Growth of Larval *Agrilus planipennis* (Coleoptera: Buprestidae) and Fitness of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Blue Ash (*Fraxinus quadrangulata*) and Green Ash (*F. pennsylvanica*)

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ABSTRACT Emerald ash borer (*Agrilus planipennis* Fairmaire) is an invasive primary pest of North American ash (*Fraxinus* spp.) trees. Blue ash (*F. quadrangulata*) is less susceptible to emerald ash borer infestations in the forest than other species of North American ash. Whereas other studies have examined adult host preferences, we compared the capacity of emerald ash borer larvae reared from emerald ash borer eggs in the field and in the laboratory to survive and grow in blue ash and the more susceptible green ash (*F. pennsylvanica*). Emerald ash borer larval survivorship was the same on both ash species. Mortality due to wound periderm formation was only observed in living field grown trees, but was low (<4%) in both green and blue ash. No difference in larval mortality in the absence of natural enemies suggests that both green and blue ash can support the development of emerald ash borer. Larvae reared from eggs on blue ash were smaller than on green ash growing in the field and also in bolts that were infested under laboratory conditions. In a laboratory study, parasitism rates of confined *Tetrastichus planipennisi* were similar on emerald ash borer larvae reared in blue and green ash bolts, as were fitness measures of the parasitoid including brood size, sex ratio, and adult female size. Thus, we postulate that emerald ash borer larvae infesting blue ash could support populations of *T. planipennisi* and serve as a potential reservoir for this introduced natural enemy after most of the other native ash trees have been killed.

KEY WORDS Agrilus planipennis, Fraxinus, Tetrastichus planipennisi, blue ash, green ash

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a primary pest of North American ash trees (*Fraxinus* spp.). First detected in 2002, this pest arrived in wood packing material from China in the early- to mid-1990s (Siegert et al. 2008, 2014; Cappaert et al. 2005). This phloem feeding beetle threatens billions of ash trees in forests (Mercader et al. 2009) and could cost tens of billions of dollars to manage in municipal areas (Kovacs et al. 2010). Although urban ash trees can be protected by insecticides (Herms et al. 2014), such treatments are impractical in forests. Thus, the survival of ash trees in North American forests is likely to depend on other emerald ash borer management strategies including host plant resistance (HPR) and biological control.

Emerald ash borers that overwinter as mature larvae, pupate to adults in the spring (Cappaert et al. 2005, Poland and McCullough 2006). Adults emerge after chewing through the bark and then fly to the canopy to feed on ash foliage to obtain nutrients necessary for maturation of eggs, mating and dispersal. Females lay up to 90 eggs individually in bark cracks or crevices. Eggs hatch within two weeks of oviposition and first-instar larvae bore directly through bark into the phloem. Larvae feed on host phloem through each of four instars, and then bore into the sapwood to form a pupal chamber. They seal the gallery leading to this chamber with frass and fold themselves into a recognizable "J-shaped larvae" that will become pre-pupae (Ulyshen et al. 2010b). Larvae that reach the J-shaped stage in the fall will pupate and emerge as adults the following growing season. Although most larvae complete this cycle in a single year, some overwinter as earlier stage larvae and take another year to develop. Temperature and host plant quality determine whether emerald ash borer take one or more years to develop to adults (Wei et al. 2007, Cappaert et al. 2005, Tluczek et al. 2011). In North America, adult emerald ash borer begin to emerge after the accumulation of 230 to 290 growing degree days from January 1 using a threshold temperature of 10°C (Herms et al. 2014).

Most North American ash species are far more susceptible to emerald ash borer than their Asian congeners (Anulewicz et al. 2008, Rebek et al. 2008, Pureswaran and Poland 2009, Tanis and McCullough 2015). In a common garden experiment, North

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American white ash (*F. americana* L.) and green ash (*F. pennsylvanica* Marsh) suffered high mortality rates >75%, while only 20% of Asian Manchurian ash (*F. mandshurica* Rupr.) died (Rebek et al. 2008). In North America, emerald ash borer infests and preferentially feeds on green ash, then black ash (*F. nigra* Marshall), white ash, and blue ash (*F. quadrangulata* Michx.) (Anulewicz et al. 2006, 2007; Pureswaran and Poland 2009, Chen and Poland 2010; Tanis and McCullough 2012, 2015; Carson 2013). A recent study of mixed ash tree forests in Michigan reported one site with 71% of blue ash and 16% white ash surviving, whereas in a second site only 63% of the blue ash and none of white ash survived (Tanis and McCullough 2012). In a Canadian forest, 82% of blue ash crowns retained >90% of their foliage after the initial wave of emerald ash borer infestation, while only 3.2% of green ash trees retained 70% of their foliage (Carson 2013). Thus, in North America blue ash is considered the least susceptible and least preferred North American ash host for emerald ash borer.

Mechanisms of HPR to emerald ash borer are not well understood. Current research focuses on plant factors that contribute to antixenosis (Groot et al. 2008, Pureswaran and Poland 2009, Rigsby et al. 2014), or to antibiosis (Eyles et al. 2007; Cipollini et al. 2011; Whitehill et al. 2011, 2012; Chakraborty et al. 2014). Antixenotic properties of host plants that affect behavior of adult herbivorous insects can be mediated by green leaf volatiles (Dickens 2001, Cosse et al. 2006, Fernandez et al. 2007, Groot et al. 2008). Differences in the emissions of volatile organic compounds may also mediate the differential attraction of emerald ash borer to the various *Fraxinus* species. For example, it appears that North American ash species, such as black, green, and white ash differ in their constitutive foliar chemistry from the less susceptible Manchurian ash (Pureswaran and Poland 2009). In fact, differences in phytochemicals may be responsible for the feeding preference of adult emerald ash borer on some ash species (Pureswaran and Poland 2009, Chen and Poland 2010). Volatile organic compounds may also influence the oviposition preference of gravid female emerald ash borer on North American ash trees over Manchurian ash (Pureswaran and Poland 2009, Rigsby et al. 2014). Alternatively, antibiotic properties of constituent or induced compounds produced in host plants can slow larval development or reduce adult fecundity of insect pests (Meisner and Skatulla 1975, Mansour 1982, Fung and Herrebout 1987, Silva et al. 2006). Although certain complex polyphenolics produced by Manchurian ash in response to emerald ash borer attack can reduce emerald ash borer larval growth, identifying unique compounds associated with resistance has been difficult (Poland et al. 2015, Chakraborty et al. 2014).

Some volatile and nonvolatile phytochemicals not only ward off or defend against herbivory, they may also function as attractants for natural enemies (e.g., predators and parasitoids) and thus mediate indirect defense (Eyles et al. 2010). Nevertheless, the extent to which larval parasitoids are directly affected by HPR to emerald ash borer is also unknown. Natural enemies may benefit from HPR that slows development of pest insects and increases the opportunity for natural enemies to attack (Haggstrom and Larsson 1995, Benrey and Denno 1997, Williams 1999, Havill and Raffa 2000). Yet, HPR, specifically antibiosis, may hinder biological control by increasing development time, reducing size, and decreasing survivorship of parasitoids on affected insect hosts in forest ecosystems (Kruse and Raffa 1997, Werren et al. 1992). In North America, emerald ash borer is attacked by many natural enemies including native (Duan et al. 2012b, Abell et al. 2012, Lyons 2015, Tanis and McCullough 2015) and introduced parasitoids (Duan et al. 2013a, Jennings et al. 2013, Mapbiocontrol 2015).

Tetrastichus planipennisi Yang (Hymenoptera: Eulophidae) is a larval parasitoid introduced from China to North America as part of an emerald ash borer classical biological control program (Bauer et al. 2015). This gregarious endoparasitoid has become established in US populations of emerald ash borer with parasitism rates of up to 28% in some areas (Mapbiocontrol 2015, Duan et al. 2013a, Jennings et al. 2013). It attacks second through fourth-instar larvae in naturally infested ash, and is capable of attacking J-shaped and prepupal stages under laboratory conditions (Ulyshen et al. 2010a,b). Parasitoid larvae feed on live emerald ash borer larvae for 7–10 d until pupation (Duan et al. 2011b). Eclosing adults chew through the body of the dead larval host after a 15 d pupation period.

No studies to date have attempted to examine the extent to which blue ash affects emerald ash borer larval survivorship and growth compared to the more susceptible native hosts (e.g., green ash). Additionally, the effect of HPR on the fitness of emerald ash borer parasitoids is unknown. In this study, we evaluate growth and survival of emerald ash borer larvae in blue ash and green ash and evaluate the suitability of emerald ash borer larvae reared in blue ash as hosts for the introduced parasitoid, *T. planipennisi*. We hypothesize that blue ash, being less susceptible to emerald ash borer, negatively affects both emerald ash borer growth and survival, as well as its suitability as a host for *T. planipennisi*.

Materials and Methods

Field Sites and Host Trees. Existing stands of green ash in West Lafayette, Tippecanoe County (Celery Bog), IN, and blue ash in Peru, Miami County (Mississinewa reservoir), IN, were selected as field sites and as a source of host material for emerald ash borer larval growth studies in April 2013. Ash trees measured at 1.5 m above the soil line had a diameter at breast height (DBH) between 8–12 cm in the field studies and 3–6 cm in the laboratory studies. Selected trees had no obvious signs of emerald ash borer infestation (e.g., vertical bark splits, epicormic shoots, D-shaped exit holes, canopy thinning, or woodpecker holes).

Field Studies. *Infestation of Ash Trees With Emerald Ash Borer Eggs.* Field studies were conducted to determine the extent to which living green

and blue ash host trees affected survival and development of emerald ash borer larvae and *T. planipennisi*, and rates of emerald ash borer parasitism. A total of 51 trees were selected in the field and infested with emerald ash borer eggs acquired from the USDA Forest Service, Northern Research Station, East Lansing, Michigan. In the Michigan laboratory, eggs were laid on filter paper by gravid females reared from tropical ash logs, *Fraxinus uhdei* (Wenzig), and fed tropical ash leaves (Duan et al. 2013b). Filter paper discs with eggs were harvested daily. Young eggs were allowed to develop at 24°C until they reached 8–12 d of development, at which point all eggs were stored at 10°C and collected for refrigerated shipment to West Lafayette, Indiana. Upon receiving egg-laden filter paper discs in Lafayette, cohorts were incubated at 24°C, 75% humidity, and a photoperiod of 16:8 (L:D) h until 13 d old, three days before expected eclosion.

Emerald ash borer egg covered filter paper discs were attached to trees between 28 May and 6 June 2013 using methods described by Abell et al. (2012). Each tree had a density of 54 eggs/m^2 along a 1.5 m length of main stem beginning at 0.3 m above the soil surface. Extra eggs were maintained in the laboratory under incubator conditions for two weeks to determine hatch rates by monitoring for neonate exit holes with a dissecting microscope. Hatch rate was used to estimate the number of viable eggs transferred to ash trees in our experiments. Four weeks after infestation (8–12 July), the filter paper discs were removed. Organdy cloth and wire cages were constructed as described by Ulyshen et al. (2010a,b) and placed around the infested stem length to protect the emerald ash borer larvae from natural enemies.

Emerald Ash Borer Larval Survival and Growth in Infested Ash Trees. On 4 December 2013, five green and five blue ash trees we infested were felled at ground level to determine emerald ash borer survival and growth before winter. The lower 2 m of the main trunk section that encompassed the caged area was harvested and transported to the laboratory at Purdue University. Logs were stored in a walk-in cooler set at 5.5°C until removed for dissection. Additionally, the lower 2 m from six caged green and six caged blue ash were harvested on April 11, 2014 and stored to determine emerald ash borer survival and growth through early spring. To determine the potential for emerald ash borer development between the fall and spring harvest dates, accumulated growing degree days were calculated during this period using the sine wave method with a base of 10°C from data acquired at nearby weather stations (Weather Channel 2015)

Emerald ash borer larvae were recovered from ash logs using a drawknife to remove bark and phloem until emerald ash borer galleries were revealed. When an individual larva was encountered a chisel was used to carefully remove enough remaining bark to extract the larva unharmed with a soft forceps. Each larva was placed into a single cell of a 24-cell well plates (Costar 3526, Corning Incorporated, Corning, NY) for subsequent evaluation. The terminal width of the emerald ash borer gallery (mm) at the point where the larva was collected was measured to estimate larval development: < 2 mm wide for L2, 2–3 mm for L3, and < 3 mm for L4 (Duan et al. 2014). The health of emerald ash borer larvae was evaluated by noting whether individuals were creamy white in color as expected of larvae protected from parasitism by the caging material, or dead if discolored (e.g., black), covered with fungal fruiting bodies, or engulfed in callus tissue formed by the tree. Additionally, we noted whether individuals were larvae or prepupae, or folded into a "J-shape" within the sapwood. Weight (g) was also recorded for emerald ash borer larvae from trees that were peeled in April 2014.

Release and Recovery of Tetrastichus planipennisi. On 5 September 2013, 1500 female *T. planipennisi* were released into 14 caged green ash trees growing at Celery Bog that were previously infested with emerald ash borer as described above. Similarly, *T. planipennisi* were released into 15 caged blue ash trees previously infested with emerald ash borer in Mississinewa on 21 May 2014. Due to reports of resistance in the literature, the release *T. planipennisi* on blue ash was delayed to allow the emerald ash borer larvae sufficient time to grow to a size the wasps would attack. The parasitoids were reared by the USDA Emerald Ash Borer Parasitoid Rearing Facility in Brighton, MI, and placed in plastic cups, each containing approximately 50 *T. planipennisi* adult females and at least 5 adult males. Cups were shipped overnight in an insulated container.

For each experimental tree, 50 female wasps were introduced from plastic cups into previously constructed cages. Prior to releasing the wasps, small amounts of honey were streaked with a paint brush along two 7.5-cm bands on the bark of each tree within the cages to provide *T. planipennisi* adults with food. Two additional streaks of honey were applied to the exterior of each cage one week after release as supplemental food for *T. planipennisi*.

Two weeks after *T. planipennisi* were released, all 14 green ash (19 September 2013) and all 15 blue ash (5 June 2014) trees were felled. The caged portion of each tree was transported to the laboratory. Logs were stored and later dissected as described previously to determine growth by measuring size and determining developmental stages of uncovered emerald ash borer larvae. Larvae that had been parasitized by *T. planipennisi* were counted and recognized by a distinct braided appearance when filled with numerous parasitoid larvae or as a cluster of white parasitoid pupae that had completely consumed their host (Duan et al. 2011b). Parasitized emerald ash borer larvae found in logs were removed, placed in 24 cell well plates (Costar 3526, Corning Incorporated, Corning, NY), which were then covered with parafilm (Bemis Company, Inc., Neenah, WI) to reduce desiccation, limit pathogens, and isolate parasitoids. Well plates were monitored for the emergence of adult parasitoids for up to seven weeks (25 September–15 November 2013 for green ash; 7 June–26 July 2014 for blue ash). We determined the sex ratio (female: male) of all parasitoids that developed to adults in well plates, and calculated the

parasitism rate and the number individuals per brood. Adult specimens were stored in 75% ethanol. Voucher specimens of emerald ash borer were deposited in the Purdue Entomological Research Collection (PERC).

Laboratory Studies. *Infesting Ash Bolts With Emerald Ash Borer.* In order to better control ambient conditions, we conducted studies of emerald ash borer survival and development on cut bolts of green and blue ash in the laboratory. On 19 February and 15 April 2014, emerald ash borer eggs between 8 and 12 d old were obtained from USDA Forest Service, Northern Research Station, East Lansing, Michigan and USDA ARS Beneficial Insects Introduction Research Unit (Newark, DE), respectively. Eggs were incubated at 24°C until they reached 12 days of development, at which point all eggs were stored at 10°C until used in experiments. On 24 February and 11 April, four blue and four green ash trees between 3.0 and 6.0 cm in diameter at 1.5 m above the soil line were felled at both Mississinewa and Celery Bog sites. Main stems were then cut into 2 m lengths and transported back to the laboratory. In February, these ash trees were cut into 42 blue and 42 green ash bolts, whereas in April they were cut into 13 blue and 13 green ash bolts, each 25 cm in length. To sterilize the bolts, we soaked the bolts in a 10% sodium hypochlorite (bleach) solution for 30 min within four days after they were cut into sections (Duan et al. 2011a). Bolts were then rinsed with water for 15 min to remove the bleach and then left to dry at ambient temperature for 12 h (Ulyshen et al 2010a). Individual eggs cut out from clutches laid on coffee filters were used to infest bolts. We attached a total of 13 eggs from at least three different clutches, either individually or in pairs, to each bolt by securing them with parafilm strips. Extra eggs were incubated in the laboratory and monitored to estimate hatch rate and determine the number of viable eggs transferred to ash trees as discussed in the field studies.

Bolts containing emerald ash borer eggs were kept moist by inserting one end approximately 3.8 cm deep into water soaked floral foam (Oasis floral foam deluxe, Smithers-Oasis Company, Kent, OH). Three bolts were inserted into each floral block and kept in uncovered 5.7-liter clear plastic bins to allow air circulation. Floral blocks were kept moist by adding water to the floral foam until saturated on Monday, Wednesday, and Friday of each week. Ash bolts were kept inside growth chambers at 27°C and a photoperiod of 16:8 (L:D) h, with 70% RH.

Emerald Ash Borer Growth in Ash Bolts. Forty-two blue and 42 green ash bolts were maintained in growth chambers for up to five weeks to track the size (gallery width and larval weight) of emerald ash borer larvae older than 2nd instar (Duan et al. 2013b) (1 March–7 April 2014). Ten green and ten blue ash bolts were removed at days 23, 30, and 37 (24, 31 March and 7 April, respectively), and emerald ash borer larvae were harvested using the peeling method previously described. Twelve green and 12 blue ash bolts were peeled on day 33 (16 bolts) and 34 (8 bolts) (3–4 April, referred to as day 33.5). The size and health of emerald ash borer larvae were recorded as previously described in field studies.

Tetrastichus planipennisi *Parasitism and Fitness.* Thirteen green and 13 blue ash bolts were infested with 12-d-old emerald ash borer eggs as previously detailed. Twenty-one days later these bolts were used in a laboratory experiment to determine the relative suitability of emerald ash borer larvae as hosts for *T. planipennisi*. Two ash bolts were inserted per wet floral foam block described previously. Each bolt was covered with a two liter plastic soda bottle that had the top five cm removed and the cut end was inserted into the foam block. In order to contain the emerging *T. planipennisi* and provide air circulation, two holes (~5 cm in diameter) were cut into the side wall of each bottle and covered with organdy cloth and sealed with hot glue along the edges. Ten *T. planipennisi* females and two males were added to each 2-liter bottle on 21 May 2014. Additionally, honey was added to the organdy cloth fabric as food for parasitoids. These parasitoids were shipped via FedEx overnight from the USDA APHIS Brighton Rearing Facility (Brighton, MI) in ventilated cups contained in a cooler with one or two (90 ml) cold packs inside.

Bottles were monitored daily for the presence of live female parasitoids. After two weeks (4 June), the bark of each ash bolt was removed with a hand chisel to reveal emerald ash borer. Larval size and health were recorded as previously described. All emerald ash borer larvae were put into 24-cell well plates to determine parasitism rate, sex ratio (female: male) per brood, and individuals per brood as described for the field experiments. Length of the left hind tibia (mm) of five adult females was measured and recorded with a Leica EC3 camera equipped with Leica Application Suite software. Voucher specimens of *T. planipennisi* were deposited in the PERC.

Statistical Analysis. Data from field and laboratory studies were analyzed using SAS (SAS® 9.4 Institute Inc., Cary, NC, 2014). A 2×2 contingency Chi squared test was conducted to determine if survivorship to the next life stage was independent of host plant species. This test was conducted on the number of emerald ash borer larvae recovered from viable transferred eggs (field and laboratory), healthy emerald ash borer larvae recovered in the field, and individuals developing J-shaped larval stage in the field. PROC GLIMMIX for Generalized Linear Effect Mixed Models was used to determine the extent to which ash species affected gallery width as a proxy for larval size (field and laboratory), weights of J-shaped larvae collected during the April harvest of emerald ash borer (field), and weight of growing emerald ash borer larvae (laboratory). Fixed effects were ash species, time (harvest times), and interaction of these two effects, while variation among ash logs nested within ash species was considered to be a random effect. Harvest time was not included in the model for weights of live emerald ash borer larvae collected from field grown ash, as this was recorded only once. Emerald ash borer weights were square root transformed to correct for nonnormality based on the shape of residual plots.

For the *T. planipennisi* experiments, 2×2 contingency Chi squared tests were used to compare rates of parasitism (laboratory) in green and blue ash bolts. We compared *T. planipennisi* sex ratios, brood sizes and female tibia lengths in a mixed effect model analysis of variance using PROC GLIMMIX to determine if these life history parameters were affected by ash species. The fixed effect was ash species with a random effect of ash log nested within ash species. PROC GLIMMIX was also used to determine if the brood size of *T. planipennisi* was affected by ash species and emerald ash borer larval instar. Fixed effects were ash species, emerald ash borer larval stage (3rd and 4th instars), and their interactions. There was also a random effect of ash bolt nested within ash species.

Results

Emerald Ash Borer Growth in Ash Trees in the Field. In the field experiments, 8.9 Growing Degree Days base 10°C ($GDD_{10°C}$) accumulated at the Celery Bog site, while 7.8 $GDD_{10°C}$ accumulated at the Peru site between the December 2013 and April 2014 harvests. Out of 550 eggs transferred to trees, 86.7% were estimated to be viable (Table 1). In total, 268 live emerald ash borer larvae were recovered during December and April from the 477 viable eggs placed on blue and green ash trees. Total emerald ash borer larvae recovered from viable eggs did not differ between green ash (52.59%) and blue ash (60.17%) (Table 1). The percentage of surviving emerald ash borer larvae did not differ between green and blue ash when harvested in December 2013 and in April 2014 (Table 2). In the December harvest, two (3.17%) of the recovered larvae on green ash and three (3.95%) of those recovered on blue ash, died in the callus tissue. In the April harvest, only two larvae (2.9%) recovered on green and one larva (1.67%) recovered on blue ash died in this manner. The percentage of larvae developing to the J-shaped stage in green ash (81.82%) was higher, but not significantly different from blue ash (66.18%) (Table 1). Emerald ash borer galleries in blue ash were significantly smaller ($P < 0.001$), while J-shaped larval weights in blue ash were only marginally smaller ($P = 0.071$) than those in green ash (Table 3).

Emerald Ash Borer Growth in Ash Bolts in the Laboratory. Out of 1,092 eggs placed on ash bolts, 91.9% were estimated to be viable. Over four sampling periods, 445 emerald ash borer larvae (1st instars through J-shaped stages) were recovered in similar proportions on green ash (45.62%) and blue ash (43.03%) (Table 4). Similarly, the number of live emerald ash borer larvae recovered did not differ between green and blue ash (Table 4). None of the larvae collected on green or blue ash bolts were killed by callus tissue. Emerald ash borer larvae on blue ash had significantly smaller gallery widths ($F = 19.43$; df = 1, 76; $P = < 0.001$; Fig. 1) and larval weights ($F = 10.99$; df = 1, 76; $P = 0.001$; Fig. 2) than those on green ash. Gallery width ($F = 42.69$; df = 3, 76; $P = < 0.001$) and larval weight ($F = 60.66$; df = 3, 76; $P = < 0.001$) increased significantly over time in both ash species. There was no significant interaction between harvest time and ash species for gallery width ($F = 1.37$; df = 3, 76; $P = 0.258$) and larval weight ($F = 2.13$; df = 3, 76; $P = 0.103$).

***Tetrastichus planipennisi* Recovered from Emerald Ash Borer-Infested Trees in the Field.** When green ash logs were harvested in September 2013, 100% of the emerald ash borer were in the larval stage (Table 5). In contrast, 20.1% of emerald ash borer were in the larval stage in blue ash logs when harvested in June 2014. Brood size averaged 68.6 *T. planipennisi* per emerald ash borer host with a sex ratio of 4.8:1.0 (female: male). Only one parasitized emerald ash borer larva was found in blue ash; however, this host desiccated before parasitoids could develop to adults. The parasitoids is assumed to be *T. planipennisi* because all other species were excluded by the cage.

***Tetrastichus planipennisi* Recovered From Emerald Ash Borer-Infested Ash Bolts in the Laboratory.** *Tetrastichus planipennisi* parasitism rates, brood sizes, sex ratios, and tibia lengths did not differ between emerald ash borer infesting green and blue ash (Table 6). Parasitoid brood size was also not significantly affected by ash species or the interaction between ash species and emerald ash borer larval stage (Table 7). However, parasitoid brood size was significantly greater on the 4th instars when compared to those on 3rd instars ($F = 6.30$; df = 1, 6; $P = 0.046$).

Table 1. Survival and development of a cohort of emerald ash borer larvae hatched from eggs placed on caged green and blue ash trees in West Lafayette and Peru, Indiana, during May and June 2013 and sampled in December 2013 and April 2014

Hosts	Eggs placed	Viable eggs[a]	Live larvae[b] (% viable eggs)	J-shaped larvae (% live larvae)
Green ash	289	251	132 (52.59)	108 (81.82)
Blue ash	261	226	136 (60.17)	90 (66.18)
			$\chi^2 = 0.78$ df = 1 $P = 0.378$	$\chi^2 = 1.28$ df = 1 $P = 0.256$

[a] Estimated from inspection of a subsample of eggs.
[b] Larvae lost as neonates unable to penetrate bark are excluded from this count.

Discussion

In our study, emerald ash borer was able to survive and develop on blue ash in the laboratory and in the field, even though larval growth (as measured by gallery width and larval weight) was initially reduced, the gap narrowed before pupation (Fig. 1 and 2). This suggests that while blue ash may not be as preferred by emerald ash borer as other ash species, it could become an important host for emerald ash borer. As such it could serve as a refuge for the introduced parasitoid *T. planipennisi*, if the ash forest species composition changes under the pressure of the emerald ash borer invasion.

In the laboratory, larvae in blue ash gained less weight, and produced narrower galleries than in green ash. This result is similar to those of other studies that found smaller larvae produced in resistant Manchurian ash when compared with black ash (Chakraborty et al

Table 2. Pre- and postwinter survival of a cohort of emerald ash borer larvae placed on caged green and blue ash trees in West Lafayette and Peru, Indiana, during May and June 2013.

Hosts	Sample time	Larvae recovered[a]	Live larvae (% recovered)[b]	
Green ash	December 4, 2013	63	61 (96.83)	$\chi^2 = 0.0001$; df = 1
	April 11, 2014	69	67 (97.10)	$P = 0.991$
Blue ash	December 4, 2013	76	73 (96.05)	$\chi^2 = 0.0091$; df = 1
	April 11, 2014	60	59 (98.33)	$P = 0.924$

[a] Larvae lost as neonates unable to penetrate bark are excluded from this count.
[b] Dead larvae were killed by callus tissue.

Table 3. Mean (±SE) emerald ash borer larval weights and gallery widths hatching from a cohort eggs placed on caged green and blue ash trees in West Lafayette and Peru, Indiana, in May and June 2013

Host	Gallery width (mm)[a]	J-shaped larvae weight (mg)[b]
Green ash	3.90 ± 0.06	67.9 ± 1.5
Blue ash	3.42 ± 0.08	61.9 ± 1.4
	$F = 24.52$	$F = 4.07$
	df = 1, 18	df = 1, 10
	$P = < 0.001$*	$P = 0.071$

[a] Include December 2013 and April 2014 samples.
[b] Only measured in April 2014.
* Indicates significant differences, $P < 0.05$.

Table 4. Survival of laboratory-reared emerald ash borer larvae hatched from eggs placed on green and blue ash bolts collected from West Lafayette and Peru, Indiana, and reared in the laboratory during 2014

Host	Eggs placed	Viable eggs[a]	Live larvae (% Viable eggs)[b]
Green ash	546	502	229 (45.62)
Blue ash	546	502	216 (43.03)
			$\chi^2 = 0.68$
			df = 1
			$P = 0.409$

[a] Estimated from inspection of a subsample of eggs.
[b] Larvae lost as neonates unable to penetrate bark are excluded from this count.

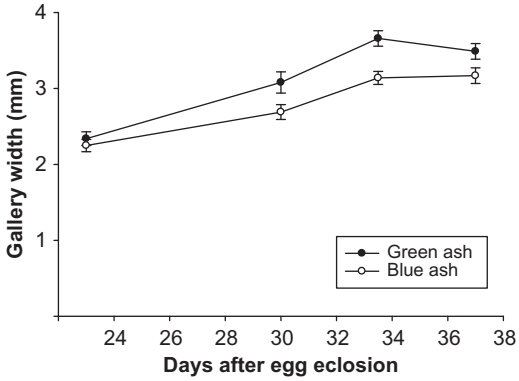

Fig. 1. Mean (±SE) gallery widths of emerald ash borer larvae reared in the laboratory on green and blue ash bolts on 4 dates after egg eclosion.

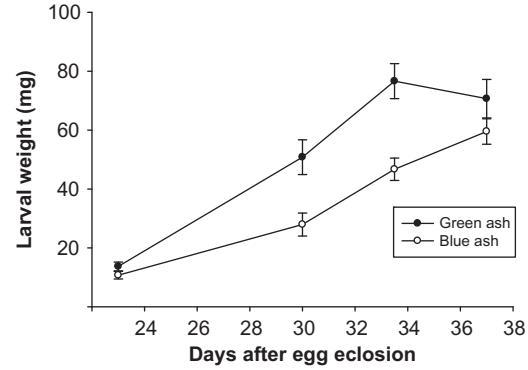

Fig. 2. Mean (±SE) emerald ash borer larval weight from laboratory green and blue ash bolts collected from West Lafayette and Peru, Indiana field sites in 2014.

2014). Although the mechanism that reduced larval growth has not been determined in blue or Manchurian ash, several candidate constituent or induced secondary metabolites have been implicated (Whitehill et al. 2012, Chakraborty et al. 2014, Poland et al. 2015). For example, the lignin, pinoresinol dihexoside, is unique to Manchurian ash phloem and may contribute to emerald ash borer resistance in this species (Eyles et al. 2007, Whitehill et al. 2012). Furthermore, in response to emerald ash borer larval feeding, Chakraborty et al. (2014) found a higher accumulation of pinoresinol A and lower total larval biomass in Manchurian ash than in black ash.

Although emerald ash borer larval densities on blue ash were lower than those reported on other North American ash species (Anulewicz et al. 2007, Tanis and McCullough 2015), mechanisms for these lower densities are unclear. In our field and laboratory studies of emerald ash borer in green and blue ash, we excluded parasitoids and woodpeckers and found no differences in larval mortality between species regardless of season (December vs April). Levels of larval recovery from the initial cohort of eggs were similar to those obtained by others using the same infestation procedures (Jennings et al 2013). In this field study, both blue and green ash encapsulated larvae by wound formation at very low rates (<4%). No encapsulation of emerald ash borer larvae was observed in bolts monitored in our laboratory study. These result are consistent with others who have found that field grown blue ash and other less

Table 5. Parasitism of emerald ash borer by *Tetrastichus planipennisi* confined in cages on green ash and blue ash trees in West Lafayette and Peru, Indiana

Host (parasitoid release date)	No. emerald ash borer recovered (larvae to adult stages)	No. emerald ash borer larvae recovered before J-shaped stage[a] (% total larvae)	Larvae parasitized (%)	Parasitoid brood size	Sex ratio (F:M)
Green ash (Sept. 2013)	151	151 (100%)	17 (11.3%)	68.6 ± 12.9	4.8:1 ± 1.4
Blue ash (May 2014)	210	43 (20.5%)	1 (2.3%)	No data[b]	No data[b]
		$\chi^2 = 223.45$			
		df = 1			
		$P = < 0.001$*			

Mean (±SE) of emerald ash borer larvae recovered from trees two weeks after exposure to parasitoids, percentage emerald ash borer larvae parasitized by *T. planipennisi, T. planipennisi* brood sizes and sex ratios.

[a] Emerald ash borer in J-shaped larval stage are too deep in sapwood to be parasitized by *T. planipennisi*.

[b] Emerald ash borer host desiccated before parasitoid developed to adult.

* Indicates significant difference, $P < 0.05$.

Table 6. Parasitism of emerald ash borer larvae by *Tetrastichus planipennisi* on green and blue ash bolts in the laboratory during 2014 and their mean (±SE) tibia lengths, brood sizes, and sex ratios

Host	Larvae recovered	Larvae parasitized (% larvae recovered)	Tibia length (mm)	Parasitoid brood size	Sex ratio (F:M)
Green ash	62	30 (48.39)	0.614 ± 0.0077	36.62 ± 4.52	3.04 ± 0.74
Blue ash	53	29 (54.72)	0.603 ± 0.0086	33.04 ± 3.52	2.81 ± 0.49
		$\chi^2 = 0.4583$	$F = 0.62$	$F = 0.39$	$F = 0.07$
		df = 1	df = 1, 197	df = 1, 17	df = 1, 17
		$P = 0.498$	$P = 0.433$	$P = 0.541$	$P = 0.799$

Table 7. Mean (± SE) *Tetrastichus planipennisi* brood size emerging from second, third, and fourth emerald ash borer larval instars on green and blue ash bolts reared in the laboratory during 2014

Hosts	Second instar (*n*)	Third instar (*n*)	Fourth instar (*n*)
Green ash bolts	No data (0)	22.20 ± 3.80 (5)	40.84 ± 5.73 (19)
Blue ash bolts	12.5 ± 0.50 (2)	22.83 ± 2.85 (6)	38.72 ± 4.35 (18)
Sources of variation			
Host plant	$F = 0.07$; df = 1, 17; $P = 0.795$		
Host plant × instar	$F = 0.04$; df = 1,6; $P = 0.848$		

susceptible ash species fail to encapsulate emerald ash borer larvae (Tanis and McCullough 2015), but contrasts greatly with studies of a native congener of emerald ash borer, the bronze birch borer (*Agrilus anxius* Gory), where larvae are commonly killed by putative defenses and encapsulation on its North American hosts (*Betula* spp.) (Muilenburg and Herms 2012). Thus, it appears that putative defenses in the phloem do not increase larval mortality on blue ash. Rather, lower emerald ash borer densities in blue ash more likely results from the nonpreference of adult females to lay eggs or feed on the leaves of this tree species (Anulewicz et al. 2006, 2007; Pureswaran and Poland 2009; Chen and Poland 2010; Tanis and McCullough 2012, 2015; Carson 2013).

Our field study of parasitism on caged live ash trees failed to adequately test effects of host species on the capacity of emerald ash borer larva to serve as hosts for *T. planipennisi*. Our late parasitoid release on blue ash occurred after nearly 80% of the emerald ash borer larvae had matured into the J-shape stage and were too deep in the sapwood to be attacked by *T. planipennisi* (Ulyshen et al. 2010b). In contrast, none of the emerald ash borer larvae inside field grown green ash had entered the J-shape stage when *T. planipennisi* were released on these hosts eight months earlier. Thus, while the single parasitized larva reported on a caged emerald ash borer larvae in a live blue ash tree is interesting, our experiment was not an adequate comparison of *T. planipennisi* performance on emerald ash borer larvae in different ash hosts.

In contrast, our laboratory study of parasitism by *T. planipennisi* was conducted with equal numbers of susceptible larvae in blue and green ash hosts and allowed us to test host plant effects on parasitoid fitness. In fact, observed parasitism rates were consistent with attack rates reported in laboratory reared green and tropical ash (Duan and Oppel 2012). Sex ratios we observed in the laboratory (~3:1) are consistent with those found in field populations of *T. planipennisi* in China and the US (Liu and Bauer 2007, Duan et al. 2011b, 2012a). The numbers of *T. planipennisi* emerging from 3rd and 4th instars (Table 6) were not affected by ash species, yet were consistent with brood sizes in naturally infested green ash reported by Ulyshen et al. (2010b). Finally, tibia length, our proxy measure of

adult female size and fitness, did not differ between ash species in our laboratory study. Thus, ash species did not affect the suitability of emerald ash borer larvae as hosts for *T. planipennisi* in the laboratory.

If *T. planipennisi* can locate and attack emerald ash borer larvae in blue ash, then this tree species may contribute to this parasitoid's persistence in North American forests when more susceptible ash host species are dead and emerald ash borer larvae become scarce. In their native range, emerald ash borer and *T. planipennisi* persist at low levels in resistant Asian species including Manchurian ash (Liu et al. 2003). Likewise, emerald ash borer densities in blue and Manchurian ash are low in North America (Anulewicz et al. 2007, Rebek et al. 2008, Duan et al. 2012a, Tanis and McCullough 2015). In our studies, *T. planipennisi* thrived on emerald ash borer larvae when constrained to blue ash. Although the ability of *T. planipennisi* to locate larvae in uncaged blue ash has yet to be determined, other parasitoids have been reported to attack emerald ash borer in blue and Manchurian ash (Tanis and McCullough 2015).

Blue ash and possibly other less susceptible species may have the potential to serve as a refuge for parasitoids after the initial emerald ash borer invasion kills or greatly reduces the remaining ash tree species. Even though blue ash hosts are less suitable to emerald ash borer larvae compared with its more preferred green ash hosts, it can be hypothesized that blue ash persistence in forests is explained by defensive mechanisms that impeded emerald ash borer fitness and reduce the likelihood of adult feeding and oviposition (Pureswaran and Poland 2009, Rigsby et al. 2014, Tanis and McCullough 2015). These factors have the potential to act together to slow the rate of emerald ash borer population growth and prolong the time during which emerald ash borer densities are below levels that overwhelm plant defenses and increase the opportunity for control by biological control agents (MacQuarrie and Sharbach 2015). Nevertheless, the capacity of *T. planipennisi* to attack and develop on emerald ash borer larvae in blue ash with no fitness loss shows some promise for the persistence of classical biological control agents introduced to control emerald ash borer.

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