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Larval Performance of a Major Forest Pest on Novel Hosts and the Effect of Stressors

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Abstract

Novel hosts lacking a coevolutionary history with herbivores can often support improved larval performance over historic hosts; e.g., emerald ash borer [*Agrilus planipennis* (Fairmaire) Coleoptera: Buprestidae] on North American ash (*Fraxinus* spp.) trees. Whether trees are novel or ancestral, stress on plants increases emerald ash borer preference and performance. White fringetree [*Chionanthus virginicus* (L.) Lamiales: Oleaceae] and olive [*Olea europaea* (L.) Lamiales: Oleaceae] are closely related non-ash hosts that support development of emerald ash borer to adulthood, but their relative suitability as hosts and the impact of plant stress on larval success has not been well studied. In a series of experiments, survival and growth of emerald ash borer 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 [*Fraxinus pennsylvanica* (Marshall) Lamiales: Oleaceae] and several adults successfully emerged from larger olive 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 emerald ash borer attacked and mechanically girdled plants than in healthy stems or stems treated with the defense hormone, methyl jasmonate. Our results demonstrate that these non-ash hosts are less suitable for emerald ash borer than preferred ash hosts, but previous emerald ash borer 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.

Key words: host range expansion, stressed host, emerald ash borer, white fringetree, olive

Many woodboring insects perform better on and prefer stressed over healthy hosts (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) (Coleoptera), 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 (*Agrilus anxius*) on novel birch hosts (*Betula* spp.) from Eurasia (Muilenburg and Herms 2012), goldspotted oak borer [*Agrilus auroguttatus* (Schaeffer) Buprestidae: Coleoptera] on novel oak hosts in California (*Quercus* spp.; Coleman and Seybold 2008), and the most well-studied buprestid, emerald ash borer [*Agrilus planipennis* (Fairmaire) Coleoptera: Buprestidae] on novel North American and European ash hosts (*Fraxinus* spp., Lamiales: Oleaceae). 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 emerald ash borer, the accidental introduction into North America 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 generally higher susceptibility of North American ash trees to emerald ash borer compared to the ancestral host, Manchurian ash [*Fraxinus mandshurica* (Rupr.) Lamiales: Oleaceae, Liu et al. 2003, Rebek et al. 2008, Herms 2014] which appears to have defenses targeted for emerald ash borer (Whitehill et al. 2012, Rigsby et al. 2015, 2016). After reaching a new geographic range, herbivorous insects may encounter novel 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 hosts and what factors affect their relative suitability?

Emerald ash borer, the most costly North American forest pest (Aukema et al. 2011), provides a model for the role of stress in the ability of an invasive insect to use novel hosts. Previously, emerald ash borer 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 is white fringetree [*Chionanthus virginicus* (L.) Lamiales: Oleaceae] which is closely related to *Fraxinus* (Wallander and Albert 2000). Since the initial discovery of this tree as a host for emerald ash borer (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, attack rates by emerald ash borer 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, emerald ash borer larvae generally grow more poorly on 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 emerald ash borer is cultivated olive [*Olea europaea* (L.) Lamiales: Oleaceae]. This plant species is a close relative of white fringetree and ash (Wallander and Albert 2000) and cut stems can support emerald ash borer development from egg to adulthood (Cipollini et al. 2017). Although never directly compared, larval development appears to be slower on olive than on preferred ash hosts (Cipollini et al. 2017). Larvae, for example, fed for over 69 d in cut stems of olive before reaching mature fourth instars (Cipollini et al. 2017), compared to 37–40 d to reach the same stage in 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 emerald ash borer.

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 emerald ash borer larvae or that are affected by 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 emerald ash borer can routinely feed and reach adulthood on live intact white fringetrees 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). Potted white fringetrees with intact stems kill larvae at a much higher rate than does potted white ash, *F. americana* (L.) (Lamiales: Oleaceae) (Rutledge and Arango-Velez 2017). In olive, larval survivorship in cut stems appears to be lower (Cipollini et al. 2017) than that observed in cut stems of preferred ash (Peterson et al. 2015, Cipollini and Rigsby 2015), but the relative performance of emerald ash borer in these hosts has not been directly compared. Moreover, the observation of substantial larval development in an intact potted olive tree has been limited to a single tree (Cipollini and Peterson 2018).

A variety of abiotic and biotic factors can affect the performance of emerald ash borer on its hosts (Tluczek et al. 2011, Chakraborty et al. 2014, Whitehill et al. 2014, Rutledge and Arango-Velez 2017,

Rigsby et al. 2019). Factors such as drought, which is predicted to increase in severity and/or frequency under most climate change (Williams et al. 2013), may expand opportunities for invasive insects to use novel hosts. Larval performance increases in ash trees when they are droughted, mechanically girdled, or attacked previously by emerald ash borer (Tluczek et al. 2011, Chakraborty et al. 2014, Rigsby et al. 2019). In contrast, larval performance can be hampered from a treatment of methyl jasmonate (MeJA), a compound involved in the induction of secondary metabolites in response to pathogen or insect attack (Erbilgin et al. 2006, Ballaré 2011). In the emerald ash borer 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 emerald ash borer is expected to increase in stressed hosts and decrease in MeJA-treated plants, as observed in ash trees. Rutledge and Arango-Velez (2017) investigated how larval performance was affected by drought stress in white ash and white fringetree, predicting higher survivorship in drought stressed plants. This was indeed the case on white ash, but drought stress unexpectedly led to lower larval survival of emerald ash borer in white fringetrees. The authors suggested that, when under drought stress, the availability of nitrogen or carbon compounds were reduced, 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 emerald ash borer 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 emerald ash borer and the extent to which stress can alter it, we conducted a series of field and laboratory experiments. In the first experiment, the survival and growth of emerald ash borer were evaluated in cut stems of mature olive and green ash *F. pennsylvanica* (Marshall), which have never been compared. We predicted that survival and growth will be poorer in the non-ash host. The other experiments used live, intact plants. In two experiments with field-grown white fringetree, we examined the effect of previous attack by emerald ash borer, girdling, and MeJA application on growth and survival. Larvae were predicted to survive better and grow larger on previously attacked (stressed) and mechanically girdled (stressed) white fringetrees compared to uninjured 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 emerald ash borer in young potted olive trees. We hypothesized that larvae would grow larger and survive at higher rates in girdled and stressed stems in contrast to healthy olive trees.

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 3 to 6 d before being sent overnight to Wright State University. Eggs were stored in a growth chamber (25°C 16:8 (L:D) h cycle) until they reached ~12–14 d old, or about 2 to 3 d 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 Versus Ash

Emerald ash borer eggs laid between 26 and 30 November 2016 were sent overnight to Wright State University on 30 November 2016 and stored in a growth chamber (25°C 16:8 (L:D) h 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 mature trees used in commercial olive production. These logs were cut to lengths between 45 and 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 emerald ash borer eggs attached on 6 December 2016 and stored in a growth chamber (25°C 16:8 (L:D) h 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). Distilled water was added as needed to maintain the depth of water and moisture. Upon inspection, the majority of eggs hatched by 12 December. At 37 d (18 January 2017), 44 d (25 January), and 51 d (1 February) after egg hatch, one ash, three Barouni, and three Manzanilla stems were debarked using wood chisels to reveal emerald ash borer larvae and feeding galleries. Digital calipers were used to measure maximum gallery width of each gallery (mm; Fig. 1A) and survivorship was recorded, as described in Peterson et al. (2015) and Cipollini et al. (2017). Gallery width was also used to estimate larval size and life stages:

Fig. 1. (A) Width of prothoracic (yellow) and gallery (red) measured to assess larval performance and size of emerald ash borer in white fringetree (*Chionanthus virginicus*) and olive (*Olea europaea*) trees. (B) Correlation of prothoracic width (mm) and gallery width (mm) of emerald ash borer larvae (*Agilus planipennis*) based on performance in cut and live stem assays in ash (*Fraxinus* spp.) from Peterson et al. 2015 and DLP unpublished data.

<1 mm = 1st instar, 1–2 mm = 2nd instar, 2–3 mm = 3rd instar, and >3 mm = 4th instar (Duan et al. 2014, Peterson et al. 2015). 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 whether 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 10°C in an incubator starting on 14 March and then transitioned to 4°C on 13 April, 10°C on 12 May, and then returned to ~23°C 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 3 mo for adult beetle emergence and then debarked to determine fates of any remaining larvae. Emerged adults were kept in a growth chamber (25°C, 16:8 (L:D) h) in clear plastic containers (72 oz.) and provided fresh green ash foliage every 3–4 d to examine their viability. 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 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), 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 1.3 m above the soil. On 20 June, emerald ash borer eggs were received from the emerald ash borer rearing facility and 10 were attached to each olive tree on 22 June as previously described. Girdled trees had four eggs placed above the girdled and the other six eggs were placed below. Olive trees were watered 3 d a week. On 28 September, olive trees were debarked and larval gallery width and survivorship were assessed. Galleries that were formed by emerald ash borer were small so we did not debark the entire stem of the plants in order to use the trees for experiment 3. Gallery establishment rate was also assessed, the number of galleries formed. A digital caliper was used to measure the terminal gallery widths of larvae, which is strongly correlated with larval prothoracic size, and related to larval instar (Fig. 1B).

Experiment 3: 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 infested with emerald ash borer in 2018 when only a small area of bark encompassing a few square centimeters was removed to assess the performance of each beetle (six to eight areas per tree) on each tree. This mechanical damage provided a chance to further examine the impact of stress on emerald ash borer performance. Ten trees used in this experiment were previously healthy trees that had been inoculated with emerald ash borer eggs and evaluated 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, and inoculated with emerald ash borer eggs and evaluated in 2018. In 2019, these 16 trees were exposed to the same inoculation procedure and examined in the same manner as in 2018. On 17th June, emerald ash borer eggs were attached to olive trees and on 26th July, stems were debarked to assess larval survivorship, gallery widths, and number of galleries established.

Experiment 4: Larval Performance on Previously Attacked Versus Healthy White Fringetrees in the Field

On 22 June 2016, white fringetrees ($n = 16$) with lateral branch diameters between 2.5–5.0 cm were selected to be infested with emerald ash borer eggs at Spring Grove Cemetery and Arboretum in Cincinnati, Ohio. White fringetrees were split into two treatments: those that were previously attacked by emerald ash borer ($n = 8$) and uninfested, healthy trees ($n = 8$). The trees were previously recorded by Cipollini and Rigsby (2015) and Peterson and Cipollini (2017) as attacked or healthy based on signs and symptoms of emerald ash borer 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 selected stem spaced approximately 10 cm apart, using the same inoculation procedures as for cut stems and potted trees. Emerald ash borer larvae fed for the remainder of summer 2016. Stems were harvested on 4 November 2016 with care to cut 30 cm above and below the terminal placement of emerald ash borer eggs and transported to Wright State University. Bark was removed to reveal larval survivorship and galleries using wood chisels.

Experiment 5: 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 emerald ash borer 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 emerald ash borer. We imposed three treatments to three separate stems on each tree consisting of mechanical girdling ($n = 9$), application of MeJA ($n = 9$), and a control with 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 attachment of the stem to the main trunk. MeJA 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. Control stems received no treatment. One week after MeJA and girdling treatments were imposed, six emerald ash borer 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.

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 gallery width in experiment 1, with the fixed effects of plant species or cultivar, harvest times, and their interaction. Tukey's post-hoc test were used to determine differences among species. ANOVAs/T-tests (PROC GLM) were used for experiments two through four to compare the average gallery width among treatments. Chi-squared tests (Fisher) were used to determine differences of survivorship for the four experiments, gallery establishment rate for experiment four and egg hatch rate for experiment one. Hatch rate and gallery

establishment rates are not presented for the other experiments since there were no significant differences among treatments. When data was non-normal (Experiment three), we used a Wilcoxon signed-rank test (PROC NPAR1WAY) to determine whether 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 experiments 3, 4, and 5.

Results

Experiment 1: Larval Performance in Cut Stems of Olive Versus Ash

The egg hatch rate was significantly higher for eggs placed on green ash stems (93.3%; $n = 14$) compared to those eggs on Barouni stems (62.1%; $n = 59$; $\chi^2 = 5.66$; $df = 1$; $P = 0.012$) and Manzanilla olive (55.5%; $n = 35$; $\chi^2 = 7.40$; $df = 1$; $P = 0.007$). Hatch rates were the same among the two olive treatments ($\chi^2 = 0.67$; $df = 1$; $P = 0.094$). Overall, gallery width varied among plant species (Fig. 2; $F = 27.73$; $df = 2$; $P \leq 0.001$) 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 produced larger galleries in green ash stems than in the two olive cultivars, which were of the same size. Survival of larvae was significantly different between species ($\chi^2 = 6.41$; $df = 2$; $P = 0.002$). Larvae survived at a significantly greater rate in green ash (100%, $n = 14$) than in Barouni olive (65.9%; $n = 29$; $\chi^2 = 6.44$; $df = 1$; $P = 0.007$). Survival in Manzanilla olive (72.7%; $n = 8$) did not vary from that in either green ash ($\chi^2 = 4.33$; $df = 1$; $P = 0.07$) or Barouni olive ($\chi^2 = 0.18$; $df = 1$; $P = 0.263$). After the artificial overwintering period, three male emerald ash borer emerged from Manzanilla stems (20% of hatched eggs) with no emerald ash borer reaching adulthood from Barouni stems. The adults that emerged from olive survived on green ash foliage for at least 10 d before ash leaf quality collected from the field declined with natural autumn senescence.

Experiment 2: Larval Performance on Young Live Girdled and Healthy Olive Trees (2018)

Larval gallery widths in girdled olive trees, 0.81 ± 0.04 mm, were the same size as those in control stems, 0.79 ± 0.05 ($t = 0.09$;

Fig. 2. Average gallery width (mm $\pm$ SE) of emerald ash borer (*Agrilus planipennis*) larvae over time in cut stems of green ash (GA, *Fraxinus pennsylvanica*) and two cultivars of olive (*Olea europaea*); Barouni (Bar) and Manzanilla (Man) in a growth chamber for 51 d after egg hatch at Wright State University, Dayton, Ohio. Different letters indicate significant differences using analysis of variance (ANOVA) and Tukey's HSD.

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%; $n = 15$) compared to control olives (12.5%; $n = 8$; $\chi^2 = 3.93$; df = 1; $P = 0.0473$). Gallery widths of larvae above the girdle were 0.83 ± 0.04 mm, which was the same as those below the girdle, 0.78 ± 0.06 ($t = 1.77$; df = 13; $P = 0.563$).

Experiment 3: Larval Performance on Young Live, Previously Infested Olives (2019)

Larval galleries were the same size in the wounded trees, 0.88 ± 0.12 mm as in the wounded, but previously girdled trees, 0.80 ± 0.08 mm ($t = 0.32$; df = 30; $P = 0.571$). Gallery establishment was the same between the girdled, wounded olive (56.5%; $n = 13$) and wounded olive (67.6%; $n = 25$; $\chi^2 = 0.745$; df = 1; $P = 0.421$). However, gallery establishment significantly increased in 2019 overall (63.3%; $n = 38$) compared to that in 2018 (19.2%; $n = 23$; $\chi^2 = 34.837$; df = 1; $P \leq 0.001$).

Experiment 4: Larval Performance on Previously Attacked Versus Healthy White Fringetrees in the Field

Emerald ash borer larvae in previously attacked white fringetrees were 1.3 times larger than in healthy trees (Fig. 3A; $t = 2.29$; df = 71; $P = 0.025$). Similarly, larvae produced galleries that were twice as long in previously attacked trees than in healthy control trees (Fig. 3B; $t = 2.27$; df = 71; $P = 0.027$). Significantly more emerald ash borer larvae were found alive (15.3%; $n = 6$) in previously attacked white fringetree than healthy white fringetrees, in which no larvae were found alive ($\chi^2 = 5.08$; df = 1; $P = 0.032$). Out of the six surviving larvae in previously infested trees, three reached the prepupal stage indicating those beetles will emerge as adults the following spring.

Experiment 5: 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. 4; $F = 3.97$; df = 2, 61; $P = 0.0238$). Survivorship to the end of the experiment was low in all treatments, but differed among treatments ($\chi^2 = 6.98$; df = 2; $P = 0.034$). Larval survival was highest in girdled stems (16%; $n = 4$) and no survival was seen in MeJA-treated and control trees. Within the girdled stems, no larvae survived above the girdle and those above the girdle 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; $P = 0.050$).

Discussion

In a series of experiments in the field and laboratory, we examined the survival and growth of emerald ash borer 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 emerald ash borer. 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

Fig. 3. (A) Average gallery width (mm $\pm$ SE) and (B) gallery length (cm $\pm$ SE) of emerald ash borer (*Agrilus planipennis*) larvae that fed on previously attacked by emerald ash borer (IN) and healthy (UN) white fringetree (*Chionanthus virginicus*) in summer 2016 at Spring Grove Arboretum and Cemetery, Cincinnati, Ohio. Different letters indicate significantly different means using a T-test.

Fig. 4. Average gallery width (mm $\pm$ SE) of emerald ash borer (*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. Different letters indicate significantly different means using analysis of variance (ANOVA) and Tukey's HSD.

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 emerald ash borer in our study. Similarly, variation in oleuropein concentration and/or nutritional qualities may have also contributed to the lower survival of emerald ash borer 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. Manzanilla (Cipollini et al. 2017) and in both instances of the successful emergence, the olive stems were greater than 11.9 cm in 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, the 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 success 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 a live, healthy tree of the same cultivar (Cipollini and Peterson 2018). Similarly, no larvae survived in recently girdled olive which was unexpected since stress generally has led to better performance of emerald ash borer 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 whether 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 emerald ash borer, at least those stems with smaller diameters (<4 cm). Larvae may be affected by the age of stems due to the presence of photosynthetic/chlorophyllous tissue in the bark of young olive and ash ([Pfanzen 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 ([Pfanzen 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](#)). Chlorophyll and other associated metabolites are likely foreign to emerald ash borer and potentially toxic since larvae are adapted to use phloem tissue, generally under a thick layer of bark. For example, chlorophyll breakdown products, like chlorophyllide, are associated with insect resistance in other systems ([Hu et al. 2015](#)). 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 emerald ash borer attacks in the forest might be more susceptible to emerald ash borer because these stems have thicker bark that reduces light penetration and have lower levels of chlorophyll.

One effect of stress that we observed in olive is that larvae established galleries at higher rates in mechanically girdled olive trees compared to healthy plants. When those trees had emerald ash borer eggs attached 1 yr later, 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-associated metabolites, high light penetration, and/or higher concentrations of oleuropein in young stems. While larval success was still limited, our findings suggest that bark wounding and possibly other stresses can increase the susceptibility of young olive trees to some degree, which could open windows of opportunity for emerald ash borer to exploit this host.

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 at significant rates 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 in the native host of emerald ash borer, 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

emerald ash borer in white fringetree ([Rutledge and Arango-Velez 2017](#)). Therefore, when considering the likelihood of larval success in the field, white fringetree could be either a reservoir or population sink for emerald ash borer. If white fringetree is healthy or drought stressed, this plant kills or slows development of emerald ash borer. In contrast, when plants are girdled in some manner or stressed from previous attack, white fringetree is more susceptible to emerald ash borer and larvae can reach adulthood. Emerald ash borer appears to be a secondary pest on white fringetree since it succeeds best on stressed plants, while healthy plants appear to largely resist this plant like the historic host in Manchurian ash in East Asia ([Liu et al. 2003](#)). This is further supported by the low, observable attack rates of white fringetree observed 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.

MeJA treatment applied to trees induces the accumulation of some defense-related metabolites and decreases the success of emerald ash borer 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 worthy of future investigation.

Overall, our findings suggest that emerald ash borer larvae perform sub-optimally on the novel hosts, white fringetree and olive, relative to preferred ash species. However, their performance increases with certain types of stress including previous attack by larvae and mechanical girdling. In contrast, healthy plants and those treated with MeJA generally killed emerald ash borer. The relative lack of success in thin barked stems of young olive trees suggests they are generally toxic in some way to emerald ash borer, 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 emerald ash borer to determine to what extent this pest could threaten this economically important plant.

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