollini in prep.).

White fringetree is attacked less after peak EAB populations (Ellison et al. in review) suggesting that this host is more resistant. Furthermore, attacked fringetrees were more likely to be stressed (Ellison et al. in review) as found during peak populations of EAB (Peterson and Cipollini 2017). White fringetree appears to be less susceptible than ash trees because morality rates are drastically lower, only 1% in contrast to ash, >99% (Klooster et al. 2014). One reason such low morality was observed is due to white fringetrees having multiple stems, we more often found killed branches on this novel host and those few that were killed by EAB were single stemmed (Peterson and Cipollini 2017) similar to most ash trees. Together, these studies (Peterson and Cipollini 2017, Ellison et al. in review) demonstrate that fringetrees will not be drastically impacted by EAB compared to susceptible ash trees in North America. But, these studies were conducted retroactively during or after EAB attack waves and have not investigated natural populations of fringetree. White fringetrees grow densely (DLP pers. obs) in wild populations and infestation is likely higher as observed in a population in Wexford (~50%), PA (DLP unpublished data) and in more densely planted ornamental sites (Peterson and Cipollini 2017).

In our study of three wild fringetree populations (Peterson and Cipollini in prep), we found that the EAB attack dynamics was similar to previous studies (Peterson and Cipollini 2017, Ellison et al. in review). Near the beginning of EAB wave, beetles attacked ash trees first, with infestation of fringetrees occurring one to two years afterwards as observed in Thiemann et al. (2016) where dendrochronology revealed ornamental grown white fringetrees infested within two years of reported attacks on ash trees. Emerald ash borer attacked white fringetrees at the highest rate (36%) at our Vinton site which had the greatest number of ash and trap captures. These data suggest that beetle attack on white fringetrees correspond with EAB pressure, population levels. When compared to our Jackson site which had fewer ash trees and lower beetle populations, EAB attack was lower in white fringetree providing further evidence that beetle pressure is predictive of impact on this novel host. Monitoring at the third site, Shawnee, revealed no attack on white fringetrees or ash trees. But in the final two years of surveying, traps began capturing EAB suggesting that beetles were just arriving to this site. We would expect that EAB would begin to infest ash and fringetrees within a year or two and speculate that attack of fringetrees would be lowest at Shawnee compared Jackson and Vinton. Emerald ash borer that attacked fringetrees were more likely to display stress symptoms of epicormic branching and a higher degree of canopy thinning which supports previous studies (Peterson and Cipollini 2017, Ellison et al. in review) suggesting that EAB is likely attacking stressed plants. Plant responses such as these can be caused by EAB larvae which would indicate that we are observing a symptom of EAB infestation, not a variable that predicts likely attack. However, these traits could have been caused by other agents and attracted EAB adults as observed in stressed ash hosts (Liu et al. 2003, McCullough et al. 2009). When epicormic branching and dieback presence from 2015 data, pre-EAB attack wave, and was compared to the eventual outcome of attack by the end of 2019, we found that white fringetrees were more likely to be attacked by EAB if they had epicormic sprouting. This data suggests that at least one plant stress response predicts EAB attack on white fringetree. Before EAB was present at sites, canopy dieback was low indicating that larvae were likely the cause of the increased canopy thinning. Observable signs of attack in this study and others (Peterson and Cipollini 2017, Ellison et al. in review) rely on exit holes or opportunistic peeling of bark to reveal EAB galleries suggesting that attack rates are conservative. Thus, we would expect that attack rates are higher on fringetrees since the whole tree could not be debarked. If many larvae are dying in these stems, then it would suggest that white fringetree is more resistant to EAB than susceptible ash trees, similar to observed attack rates on these two hosts.

White fringetree suitability for emerald ash borer:

In order for EAB to complete development, larvae go through four instars while feeding on the phloem before boring deeper into the sapwood to create a pupal chamber from which they will emerge as adult the following spring (Herms and McCullough 2014). On healthy ash hosts, larvae can take two years (semivoltine) to develop, but on susceptible, stressed hosts they can reach prepupae after one summer of feeding on phloem tissue (univoltine; Herms and McCullough 2014). Initial cut stem assays revealed that larvae appear to develop quicker in white fringetree than the ancestral host, Manchurian ash (*F. mandshurica*), but not as fast as the highly susceptible green ash (Cipollini and Rigsby 2015). Cut stem assays have been used in several experiments to assess larval performance (Cipollini and Rigsby 2015, Peterson et al. 2015, Cipollini et al. 2017), but these stems are likely more susceptible than living, intact stems since inducible defenses may be reduced. This is if long distance transportation through phloem is required for plant defenses. In live white fringetrees, EAB survived at much lower levels ranging from 0-15% compared to 97% in cut stem assays (Cipollini and Rigsby 2015). The variation in attack rates was due to different treatments of white fringetrees. No larvae were recovered healthy stems and those with a defense hormone, methyl jasmonate. In contrast, mechanically girdling or previous attack by EAB caused white fringetrees to be more susceptible with larval survivorship reaching 15% (Peterson and Cipollini in review). These data show that healthy white fringetrees are highly resistant to EAB, but once stressed, they become more susceptible as observed in ash trees (Chakraborty et al. 2014, Rigsby et al. 2019). Interestingly, stress type matters because water stressed white fringetrees decrease the survivorship of larvae (Rutledge and Arango-Velez 2017) indicating that white fringetree and their inducible defenses leads to vary degrees of larval success.

Agrilus including EAB have a narrow host range and are specialists that perform better on stressed ash trees which has been demonstrated similarly on white fringetree (Peterson and Cipollini in review). Interestingly, the type of stress matters for larval success on white fringetree because we found that girdling and previous attack by EAB increased larval survival, but drought decreases survival in this host (Rutledge and Arango-Velez 2017). Differences in larval success may be due to the induced defense responses in the phloem based on the type of stress. Previous research investigating the phenolics in the phloem of white fringetree found that chemistry is mostly similar to black and Manchurian ash, however some compounds such as oleuropein and pinoresinol occur at significantly higher levels (DC unpublished data). These two compounds could be adversely effecting EAB larvae and future research should investigate these chemicals and others to determine the extent to which secondary metabolites change due to stress responses. Similar to fringetree, olive contains higher loads of oleuropein (Tóth et al. 2015) than susceptible ash trees (Whitehill et al. 2012). In drought stressed olive, levels of this compound increase (Petridis et al. 2012, Mechri et al. 2019) suggesting that if this pattern occurs in white fringetree, the decrease in survival of EAB in water stressed plants (Rutledge and Arango-Velez 2017) may have been due to increased levels of oleuropein. While in susceptible hosts like white ash, oleuropein levels, which are much lower to begin with, only have slight changes due to drought conditions as observed in other susceptible ash (Chakraborty et al. 2014).

Due to the presence of exit holes, EAB larvae clearly reach maturity on some fringetrees (Cipollini 2015, Thiemann et al. 2016) likely on stressed hosts (Peterson and Cipollini in review). However, in order for EAB to fully utilize white fringetree and possibly host switch, adults need be fit and successfully feed and lay eggs solely on this novel host. When given no choice of diet, adult beetles readily consumes white fringetree foliage at the same rate as those given foliage of green ash (*F. pennsylvanica*; Peterson et al. in prep) a preferred, susceptible host (Pureswaran and Poland 2009). The beetles also survive the same length of time and successfully mate (Peterson et al. in prep). Additionally, females consuming foliage of white fringetree were fecund with no difference in number of laid eggs or fertilization and hatching rates as those beetles with a diet of green ash. These data show that white fringetree is a suitable host for EAB adults and they have the capacity to use this host in the field. Yet, the preference of EAB for white fringetree compared to susceptible hosts is unknown. Some insights can be gained indirectly, for instance females choose to lay eggs on white fringetree (Cipollini 2015, Peterson and Cipollini 2017) but to what extent and whether adults will feed on foliage of this host in the field is unclear. Y-tube, choice olfactometer assays reveal some evidence that gravid female chose host based on volatiles, these adults choose to spend equal amounts of time with white fringetree and green ash indicating a similar preference (Peterson et al. in review). Future research is needed to determine preference in the field by conducting studies that can evaluate adult feeding and egg laying choices among susceptible ash and fringetree. These studies would contribute to determining whether EAB will be able to host switch to white fringetree.

Emerald ash borer fitness on olive:

Olive is more distantly related to ash than white fringetree (Wallander and Albert 2000, Besnard et al. 2009, Yuan et al. 2010, Hong-Wa and Besnard 2013) and EAB can also complete development on this host (Cipollini et al. 2017). This tree is an economically important agricultural plant in Mediterranean climates and olive production could adversely impacted by EAB once it reaches groves in the United States (i.e. California) and Europe (Orlova-Bienkowskaja 2014). Larval performance on olive will dictate to what extent olive will be damaged by EAB. In cut stem assays, larvae take longer to develop to reach prepupae in olive (Cipollini et al. 2017) in contrast to susceptible North American ash species (Cipollini and Rigsby 2015, Peterson et al. 2015). We confirmed the delayed development in two cultivars of olive by directly comparing with green ash (Peterson and Cipollini in review). The delayed development in olive suggests that this host is less suitable, which in the field exposes larvae for a longer period of time to natural enemies which would support the slow growth high morality hypothesis (Häggström and Larsson 1995, Havill and Raffa 2000) as suggested for blue ash (*F. quadrangulata*) and developing larvae (Peterson et al. 2015).

Yet, as observed in white fringetree stems, live trees have induced responses and these defenses could reduce larval success in olive. Infestation of live potted olives reveals larvae did not develop beyond the 1st instar with 100% mortality, regardless if the tree was healthy or stressed with a mechanical girdle (Peterson and Cipollini in review).

Stress did increase the ability of larvae to establish galleries suggesting that girdling slightly increases larval performance. Gallery establishment rate increases further when the olives treated with secondary stress of debarking. From the first olive assay, the manner in which olive trees are debarked to reveal galleries causes minor damage (<3 cm^2/larva), but causes further stress to the plants. Thus, we reinfested these olives which significantly increases the rate of gallery establishment (63.3%) in contrast to the first assay (19.2%; Peterson and Cipollini in review). This increased rate could be due to changes in chemistry in the plant caused by debarking and girdling. As neonates eclose from eggs, they bore through the cork, cork cambium and phelloderm (periderm) to reach the phloem where EAB spends the majority of their live feeding and developing in ash (Poland and McCullough 2006, Herms and McCullough 2014). All larvae died once they reached the phloem layer in both of the live olive assays demonstrating that there is a toxic defense or nutritional limitation in these layers, but those on healthy trees died more often in the periderm, not establishing a gallery in the phloem. Gallery formation increases drastically due to stress from girdling and debarking. These data indicate that the periderm is resistant to feeding larvae in some manner in a healthy olive tree, however this mechanism is hampered in olive when stressed. If the phloem and periderm were analyzed for primary and secondary metabolites, then chemical differences could be revealed to point at a mechanism of resistance in olive.

Regardless of the mechanism, the phloem layer is lethal to EAB larvae whether in stressed or healthy olive trees at least withsmall potted plants used in Peterson and Cipollini (in review). It is unknown if such high mortality rates would occur in larger stemmed trees since it was these stems that produced adult beetles previously (Peterson and Cipollini in review; Cipollini et al. 2017). Young, small diameter ash (Herms and McCullough 2014) and olive (Peterson and Cipollini in review) do not support larval development which could be due to the presence of photosynthetic/chlorophyllous tissue in the bark (Pfanz et al. 2002, Filippou et al. 2007). The chlorophyll and reactive oxygen species associated with photosynthesis (Davletova et al. 2005) may cause damage to the digestive system of larvae (Krishnan et al. 2007) which appears to occur in developing beetles in the resistant Manchurian ash (Rigsby et al. 2016). In older, larger diameter olives, the photosynthetic capacity is lower due to reduced light transmission throughout the thicker bark than in young stems (Pfanz et al. 2002, Filippou et al. 2007). The reduced rate of photosynthesis may increase the survival of larvae in older olive trees, and provide a chance for host switching to olive, if adults can survive on foliage.

Adults can consume olive foliage, but feeding rates begin low and increase through time in contrast to EAB with a diet of tropical ash leaves (*F. uhdei*; Peterson et al. in prep). During this period of feeding, morality of EAB on olive foliage happens quickly with 75% by day 10 and ~90% by day 18. In comparison, beetle morality on tropical ash was only ~10% by day 18. These data demonstrate leaves of olive are poor for adults and they contain either toxic or antinutritive compounds, similar to phloem and larval mortality in olive (Peterson and Cipollini in review). Additionally, the feeding pattern of adults with an olive diet suggests EAB are compensatory feeding due to a poor quality host (Kondoh and Williams 2001). Mortality was high but a few adults lived to an age to mate, the first observation of this, and two females laid three eggs, two of which were fertile, and only one produced a larva that moved vigorously (Peterson et al. in prep). Females on tropical ash were more fecund, laying thousands of eggs which tended to have higher fertility and hatch rates. With respect to adult fitness and female fecundity, olive is a poor host for EAB and only a small percentage would be expected to survive in the field. Coupled with larval morality (Peterson and Cipollini in review), there is limited opportunity for EAB to survive and potentially host switch to olive. If EAB can survive, then we speculate that it would be limited to larvae surviving and reaching maturity on large stemmed, older olive trees, and those few females that survive on foliage would need to lay many eggs to continue the next generation. Furthermore, this idea is premised on either that females select olive often enough over ash that are likely more attractive based off olfactometer experiments (Peterson et al. in review), or ash is eliminated by EAB and olive becomes the only available host. A more realistic scenario, EAB that emerge from susceptible ash tree will consume ash foliage, mate, and females could choose to lay eggs on olives, as observed in Cipollini and Peterson (2018) and those larvae likely would die by the time they reach the phloem layer, as observed in young olive stems (Peterson and Cipollini in review). The cause of morality for adults and larvae could be attributed to the presence of oleuropein. This compound occurs in much higher levels in olive foliage and phloem in contrast to susceptible ash trees (Chen and Poland 2010, Whitehill et al. 2012, Tóth et al. 2015). Furthermore, oleuropein is antinutritive due to the crosslinking with proteins, reducing nutritive value (Konno et al. 1999) supported by the observation that adults are compensatory feeding on foliage.

Host range expansion due to shared chemistry:

With the host range expansion of EAB to white fringetree (Cipollini 2015) and olive (Cipollini et al. 2017), there was an opportunity to better understand and study the chemical differences among resistant and susceptible ash, and novel non-ash hosts. We analyzed the similarities of volatile profiles emitted among black ash (*F. nigra*), blue ash, Manchurian ash, white fringetree, and olive. Common green leaf volatiles (GLVs), such as Z-3-hexanol, were found to be shared most which are routinely used in traps to increase trap captures (de Groot et al. 2008, Crook and Mastro 2010, Silk and Ryall 2015) and similarly used for EAB congeners gold-spotted oak borer (*A. auroguttatus*; Coleman et al. 2014) and bronze birch borer (*A. anxius*; Silk et al. 2019). Z-3-hexanol the most similar compound among the hosts (Peterson et al. in review) and other GLVs are commonly emitted among plants and they are likely a general attractant for EAB and relatives. Using previously evolved traits (ecological fitting), adult EAB are likely using GLVs to select and lay eggs on fringetree and olive (Cipollini 2015, Cipollini and Peterson 2018; Peterson et al. in review); however, the preference of gravid females was to spend more time with volatiles of white fringetree volatiles than olive in olfactometer assays (Peterson et al. in review). These data suggest that white fringetree likely shares more in common with ash than olive. One such compound, (Z)-3-hexenyl isobutyrate is emitted at higher rates by white fringetree than black ash, an highly attractive host of EAB (Rigsby et al. 2014). This compound may contribute to the postulated greater attraction of EAB to white fringetree since females spent a similar amount of time with green ash (Peterson et al. in review), which is similarly attractive as black ash. Female preference for olive is likely lower based on olfactometer assays because the volatile profile of this plant was more similar to Manchurian ash, specifically they both emit significantly different profiles of sesquiterpenes (Peterson et al. in review). Y-muurolene, for example, is a sesquiterpene that was uniquely emitted by olive compared to the other hosts and has been implicated in repelling leafcutting ants (*Atta laevigata*) and may cause similar responses in other herbivores such as EAB. This compound and others may contribute to the lower attraction of female beetles to olive.

Conclusions:

North American and European ash trees are highly susceptible to EAB (Rebek et al. 2008, Herms 2014), but olive and white fringetree are less suitable. Our studies find that white fringetree foliage supports adults, but when phloem is healthy it causes high larval morality in contrast to girdled or previously attacked by EAB where EAB had some survival. In the field, EAB began to use white fringetree quickly, within a couple of years after initial exposure. Female choice suggests that beetles prefer white fringetree and susceptible ash due the similarity of volatile profiles. It is likely volatile emissions that led to the host range expansion of EAB to the novel host. In contrast to ash hosts, the impact of EAB on white fringetree is minimal. This plant mostly loses a branch or two from larval girdling. This damage in ornamentally planted fringetrees can be aesthetically displeasing which pruning can help. However, if the plant appears to be in a state of decline due to EAB, the plant can be rejuvenated by removing the most of the above ground stems as done with the close relative lilac (*Syringa* spp.; Niemiera 2018) and other deciduous shrubs (Ball and Graper 1999). On olive, the impact of EAB is likely to be even less because larvae take longer to develop and they die quickly on young, photosynthesizing stems. Adults, similarly, did not perform well because oleuropein may cause them to compensatory feed and causing malnutrition. In North American forests, if EAB continues to destroy ash species at such high rates, EAB may be driven to use white fringetree more often. With continued use of this host, EAB is likely to adapt to better utilize white fringetree which could eventually lead to host switching.

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