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## **OVIPOSITION AND DEVELOPMENT OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) (COLEOPTERA: BUPRESTIDAE) ON HOSTS AND POTENTIAL HOSTS IN NO-CHOICE BIOASSAYS**

Andrea C. Anulewicz<sup>1</sup>, Deborah G. McCullough<sup>1,2</sup>, and Deborah L. Miller<sup>3</sup>

### **ABSTRACT**

Emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) is an invasive phloem-feeding pest native to Asia. It was first identified in North America in 2002 and has killed millions of ash (*Fraxinus* spp.) trees in southeast Michigan and Essex County, Ontario. Since then, additional populations have been discovered across Michigan and in areas of Ohio, Indiana, Illinois, Maryland, Pennsylvania and West Virginia. In Asia, *A. planipennis* reportedly colonizes other genera, including species of *Juglans*, *Pterocarya* and *Ulmus*. In North America, attacks on non-ash species have not been observed but there is concern about host switching as ash mortality progresses. From 2003 to 2005, we evaluated *A. planipennis* oviposition and larval development on 4 North American ash species: green ash (*F. pennsylvanica* Marshall), white ash (*F. americana* L.), black ash (*F. nigra* Marshall), blue ash (*F. quadrangulata* Michaux), and 6 potential alternate hosts including privet (*Ligustrum* and *Forestiera* spp.), Japanese tree lilac (*Syringa reticulata* (Blumb) Hara), American elm (*Ulmus americana* L.), black walnut (*Juglans nigra* L.), hickory (*Carya ovata* (Miller) K. Koch) and hackberry (*Celtis occidentalis* L.). In no-choice tests using cut branches in cages, female *A. planipennis* oviposited on all species tested. Larvae on green ash, white ash, black ash, blue ash and privet developed to the second instar before branches desiccated. Larvae attempted to feed on some black walnut, Japanese tree lilac, American elm and hackberry branches but died as first instars. There were no feeding attempts on hickory branches. We also conducted no-choice tests by placing adult *A. planipennis* in cages that encircled the lower 1 m of the trunk of live green ash, white ash, black walnut and Japanese tree lilac nursery trees. High densities of larvae developed on green ash and white ash nursery trees but there was no evidence of any larval survival, feeding, or development on the Japanese tree lilac or black walnut nursery trees.

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### **INTRODUCTION**

Emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) has become one of the most important invasive forest pests in North America. This phloem-feeding insect is native to northeastern China, Japan, Korea, Mongolia, the Russian Far East and Taiwan (Yu 1992, Jendek 1994). *A. planipennis* was recently synonymized with *A. marcopoli* Obenberger (in China), *A. feretrius* Obenberger (in Taiwan) and *A. marcopoli ulmi* Kurosawa (in Korea and Japan) by Jendek (1994). *A. planipennis* was likely introduced into North America in solid wood packing material by the early 1990s but was first identified in 2002 as the cause of widespread ash (*Fraxinus* spp. [Oleaceae]) mortality in southeast Michigan and Essex County, Ontario (Cappaert et al. 2005b, Poland and McCullough 2006, Siegert et al. 2007b). Since then, additional populations

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have been found across lower Michigan and in areas of Ohio, Indiana, Illinois, Pennsylvania and Maryland (EAB Info 2007).

The life cycle of *A. planipennis* in North America is generally completed in 1 yr, although some individuals may require 2 yr to complete development (Cappaert 2005a, Siegert and McCullough 2005, Petrice and Haack 2006, Siegert et al. 2007a). Studies in southeast Michigan have shown emergence of *A. planipennis* adults generally begins in mid- to late May (Brown-Rytlewski and Wilson 2005, Cappaert et al. 2005b). Adult beetles live for 3-6 wk (Cappaert et al. 2005b). Beetles feed on ash foliage for 5-7 d before mating and females feed for at least another 5-7 d before oviposition begins (Bauer et al. 2004). Females generally lay 30-60 eggs (Chinese Academy of Science 1986, Yu 1992), although individuals have produced up to 258 eggs in laboratory conditions (Lyons et al. 2004). Eggs are laid individually on or just under the bark surface and inside bark cracks and crevices (Chinese Academy of Science 1986, Yu 1992, Bauer et al. 2004). Eggs are initially creamy white, gradually changing to reddish brown after a few days and hatch in 2-3 wk. Larvae feed under the bark in the cambium and phloem from July to October in distinctive serpentine galleries that eventually score the outer sapwood. Larval galleries disrupt vascular tissue, effectively girdling and killing branches or the entire tree. Larvae pass through four instars (Cappaert et al. 2005b) and most overwinter as prepupal larvae in the outer sapwood or in the thick bark of large trees (Cappaert et al. 2005a).

Although *A. planipennis* is not considered a major pest in Asia (Akiyama and Ohmomo 2000), this invader is aggressively attacking healthy, as well as stressed ash trees in North America. More than 20 million green ash (*F. pennsylvanica* Marsh.), white ash (*F. americana* L.), black ash (*F. nigra* Marsh.) and blue ash (*F. quadrangulata* Michx.) had been killed in southeast Michigan alone as of early 2007 (EAB Info 2007). In China, the host range of *A. planipennis* is reportedly limited to *Fraxinus* sp., including *F. chinensis* Roxb., *F. mandshurica* Rupr., *F. pennsylvanica*, *F. rhynchophylla* Hance and *F. velutina* Torr. (Chinese Academy of Science 1986, Yu 1992, Liu et al. 2003). In Japan, however, *A. planipennis* has been recorded from *F. mandshurica* var. *japonica* Maxim., *Juglans mandshurica* Maxim var. *sieboldiana* Makino and var. *sachalinensis* (Miyabe et Kudo) Kitamura, *Pterocarya rhoifolia* Sied. et Zucc. (Juglandaceae) and *Ulmus davidiana* var. *japonica* (Rehd.) Nakai (Ulmaceae) (Akiyama and Ohmomo 1997 cited in Haack et al. 2002, Sugiura 1999). North American members of these genera or families, including American elm (*U. americana* L.) (Ulmaceae), black walnut (*J. nigra* L.) (Juglandaceae), and hickory (*Carya* sp.) (Juglandaceae), are common in landscapes and wooded areas in North America, including areas with high density *A. planipennis* populations. Other members of the ash family (Oleaceae), such as privet (*Ligustrum* spp.) and Japanese tree lilac (*Syringa reticulata* (Blumb) Hara), are also commonly used in landscape settings throughout much of the eastern and central U.S (Boris and Kielbaso 1999, MacFarlane and Meyer 2005, Poland and McCullough 2006).

To date, attacks on non-*Fraxinus* genera have not been observed in North America but there is concern about the potential for additional hosts to be affected by *A. planipennis*, especially as population densities increase and ash trees die. A related species, the twolined chestnut borer, *A. bilineatus* (Weber), became an important pest of oaks (*Quercus* sp; Fagaceae.) after the introduction of chestnut blight caused the demise of American chestnut [*Castanea dentata* (Marsh.) Borkh; Fagaceae] (Haack and Acciavatti 1992). If *A. planipennis* can attack additional species, environmental and economic impacts in North America will increase dramatically. Given the extensive damage already caused by *A. planipennis*, there is an urgent need to evaluate the susceptibility of other common landscape and forest trees that may serve as alternate hosts for *A. planipennis*. We conducted no-choice studies using cut branches and live trees to assess *A. planipennis* oviposition and larval development on North American ash species and potential alternate host species.

## MATERIALS AND METHODS

## Cut branch bioassays

**2003 bioassay.** Branches of green ash, white ash, American elm, black walnut, shagbark hickory [*Carya ovata* (Miller) K. Koch.] and hackberry (*Celtis occidentalis* L.), a relative of elm in the Ulmaceae family, were harvested from healthy trees at Michigan State University's W.K. Kellogg Forest, Kalamazoo Co., MI on 16 June 2003. Black ash was collected from Kensington Metropark, Oakland Co., MI on 28 May 2003. Swamp privet (*Forestiera acuminata* (Michx.) Poir.), Chinese privet (*Ligustrum sinense* Lour.) and glossy privet (*L. lucidum* Ait.) (hereafter collectively referred to as 'privet') branches were collected in Stoneville, Mississippi from the Delta Experimental Forest on 4 June 2003 and sent by overnight mail to Michigan. Branches were harvested from multiple trees of each species. The ends of the freshly cut bolts were waxed after cutting to reduce desiccation. Bolts remained in cold storage at 1-2° C with RH ≥ 80% until the bioassay began. On 2 July, 7 branches of green and white ash and 5 branches of the remaining species, each approximately 6.5 to 10 cm diam., were cut to approx. 17 cm in length (44 sections total) (Table 1). Each section was then cut in half vertically down the center. All cut surfaces were again waxed with paraffin to slow desiccation.

Each branch section was placed in a clear, plastic box (14 × 20 × 10 cm) ventilated with small holes in the sides and lid. One green ash leaf, with the petiole submerged in a vial of water to slow desiccation, was included in each box. Green ash leaves were collected from an untreated, infested tree in Washtenaw County.

Adult beetles were collected on 1 July 2003 from wild populations at Bicentennial Park in Livonia, Wayne Co., MI. Although age and mating status of the beetles were unknown, they exhibited behaviors consistent with mated adults and oviposition activity. Adult beetles were caged in a screened box overnight and provided with green ash leaves for feeding. The following day, beetles were sexed and one male/female pair was placed in each plastic box (44 pairs total). There were seven replicates (consisting of one branch section) of the ash species and five replicates of the non-ash species. Boxes were kept in a growth chamber at 24°C, 70% RH, and 16:8 light:dark photoperiod. Condensation and frass in boxes were removed at 2-3 day intervals. Growth chamber humidity was eventually reduced to 40% to prevent mold.

Boxes were checked and ash foliage was replaced twice a week until the female beetle in the box died. Number of eggs laid on the inner surface of each box was recorded. Those eggs were removed each time foliage was replaced. Upon death of the female beetle, the branch section was removed from the growth chamber then stored on a lab bench at 24°C. The first branch section was removed from its box on 9 July and the last section was removed on 11 August 2003. Branch sections were left undisturbed for 34 to 52 days after removal, allowing time for egg hatch and early instar development.

To evaluate branch sections, we first inspected the outer bark for eggs with a magnifying lens. Eggs were removed with forceps to prevent them from being recounted. Forceps or a small knife were used to chip off bark flakes to reveal eggs hidden in bark layers. After 15 min of searching, total number of eggs on the branch section was recorded.

Larval feeding was assessed by carefully peeling bark down to the wood using a knife or chisel. Number and stage of larvae on each branch section were recorded. Surface area was calculated using the vertical length and horizontal width of the sapwood surface on each branch section (Table 1). Gallery density was standardized by 100 cm<sup>2</sup> of surface area.

**2004 bioassay.** We repeated this bioassay three times in 2004 with the same eight species used in 2003, plus the addition of blue ash and Japanese

Table 1. Summary results (mean ± SE) of the 2003 no-choice cut branch bioassay for *Agrilus planipennis* early instar larval development on three North American ash species and five potential alternate host species.

	Green ash	White ash	Black ash	Privet <sup>1</sup>	American elm	Black walnut	Hickory	Hackberry
No. branch sections	7	7	4	5	5	4	5	5
Diameter (cm)	8.3 ± 0.2	8.4 ± 0.1	6.6 ± 0.3	4.8 ± 0.4	7.8 ± 0.1	8.0 ± 0.1	9.2 ± 0.1	8.9 ± 0.2
Length (cm)	16.7 ± 0.3	17.3 ± 0.3	17.3 ± 0.5	17.0 ± 0.3	17.2 ± 0.4	17.0 ± 0.1	15.2 ± 0.6	16.4 ± 0.6
Surface area (cm <sup>2</sup> )	257 ± 12	247 ± 17	232 ± 13	176 ± 14	243 ± 2	1251 ± 22	251 ± 12	259 ± 6
Total galleries	44	72	37	22	0	0	0	0
No. branch sections	3	4	3	4	0	0	0	0
Mean no. galleries/100cm <sup>2</sup>	2.3 ± 1.11 <b>ab</b>	4.2 ± 1.70 <b>a</b>	4.1 ± 1.38 <b>a</b>	2.7 ± 1.35 <b>a</b>	0 <b>b</b>	0 <b>b</b>	0 <b>b</b>	0 <b>b</b>
Total unsuccessful larval feeding attempts	0	0	0	0	3	11	0	5
No. branch sections w/ unsuccessful larval feeding attempts	0	0	0	0	1	2	0	1

Within rows, letters following the mean values indicate statistically significant differences among species (Kruskal-Wallis test and nonparametric multiple comparison procedure;  $P < 0.05$ ).

<sup>1</sup>Includes *F. acuminata*, *L. sinense*, and *L. lucidum*.

tree lilac. Branches from several trees of each species for the bioassays were collected from the same sites used in 2003 on 24 May and again on 12 July 2004. Blue ash was harvested from a private woodlot in Superior Township, Washtenaw Co., MI on the same dates. Branches of Japanese tree lilac were collected from trees on the campus of Michigan State University, Ingham Co., MI on 20 May 2004. A different species of privet, *Ligustrum amurense* Carr., was collected from Wooster, Wayne Co., OH on 25 May 2004 and sent by overnight mail to Michigan. All branches were maintained in cold storage at 1-2°C with a minimum of 80% RH until needed for bioassays. Eight branches of each species were cut to approx. 17 cm in length (Table 2) at the beginning of each bioassay; however, the branches were not cut vertically as they were in 2003. Cut ends were waxed with paraffin. Boxes were set up using the same methods as in 2003, except that 20 ml vials of water with cotton wicks were included in each box to provide water for the beetles. Additional holes were added to the sides and lids of the boxes to increase ventilation.

Adult beetles used in the 2004 bioassays were reared from bolts of infested ash trees collected in heavily infested sites in southeast MI. After emergence, similarly-aged groups of beetles (approx. 200) were placed in screen cages (60 × 60 × 60 cm) to feed on green ash foliage collected from an untreated, infested green ash tree. Cages were kept in growth chambers at 24°C, 60% RH and 16:8 light:dark photoperiod. Beetles were allowed to feed and mate for 2 wk before bioassays began.

On each day that bioassays were initiated, beetles were sexed and 1 male/female pair was placed in each box (80 pairs total). The first bioassay, conducted from 18 June to 5 August, included 2 replicates of each species (20 branch sections total). The second and third bioassays were comprised of 3 replicates of each species and were conducted from 14 July to 14 August and 20 August to 14 September, respectively. Boxes were kept in growth chambers at 24°C, 60% RH and 16:8 light:dark photoperiod. If a female beetle died during the first 3 d of the bioassay, she was replaced with a similarly-aged beetle. Thereafter, upon death of the female beetle, the branch was removed and stored on a lab bench at 24°C. The total number of eggs laid on the inside of each box was recorded at this time.

The entire surface of each branch was inspected for eggs and bark was peeled to assess larval feeding 42-45 d after the branch was removed from its box. Diameter of each branch was measured and total bark surface area was calculated (Table 2). Branches were inspected, peeled, and larval density assessed using the same methods as in 2003.

#### Caged stem bioassay

**2004 bioassay.** On 12 May 2004, green ash, white ash, Japanese tree lilac, and black walnut balled-and-burlapped trees were delivered from Poplar Farms Nursery, Waterman, IL and planted at Matthaei Botanical Gardens, Washtenaw Co., MI. Trees were planted in an open field, 3 m apart, in 5 rows of 8 trees using a randomized complete block design (40 trees total). The partially exposed root balls were covered with composted wood mulch and drip line irrigation was installed for frequent watering. Trees had an overall mean ( $\pm$  SE) diameter at breast height (DBH) of 5.8 ( $\pm$  0.1) cm (Table 3) and height of 4.0 ( $\pm$  0.2) m.

Screen cages, approx. 90 cm tall and 30 cm in diameter, were constructed to confine live beetles around the lower 1 m of the stem of each tree. Two 120 cm long pieces of contractor's lathe driven into the root ball provided support for the cage. A green plastic disc (25 cm diam.) was cut to fit the stem and served as the top of the cage. A small piece of carpet (8 cm diam.) was wrapped around the stem under the plastic disc to close any gaps and ensure beetles could not escape. The plastic disc was stapled to the lathe and 18 × 16 aluminum mesh

Table 2. Summary results (mean ± SE) of the 2004 no-choice cut branch bioassay for *Agrilus planipennis* early instar larval development on three North American ash species and five potential alternate host species.

	Green ash	White ash	Black ash	Blue ash	Privet	Japanese tree lilac	American elm	Black walnut	Hickory	Hackberry
No. branch sections	8	8	8	8	8	8	8	8	8	8
Diameter (cm)	9.0 ± 0.6	8.8 ± 0.5	7.7 ± 0.2	7.2 ± 0.3	6.4 ± 0.3	4.6 ± 0.3	9.3 ± 0.2	9.5 ± 0.1	9.7 ± 0.1	9.3 ± 0.2
Length (cm)	16.8 ± 0.3	16.8 ± 0.3	16.6 ± 0.5	16.5 ± 0.3	16.9 ± 0.2	16.9 ± 0.2	15.4 ± 1.1	15.7 ± 0.6	17.0 ± 0.3	16.6 ± 0.3
Surface area (cm <sup>2</sup> )	409 ± 25	409 ± 17	352 ± 9	317 ± 21	319 ± 15	225 ± 16	408 ± 31	393 ± 23	458 ± 8	406 ± 23
Total galleries	110	105	83	95	77	0	0	0	0	0
No. branch sections w/ galleries	6	5	7	6	6	0	0	0	0	0
Mean no. galleries/100 cm <sup>2</sup>	3.6 <b>a</b> ± 1.08	3.1 <b>a</b> ± 1.43	2.9 <b>a</b> ± 0.89	3.3 <b>a</b> ± 1.43	3.1 <b>a</b> ± 1.08	0 <b>b</b>	0 <b>b</b>	0 <b>b</b>	0 <b>b</b>	0 <b>b</b>
Mean % area w/ galleries	49.2 ± 11.65	4.8 ± 2.32	12.0 ± 7.19	23.7 ± 14.65	8.2 ± 2.20	N/A	N/A	N/A	N/A	N/A
Total unsuccessful larval feeding attempts	0	0	0	0	0	30	8	26	0	1
No. branch sections w/ unsuccessful larval feeding attempts	0	0	0	0	0	4	1	3	0	1

Within rows, letters following the mean values indicate statistically significant differences among species (Kruskal-Wallis test and nonparametric multiple comparison procedure;  $P < 0.05$ ).

Table 3. Mean ( $\pm$  SE) tree diameter at breast height (DBH), surface area and number of *Agrilus planipennis* galleries per m<sup>2</sup> for four species of trees used in no-choice caged stem bioassays in 2004 and 2005.

	Green ash	White ash	Tree lilac	Black walnut
<b>2004</b>				
DBH (cm)	6.0 $\pm$ 0.2	6.3 $\pm$ 0.2	4.6 $\pm$ 0.	16.4 $\pm$ 0.2
Surface area (m <sup>2</sup> )	0.13 $\pm$ 0.00	0.14 $\pm$ 0.01	0.09 $\pm$ 0.00	0.13 $\pm$ 0.00
Total no. of galleries	49	1	0	0
Mean no. galleries/m <sup>2</sup>	34.8 $\pm$ 24.9 <b>a</b>	0.8 $\pm$ 0.8 <b>ab</b>	<b>0 b</b>	<b>0 b</b>
<b>2005</b>				
DBH (cm)	6.8 $\pm$ 0.2	6.6 $\pm$ 0.1	5.4 $\pm$ 0.2	7.0 $\pm$ 0.2
Surface area (m <sup>2</sup> )	0.17 $\pm$ 0.01	0.17 $\pm$ 0.01	0.11 $\pm$ 0.00	0.18 $\pm$ 0.01
Total no. of galleries	136	98	0	0
Mean no. galleries/m <sup>2</sup>	78.2 $\pm$ 27.0 <b>a</b>	60.0 $\pm$ 38.4 <b>a</b>	<b>0 b</b>	<b>0 b</b>

Within rows, letters following the mean values indicate statistically significant differences among species (Kruskal-Wallis test and nonparametric multiple comparison procedure;  $P < 0.05$ ).

screen was wrapped around the circumference of the cage, stapled to the plastic disc, and sealed with caulk. Excess screen above the disc was wrapped tightly around the stem of the tree and secured with a plastic cable tie. Excess screen at the bottom of the cage overlapped the root ball and was covered with mulch to seal the bottom. The lower 25 cm of the cage, above the root ball, was covered with plastic wrap. The top of the root ball was covered with approx. 20 cm of sand to prevent beetles from reaching the mulch. A small flap (approx. 15 cm  $\times$  20 cm) was cut into the screen 50 cm above the base of the cage and secured with Velcro® to provide access into the cage. A 5 cm long piece of garden hose was attached vertically to one piece of lathe inside the cage to hold shoots of green ash foliage collected from untreated, infested green ash trees. Stems of ash foliage were placed in plastic floral water pics to slow desiccation and foliage was changed every 2-3 d as needed.

Beetles used for this study were reared from infested logs following methods described in the 2004 bioassays. After 2 wk of feeding, beetles were sexed and 3 male/female pairs were placed in each tree cage (six beetles per cage). Cages were checked and foliage misted with water daily. Beetles that died were replaced with similarly-aged beetles of the same gender 2-3 times between 10 June and 13 September 2004 (586 beetles total). Number of live female beetles in each cage was tallied daily to determine total number of adult female days. For example, if 3 female beetles were alive in a cage for 5 d, the number of beetle days for that cage was 15 d.

Four of the 10 blocks of trees, selected randomly, were harvested on 1 and 2 November and returned to the laboratory. The portion of the stem that had been caged was dissected between 1 and 7 November 2004. In the lab, bark was inspected for eggs then carefully peeled down to the sapwood using a drawknife. Number and stage of larvae were recorded and gallery density was standardized by m<sup>2</sup> of surface area. Remaining trees were felled and dissected in March 2005.

For all trees, relative roughness of the bark on the caged portion of the stem was qualitatively estimated as low (very few to no cracks or crevices in the bark), medium (moderate abundance of cracks and crevices for oviposition) or high (abundant cracks and crevices).



**2005 bioassay.** The study was repeated in 2005 using the same four species supplied by the same Illinois nursery. Root balls left from the 2004 trees were removed and new trees were planted in the existing holes on 13 May 2005. Exposed root balls were again covered with mulch and irrigation was re-installed. Trees had an average DBH of 7.1 ( $\pm$  0.2 SE) cm (Table 3).

Cages were constructed using methods from 2004. In addition, the stems of each tree were partially wrapped with thin strips of white, plastic Easy Gardener® Tree Wrap 513 (Easy Gardener Products, Inc., Waco TX) to provide beetles with additional crevices for egg laying. The wrap was cut into 2.5 cm wide strips and wrapped around the stems leaving 3-4 cm of exposed bark between each strip of tree wrap. Small, potted green ash trees (approx. 50 cm tall) were placed in each cage to provide foliage for feeding.

Beetles were reared and caged using methods from 2004. Beetles were replaced as they died, typically 1-2 times between 31 May and 19 August 2005 (408 beetles total). The caged portion of the ash stems were cut and dissected on 9 August 2006, after adult emergence was complete. Walnut and tree lilac stems were cut and dissected on 15 November 2006. Stems were peeled using methods from 2004.

**Statistical analysis.** Data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Variables were not normal and could not be normalized with transformations. Therefore, the nonparametric Kruskal-Wallis test was used to determine significant differences among species (Kruskal and Wallis 1952) using SAS statistical software (PROC NPAR1WAY, SAS Institute, Inc. 2003). When the Kruskal-Wallis test was significant, nonparametric multiple comparisons tests ( $P < 0.05$ ) were used to identify differences among species (Conover 1971, Zar 1984).

## RESULTS

### Cut branch bioassays

**2003 bioassay.** Adult *A. planipennis* lived from 8 to 43 d in the boxes and eggs were laid on branch sections from all tree species included in the test. We found a total of 525 eggs, including 323 eggs on 29 of the 42 branch sections we examined (1 black ash and 1 black walnut branch section with abundant mold were removed from the growth chamber and destroyed). Number of eggs found in 15 min of searching per branch section was highly variable, ranging from 0 to 65 with a mean of 12.5 ( $\pm$  2.5) eggs per branch section. There were 202 eggs laid on the lid, sides, and bottom of 20 of the 44 plastic boxes. Number of eggs on the plastic boxes (not on the branch sections) ranged from 0 to 93 per box with an average of 4.8 ( $\pm$  2.2).

White ash, black ash, and privet branches had significantly higher densities of larval galleries (number of galleries per 100 cm<sup>2</sup>) than elm, walnut, hickory, and hackberry, while densities on green ash were intermediate ( $\chi^2 = 17.53$ ,  $df = 7$ , 34,  $P = 0.0143$ ) (Table 1). Fifty-three percent of the larvae found on ash were first instars, 33% were second instars and 14% were third instars. All larvae were either dead or dying when the branches were peeled because of the relatively rapid desiccation of the small branch sections. A total of 22 galleries were excavated on four of the five privet sections (Table 1). Four galleries contained second instar larvae, while the others had first instar larvae. When only branch sections with galleries (ash and privet) were analyzed, gallery density did not differ significantly ( $\chi^2 = 1.19$ ,  $df = 3$ , 19,  $P = 0.75$ ).

Although there were no actual galleries on elm, walnut, or hackberry sections, first instar larvae did occasionally attempt to feed on these species. Unsuccessful feeding attempts were observed on 1 elm, 2 walnut and 1 hackberry sections (Table 1). These feeding attempts consisted of galleries less than 1 mm

wide and 5 mm to 10 cm long on the sapwood surface. All larvae that attempted to feed on these species, however, died without any development or growth. No evidence of any feeding attempts were found on hickory sections. Density of unsuccessful feeding attempts among elm, walnut, hickory, and hackberry did not differ significantly ( $\chi^2 = 3.53$ ,  $df = 3$ ,  $15$ ,  $P = 0.09$ ).

**2004 bioassay.** Adult *A. planipennis* lived from 8 to 63 d in the boxes. Females laid eggs on all 10 of the species tested in 2004. We found a total of 577 eggs on 48 of the 80 branches. Number of eggs found per branch section in 15 min of searching ranged from 0 to 73, with an average of  $7.2 (\pm 1.3)$ . A total of 79 eggs were laid on the insides of 8 of the 80 boxes. Number of eggs laid on the boxes (not on branches) ranged from 0 to 47 with an average of  $1.0 (\pm 0.6)$ .

We found galleries on 24 of the 32 ash branches and on 6 of the 8 privet branches (Table 2). As in 2003, all larvae that were recovered were either dead or dying because of branch desiccation. Overall, there was a total of 393 galleries on 24 ash branches and gallery density averaged across all four ash species was  $12.3 (\pm 2.3)$  galleries per branch. The four ash species and privet had significantly higher gallery densities than tree lilac, elm, walnut, hickory and hackberry ( $\chi^2 = 38.11$ ,  $df = 9$ ,  $70$ ,  $P < 0.0001$ ) (Table 2). Sixty-two percent of the larvae on ash were first instars, 24% were second, 12% were third and 2% were fourth instars at the time of branch dissection. A total of 77 galleries were excavated on 6 privet branches (Table 2). There were 51 first instars, 7 second instars and 14 third instar larvae.

Four tree lilac, 1 elm, 3 walnut and 1 hackberry branches had unsuccessful *A. planipennis* feeding attempts made by first stage larvae (Table 2). No galleries or feeding attempts were found on hickory branches. As in the 2003 bioassay, when larvae attempted to feed on species other than ash and privet, galleries were abnormally small and resulted in larval death. Similarly, the density of unsuccessful feeding attempts did not differ significantly among these species ( $\chi^2 = 8.05$ ,  $df = 5$ ,  $35$ ,  $P = 0.08$ ).

#### Caged stem bioassays

**2004 bioassay.** Overall, the mean number of female beetle days per cage was  $40 (\pm 2)$  d. Individual female beetles lived from 1 to 34 d in cages. A total of 49 galleries were excavated on 4 green ash stems and a single gallery was excavated on 1 white ash stem. No galleries or feeding attempts were found on the other 6 green ash or 9 white ash stems, nor on any of the walnut or tree lilac stems (Table 3). Green ash stems had significantly greater mean gallery densities than black walnut and tree lilac stems, but did not differ significantly from white ash stems ( $\chi^2 = 10.08$ ,  $df = 3$ ,  $36$ ,  $P = 0.0179$ ) (Table 3).

Bark roughness was ranked as high on all of the black walnut stems, while all of the Japanese tree lilac stems had medium bark roughness. Four of the 10 green ash stems had high bark roughness. All of the white ash stems and the remaining 6 green ash stems had low bark roughness. Three of the 4 green ash stems that had eggs and galleries had rough, flaky bark; the other infested green ash stem had smooth bark.

**2005 bioassay.** Overall, the mean number of beetle days per cage was  $17 (\pm 2)$ . Individual female beetles lived from 1 to 29 d in cages. We found a total of 136 galleries on 8 green ash stems and 98 galleries on 8 white ash stems (Table 3). Thirty-two small unsuccessful feeding attempts were found on 6 of the tree lilac stems. These feeding attempts were similar to those observed on cut branch sections. Unsuccessful feeding attempts were no more than 1 mm wide and 3 cm long and consistently resulted in death of first instar larvae. No galleries or feeding attempts were found on walnut stems (Table 3). Green and white ash stems had significantly higher gallery densities than black walnut and tree lilac stems ( $\chi^2 = 26.17$ ,  $df = 3$ ,  $36$ ,  $P < 0.0001$ ) (Table 3).

As in 2004, all of the black walnut stems had high bark roughness, while all Japanese tree lilac stems had medium bark roughness. All white ash stems had low bark roughness. Bark roughness was ranked as high on 5 of the 10 green ash stems and as low on the other 5 green ash. The 2 green ash stems without galleries and all of the white ash stems had smooth bark.

## DISCUSSION

Our first goal was to determine if *A. planipennis* would oviposit on non-ash species in a no-choice situation. We found eggs on all species used in the bioassays with cut branch sections, indicating that female beetles will lay eggs on species other than ash. Our results were at least partly attributable, however, to the no-choice setting presented to the beetles, given that eggs were also laid on the plastic boxes. In related studies conducted in field settings, *A. planipennis* females occasionally oviposited on cut logs or live trees of non-ash species, but at densities considerably lower than on ash species (Anulewicz 2006, Anulewicz et al. 2007).

Our ability to find eggs varied among species and branch sections and was particularly affected by bark texture. For example, privet, tree lilac and hackberry branch sections had relatively smooth bark with few crevices for females to hide eggs; eggs were easily detected with little manipulation of the bark. In contrast, the ash, elm, walnut and hickory sections often had rough bark and finding eggs in crevices and under bark layers was difficult.

When beetles were caged on live trees in 2004, they spent a substantial amount of time on the tops and sides of the cages, which likely reduced oviposition activity. Other studies have also reported poor oviposition when buprestids were reared in cages (Nash et al. 1951, Barter 1957, Akers and Nielson 1990). In 2004, most of the eggs that were laid on the stems of the live trees were on the green ash trees that had rough, flaky bark. We typically found eggs hidden in cracks and under bark flakes on these trees. We found only 2 eggs on smooth-barked white ash trees and only 1 yielded a viable larva. That egg was positioned at the site of an old, healed-over branch junction where the bark was creased and roughened. Barter (1957) reported that *A. anxius* Gory also favored rough bark for oviposition and Loerch and Cameron (1984) found that almost 70% of *A. anxius* eggs were laid in rough crevices in the dark triangular patch at branch origins.

When we repeated the study with live trees in 2005, we wrapped the stems of the trees with tree wrap. This provided additional crevices for egg laying and more oviposition occurred. Additional research needs to be done to further examine the influence of bark texture or tactile stimuli on *A. planipennis* oviposition behavior.

Our second goal was to determine whether early instar *A. planipennis* larvae would develop on non-ash species. In the cut-branch bioassays, all larvae were dead or dying when we dissected the branch sections simply because the small sections had dried out. Eighty-six percent of larvae on ash sections were first or second instars when branches were peeled. More larvae reached later instars on black ash and blue ash sections than on green or white ash sections, probably because the black and blue ash retained higher levels of moisture over time.

Unlike any of the other non-ash species studied, approx. 25% of the larvae on the privet branch sections developed to the second or third instar. In a previous study, male beetles survived 20 d on privet (*Ligustrum* sp.) and 13 d on swamp privet (*Forestiera* sp.) when caged with small discs of foliage. Male beetles survived 17 to 21 d on discs from green ash, black ash, evergreen ash (*F. uhdei* (Winzig) Lingelsh.) and velvet ash (*F. velutina* Torr.) leaves (Haack et al. 2004). Female longevity in this study was not reported. In two-choice and

multiple-choice tests with small discs of foliage in confined conditions in the laboratory, *A. planipennis* fed readily on privet foliage (*Ligustrum* and *Forestiera* spp.) in the presence of ash foliage (Haack and Petrice 2005). In a replicated study with intact leaves on shoots, mortality rates of male and female adult *A. planipennis* did not differ significantly between green ash or privet foliage over a 9 d bioassay, although beetles consumed significantly more green ash foliage than privet foliage (unpublished data). From 2003 to 2006, however, we intensively examined live privet shrubs growing in areas with high densities of *A. planipennis*. We have not observed any evidence of *A. planipennis* feeding on privet leaves or colonizing privet phloem, even in locations where ash trees growing near or adjacent to privet shrubs were killed by *A. planipennis*. Moreover, results of current research indicate *A. planipennis* larvae are unable to complete development on live privet (unpublished data). Further monitoring of privet in field settings and additional evaluation of live and cut privet would be useful, however, to identify secondary compounds or nutritional variables that may inhibit or support successful development of *A. planipennis* larvae.

Some first instar larvae attempted to feed on black walnut, American elm and hackberry but invariably failed to develop or survive and there was no evidence of any feeding on hickory. These North American species appear to be unsuitable hosts for *A. planipennis*.

In China, the host range of *A. planipennis* is apparently limited to *Fraxinus* spp. (Chinese Academy of Science 1986, Yu 1992, Liu et al. 2003) but attacks on *Juglans*, *Pterocarya* and *Ulmus* reportedly occur in Japan or Korea (Sugiura 1999, Akiyama and Ohmomo 2000). The apparent variation in host range within Asia may reflect difficulties in distinguishing the systematic relationships of *A. planipennis* and closely related species. Several previously described Asian beetles including *A. marcopoli* Obenberger, *A. molco-poli* [sic!]: Miwa and Chujo, *A. feretrius* Obenberger and *A. marcopoli* var. *ulmi* Kurosawa were synonymized as *A. planipennis* by Jendek (1994). Host range for *A. planipennis* subsp. *ulmi*, however, included *Juglans* sp. and *Ulmus* sp., as well as ash, in China, Japan, Korea and Mongolia (Akiyama and Ohmomo 2000). In Japan, *Ulmus propinqua* Koidz was reported to be a host for *A. molco-poli* Miwa (Kurosawa 1956). To-date, *A. planipennis* attacks in North America have only been observed on *Fraxinus* species, which may reflect a founder effect associated with an accidental introduction of relatively few colonists from one origin. An increased understanding of relationships among the geographically distinct *A. planipennis* populations in Asia would be useful in assessing the potential ability of *A. planipennis* populations in North America to shift or expand their host range in areas where virtually all ash trees have succumbed. Additional studies to evaluate development of North American *A. planipennis* beetles on the Asian tree species reported as hosts for *A. planipennis* in the literature would also be valuable.

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