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HOST RANGE AND PREFERENCE OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA:BUPRESTIDAE), IN NORTH AMERICA

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ANDREA CHRISTINE ANULEWICZ

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HOST RANGE AND PREFERENCE OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE), IN NORTH AMERICA

By

Andrea Christine Anulewicz

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ABSTRACT

HOST RANGE AND PREFERENCE OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE), IN NORTH AMERICA

By

Andrea Christine Anulewicz

Oviposition and early instar development of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) was evaluated for four North American ash and six potential alternate host species from 2003 to 2005. In no-choice tests, female *A. planipennis* oviposited and larvae developed to at least the second instar on green ash, white ash, black ash, blue ash, and privet. Unsuccessful larval feeding attempts were found on black walnut, Japanese tree lilac, America elm, and hackberry, but not on hickory. With the possible exception of privet, the non-ash species appear to be unsuitable for larval development.

In multiple-choice tests with cut logs and live trees in the field, female *A. planipennis* consistently laid more eggs on ash than non-ash species. Larvae completed development on all ash species, although green and white ash logs may be more preferred than black or blue ash logs. Unsuccessful larval feeding attempts were found on black walnut and Japanese tree lilac.

Field surveys of *A. planipennis* density and canopy dieback were conducted to assess host preference among three North American ash species. Canopy dieback and density of *A. planipennis* were significantly greater on green ash street trees than white ash street trees and increased in both species over time. *A. planipennis* density was also greater in white ash woodlot trees than blue ash woodlot trees.

DEDICATION

I would like to dedicate this thesis to my mother, Frances Elizabeth Agius, who passed away before I began graduate school. She was a remarkable woman who showed me how to appreciate the beauty of nature and taught me to fight for things that couldn't fight for themselves. She has always been and always will be a guiding force in my life.

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PREFACE

Emerald ash borer was discovered attacking ash trees in southeast Michigan in June of 2002. Reared beetles were immediately identified as a member of the genus *Agrilus*, but experienced taxonomists could not determine the species. Specimens were sent to specialists around the United States and Europe and, within two weeks of its initial discovery, it was identified as *A. planipennis* Fairmaire by an *Agrilus* specialist in Bratislava, Slovakia.

Initially, literature available on this species was limited to taxonomic descriptions and several paragraphs published in Chinese literature. Research efforts by scientists across the state of Michigan immediately focused on increasing our understanding of the beetle's life history traits and ecology, developing tools for detection and monitoring, and minimizing the impact of this pest.

This thesis focused on evaluating the host range and preference of *A. planipennis*. *Agrilus planipennis* in Michigan had only been observed attacking ash trees (*Fraxinus* sp.), but previous literature reported other hosts in several genera including *Fraxinus, Ulmus*, *Juglans*, and *Pterocarya*. In Chapter One, no-choice bioassays were conducted to evaluate *A. planipennis* oviposition and early instar development on cut branches of green, white, black, and blue ash, American elm, black walnut, privet, Japanese tree lilac, hickory, and hackberry and on live specimens of green and white ash, black walnut, and Japanese tree lilac.

Studies reported in Chapter Two were designed to evaluate *A. planipennis* oviposition preference and larval development on the same ten tree species, using logs attached to t-posts and infested ash trees in multiple-choice field experiments. Host

preference was also evaluated using nursery trees planted in an area heavily infested with *A. planipennis*. Chapter Three reports on field surveys of *A. planipennis* density and canopy dieback at multiple sites with a mix of ash species. My goal was to obtain a better understanding of *A. planipennis* and some of the factors that may be influencing its host range and preference in North America. Each chapter in this thesis is intended to be prepared as a manuscript for publication.

Appendix 2 contains the methods and results of another study designed to evaluate *A. planipennis* adult leaf-feeding preference among four North American species of ash using two-choice bioassays. I felt that this study didn't quite fit in with the contents of the other three chapters and lacked substantial, definitive, or conclusive results, but still warranted reporting in some format. In 2005, we had difficulty with beetle rearing and experienced substantial mortality and reduce fecundity. Laboratory bioassays, therefore, were unsuccessful and Appendix 3 includes the methods and limited results from no-choice and two-choice oviposition bioassays conducted that year.

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CHAPTER 1

Oviposition and Early Instar Development of the Emerald Ash Borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in No-Choice Laboratory Bioassays

INTRODUCTION

Emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae), an Asian phloem borer discovered in June 2002, is established in much of southeastern Michigan and Windsor, Ontario (Cappaert et al. 2005c). More than 35 distinct, localized infestations have been found since 2003 in other areas of Michigan, Ohio, and Indiana. *Agrilus planipennis* is native to northeastern China, Korea, Japan, Mongolia, the Russian Far East, and Taiwan (Yu 1992). *Agrilus planipennis* was recently synonymized with *A. marcopoli* Obenberger (in China), *A. feretrius* Obenberger (in Taiwan), and *A. marcopoli ulmi* Kurosawa (in Korea and Japan) (Jendek 1994). This phloem-feeding species was likely introduced into North America in solid wood packing material (Cappaert et al. 2005c), such as wooden containers, pallets, or dunnage made from ash wood containing bark strips.

The life cycle of *A. planipennis* in North America is generally completed in one year, although some individuals may require two years to complete development (Siegert et al. 2005). In southeast Michigan, *A. planipennis* adult emergence begins as early as mid- to late May or early June (Brown-Rytlewski and Wilson 2005, Cappaert et al.

2005c). Adult beetles live for 3-6 weeks (Cappaert et al. 2005c). Beetles feed on ash (*Fraxinus* sp. [Oleaceae]), foliage for 5-7 days before mating and females feed for another 5-7 days before oviposition begins (Bauer et al. 2004, Lyons et al. 2004). Females generally lay 30-60 eggs (Chinese Academy of Science 1986, Yu 1992), although egg viability declines as females age (D.L. Miller, pers. comm.). Eggs are laid individually on or just under the bark surface and inside bark cracks and crevices of live ash trees, from late June to late July (Bauer et al. 2004). Eggs are initially creamy white and gradually change to reddish brown after a few days (Bauer et al. 2004). Eggs hatch in two to three weeks. Larvae feed under the bark in the cambium and phloem from July to October, in distinctive 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. 2005c) and most overwinter as pre-pupal larvae in the outer cm of the sapwood or in the thick bark of large trees (Cappaert et al. 2005a).

Although the limited reports available suggest that *A. planipennis* is not a major pest in Asia (Akiyama and Ohmomo 2000), in North America, *A. planipennis* is aggressively attacking healthy, as well as stressed ash. In Michigan, an estimated 12-15 million green ash (*Fraxinus pennsylvanica* Marsh.), white ash (*F. americana* L.), black ash (*F. nigra* Marsh.), and blue ash (*F. quadrangulata* Michx.) have been killed as of March 2006. In China, host range of *A. planipennis* reportedly includes *Fraxinus* sp., including native species of *F. chinensis* Roxb., *F. mandshurica* Rupr., and *F. rhynchophylla* Hance (Chinese Academy of Science 1986, Yu 1992). In Japan, *A. planipennis* has been recorded from *F. mandshurica* var. *japonica* Maxim., *Juglans*

mandshurica var. *sieboldiana* (Maxim.) CK Schneid (Juglandaceae), *J. mandshurica* var. *sachalinensis* (Miyabe et Kudo) Kitamura, *Pterocarya rhoifolia* Sied. et Zucc. (Juglandaceae), and *Ulmus davidiana* var. *japonica* (Rehd.) Nakai (Ulmaceae) (Akiyama and Ohmomo 1997, Sugiura 1999). North American members of these genera or families, including American elm (*U. americana* L.), black walnut (*J. nigra* L.), 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), including privet (*Ligustrum* sp.) and Japanese tree lilac (*Syringa reticulata* Bl.), are also commonly used in landscape settings.

Attacks on non-*Fraxinus* genera have not been observed in North America, but there is concern about potential host switching as *A. planipennis* densities increase and ash trees die in the infested area. If *A. planipennis* can colonize and develop in additional species, environmental and economic impacts on forest resources could increase dramatically in North America. Given the extensive damage that *A. planipennis* has already caused to the *Fraxinus* resource in southeastern Michigan, there is an urgent need to evaluate the susceptibility of other commonly planted landscape and forest trees to serve as alternate hosts to *A. planipennis*. I conducted two no-choice studies using cut branches and live trees to determine if *A. planipennis* would oviposit on potential alternate host species and to evaluate early instar development on these species.

MATERIALS AND METHODS

Cut branch bioassays

2003 bioassay. Branches of green ash, white ash, American elm, black walnut, shagbark hickory [*Carya ovata* (Mill.) K. Koch.] (Juglandaceae), and hackberry (*Celtis occidentalis* L.*)*, a relative of elm in the Ulmaceae family, were harvested from 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, Chinese, and glossy privet [*Forestiera acuminate* (Michx.) Poir.], *Ligustrum sinense* Lour., and *L. lucidum* Ait., respectively] 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 were kept in cold storage at 1-2° C with a minimum of 80% RH. On 2 July, seven branches of green and white ash and five branches of the remaining species were cut to approx. 17 cm in length, then cut in half vertically down the center (Table 1.1). All cut surfaces were again waxed with melted paraffin to slow desiccation.

Each branch section was placed in a clear, plastic box (14 × 20 × 10 cm), along with one green ash leaf collected from an untreated, infested green ash tree in Washtenaw County. Ash leaf stems were submerged in vials of water to slow desiccation. Prior to the start of bioassays, boxes were washed with soapy water, rinsed in a dilute bleach solution and rinsed again in distilled water. Boxes were ventilated with four small holes in two sides and six holes in the lid.

Adult beetles were collected on 1 July 2003 from wild populations at Bicentennial Park, in Livonia, Wayne Co., MI. Adults were kept 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.

Boxes were checked and foliage was replaced twice a week until the female beetle in the box died. Condensation and frass were wiped from boxes and growth chamber humidity was eventually reduced to 40% to help prevent growth of *Penicillium* sp., *Aspergillus* sp., and *Beauveria* sp. mold. One black ash and one black walnut branch section were removed and disposed of due to excessive mold. Twenty-two of the 44 pairs of beetles were observed mating and mating dates were recorded. Number of eggs laid on each box was recorded and eggs were removed each time foliage was replaced. Adults lived from eight to 43 days in the boxes. Upon death of the female beetle, the branch section was removed, gently rinsed with water to remove any mold, air-dried, then stored on a lab bench at 24°C. The total number of eggs laid on each box was recorded at this time. 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 eggs to hatch and first instar larvae to develop. After that time, the entire surface of each branch section was inspected for eggs and bark was peeled to quantify larval feeding. Total bark surface area was calculated using the vertical length and horizontal

width of the outer bark surface on each branch section (Table 1.1). (Because branches were originally cut in half to fit in boxes, the "back" or cut portions of the branch without bark were not included in the bark surface area). The bark surface of each branch section was first inspected with a magnifying lens to locate eggs on the surface. Hatched and unhatched eggs were removed with forceps to prevent them from being recounted. White non-viable eggs were distinguished from brown viable eggs and were not included in the overall total. Less than ten non-viable eggs were found. 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 was recorded and standardized in two ways: by 100 cm^2 of bark surface area and by the number of eggs laid per beetle per day. For example, if a female lived for 15 days in the box and laid 12 eggs on the branch section during that time, then the number of eggs per beetle per day would be equal to 0.8. Bark was carefully peeled down to the wood using a small knife or chisel and diameter of each branch was measured (Table 1.1). Number and stage of larvae on each branch section and a visual estimate of the percentage of the cambium/phloem area covered by larval galleries was recorded. Galleries were also standardized by 100 cm^2 of surface area.

2004 bioassay. I repeated this bioassay three times in 2004 with the same eight species used in 2003, plus the addition of blue ash and Japanese tree lilac. Wood from several trees of each species for the bioassays was 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 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% humidity. Eight branches of each species were cut to approx. 17 cm in length (Table 1.1) at the beginning of each bioassay, but 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 from 2003, except that 20 ml vials of water with cotton wicks were included in each box to provide drinking water for the beetles. Additional holes were added to the boxes to increase ventilation.

Instead of collecting wild beetles from the field, adult beetles were reared from bolts of infested ash trees collected in the core of the southeast MI infestation. Logs were kept in cold storage at 1-2° C until needed. Once emerged from logs, 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% humidity, and 16:8 light:dark photoperiod. Beetles were allowed to feed and mate for two weeks before bioassays began.

On each day that bioassays were initiated, beetles were sexed and one male/female pair was placed in each box (80 pairs total). The first bioassay contained two replicates of each species and was conducted from 18 June to 5 August. The second bioassay was comprised of three replicates and was conducted from 14 July to 14 August, while the third had three replicates and ran from 20 August to 14 September 2004. Boxes were kept in growth chambers at 24°C, 60% humidity, and 16:8 light:dark photoperiod. Thirty-seven of the 80 pairs were observed mating. Adults lived from eight to 63 days in the boxes. If a female beetle died during the first three days 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 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 days after the branch was removed from its box. Diameter of each branch was measured and total bark surface area was calculated (Table 1.1). Branches were inspected, peeled, and larval density assessed using the same methods as in 2003.

Caged stem bioassay

On 12 May 2004, green ash, white ash, Japanese tree lilac, and black walnut balled-in-burlap trees, with an average caliper of 5.8 (±0.1 SE) cm and height of 4.0 (±0.2 SE) m (Table 1.3), were delivered from Poplar Farms Nursery, Waterman, IL and planted at Matthaei Botanical Gardens, Washtenaw Co., MI. Ten blocks of the four species of trees were set up in a randomized complete block design (40 trees total). Trees were planted in an open field slightly above ground, 3 m apart, in five rows of eight trees. The partially exposed root balls were covered with composted wood mulch and drip line irrigation was installed for frequent watering.

Screen cages, approx. 90 cm tall and 30 cm in diameter, were constructed to confine beetles around the stem of each tree. Two 120 cm pieces of contractor's lathe were driven into the root ball to provide structure for the cage. A hole the same diameter as the tree was cut into a white, plastic disc (25 cm diam.) spray-painted green. The disc was placed around the stem of the tree, serving as the top of the cage. A small piece of carpet was first wrapped around the stem to ensure that any gaps between the plastic disc

and the stem were closed to prevent beetles from escaping. The plastic disc was stapled to the lathe and aluminum screen was wrapped around the circumference of the cage, stapled to the plastic disc, and sealed closed with caulk. Excess screen above the disc was wrapped around the stem of the tree and secured with a plastic cable tie. Excess screen around 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 wrapped in plastic wrap and filled with approx. 20 cm of sand to prevent beetles from reaching the mulch. Approximately 50 cm above the base of the cage, a small flap (approx. 15 cm × 20 cm) was cut into the screen and secured with Velcro to provide access into the cage. A 5 cm long piece of garden hose was attached vertically to one of the pieces of lathe 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 days as needed.

Beetles used for this study were reared from infested logs using methods described in the 2004 cut branch bioassays. After two weeks of feeding, beetles were sexed and three male/female pairs were place in each tree cage (six beetles per cage). Cages were checked and foliage misted with water daily. Observed mating dates were recorded. Thirty beetle pairs on 25 trees were observed mating. As beetles died, they were typically replaced 2-3 times between 10 June and 13 September 2004 (586 beetles total). The number of female beetles in each cage per day was recorded. For example, if three female beetles were alive in a cage for five days, the number of female beetles per day for that cage was 15 days. Overall, the mean number of female beetles per day per cage was 40 (±2 SE) days. Individual female beetles lived from one to 34 days in cages.

High densities of beetles were present in the area where the nursery trees were planted. The wild beetles had access to the un-caged upper portions of the trees for feeding and oviposition. This provided us with an opportunity to evaluate oviposition preference and larval development in a multiple-choice setting, as part of a related study described in Chapter Two.

Four of the ten blocks of trees were randomly selected for harvesting on 1 and 2 November and the stems were returned to the laboratory and dissected between 1 and 7 November 2004. The remaining trees were cut and dissected in March 2005. Cages were removed and stems were cut with a chainsaw just above the cage and just above the root ball. In the lab, each stem was carefully inspected for eggs for 10 min and the total number of eggs found was recorded. Bark was then carefully peeled down to the wood using a drawknife. Number and stage of larvae were recorded. Eggs and galleries were standardized by m^2 of surface area.

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), and high (abundant cracks and crevices). All of the black walnut and four of the ten green ash stems had high bark roughness. All of the Japanese tree lilac stems had medium bark roughness and all of the white ash and the remaining six green ash stems had low bark roughness.

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 (SAS Institute, Inc. 1989). 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. *Agrilus planipennis* laid eggs on all eight tree species included in this study (Table 1.2). A total of 525 eggs were found on 29 of the 42 branch sections. Number of eggs found in 15 min of searching per branch section ranged from 0 to 65 with an average of 12.5 (±2.5 SE). A total of 202 eggs were laid on the lid, sides, and bottom of 20 of the 44 plastic boxes. Number of eggs found on the boxes (not on the branch sections) ranged from 0 to 93 with an average of 4.8 (±2.2 SE). The average number of eggs on all ash sections combined was 20.5 (±4.6 SE). On average, there were at least three times as many eggs/100 cm^2 on the ash sections as on the other species, with the exception of privet, which had nearly as many eggs/100 cm^2 as green ash (Figure 1.1). Densities of eggs (number of eggs/100 cm^2) did not differ significantly among species (χ^2=9.62, df=7,34, p=0.21) (Figure 1.1), presumably because of relatively small sample size and high variation.

Beetle fecundity ranged from 0 to 2.8 eggs per day, with an average of 0.46 (±0.10 SE) (Table 1.2). Beetle fecundity varied widely and did not differ significantly among species (χ^2=10.18, df=7,34, p=0.17) (Table 1.2).

Larval galleries were most abundant on ash sections; galleries were present on ten of the 18 sections (Table 1.2). All larvae found were either dead or dying due to desiccation of the small sections. Overall, mean gallery density on all three ash species was 8.5 (±2.2 SE) galleries per branch section. A total of 22 galleries were excavated on four of the five privet sections (Table 1.2). Four of the 22 galleries on privet contained second instar larvae, while the rest contained first instar or neonate larvae. Percentage of

the cambium/phloem area covered by galleries ranged from 0 to 85% with an overall average of 10.5 (±3.1 SE) percent (Table 1.2). Black ash had three to five times more surface area covered by galleries than green ash, white ash, and privet. The amount of cambium/phloem area covered by galleries did not differ significantly among species (χ^2=13.38, df=7,34, p=0.063) (Table 1.2). Gallery density did not differ significantly among species when branch sections with normal galleries were analyzed (i.e., ash and privet) (χ^2=1.19, df=3,19, p=0.75). Overall, white ash, black ash, and privet branches had significantly higher densities of normal galleries than elm, walnut, hickory, and hackberry (χ^2= 17.80, df=7,34, p=0.0129) (Figure 1.2).

First instar larvae attempted to feed on one elm, two walnut, and one hackberry sections (Table 1.2). These feeding attempts were less than 1 mm wide, ranged from 5 mm to 10 cm long, and consistently resulted in larval death. No galleries or feeding attempts were found on hickory sections. Feeding attempts were distinguished from normal galleries for purpose of analysis. Species did not differ significantly in the density of feeding attempts (χ^2= 3.53, df=3,15, p=0.09).

2004 bioassay. Similar to 2003, *A. planipennis* laid eggs on all ten species included in this study (Table 1.2). A total of 577 eggs were found on 48 of the 80 branch sections. Number of eggs found per branch section in 15 min of searching ranged from 0 to 73, with an average of 7.2 (±1.3 SE). A total of 79 eggs were laid on the lid, sides, and bottom of eight of the 80 boxes. Number of eggs found on the boxes (not on branches) ranged from 0 to 47 with an average of 1.0 (±0.6 SE).

The average number of eggs per branch on all ash combined was 12.3 (±2.7 SE). On average, egg density was two times as high on the ash branches as on the other

species, with the exception of privet, which averaged more eggs/100cm^2 than green, white, and black ash (Figure 1.3). Egg density was significantly greater on the green ash, white ash, black ash, and privet branches than on the elm, walnut, and hickory branches (χ^2=21.78, df=9,70, p=0.0096) (Figure 1.3).

Beetle fecundity ranged from 0 to 1.6 eggs per day, with an average of 0.23 (±0.04 SE) (Table 1.2). There were significantly more eggs laid per beetle per day on black ash and privet than on tree lilac, elm, walnut, and hickory; other species had an intermediate number of eggs (χ^2=23.35, df=9,70, p=0.0054) (Table 1.2).

Twenty-four of the 32 ash branches, along with six of the eight privet branches had galleries (Table 1.2). All larvae that were recovered were either dead or dying due to desiccation of the branch sections. Overall, mean gallery density on all four ash species was 12.3 (±2.3 SE) galleries per branch. A total of 77 galleries were excavated on six privet branches (Table 1.2). Seven of the 77 galleries contained second instar larvae and 14 contained third instar larvae. Larval galleries covered 0 to 90% of the cambium surface area, with an overall average of 6.9 (±2.1 SE) percent (Table 1.2). Larval galleries covered significantly more surface area on green and black ash branches than on hickory (χ^2=31.30, df=9,70, p=0.0003) (Table 1.2).

Branch sections from four tree lilac, one elm, three walnut, and one hackberry had *A. planipennis* feeding attempts made by first stage larvae (Table 1.2). No galleries or feeding attempts were found on hickory branches. As in the 2003 bioassay, when larvae attempted to feed on other species, galleries were abnormally small and resulted in larval death. Similarly, the density of feeding attempts did not differ significantly among species for the branches with only abnormal galleries (χ^2=8.05, df=5,35, p=0.08). The

four ash species and privet had significantly higher normal gallery densities than tree lilac, elm, walnut, hickory, and hackberry (χ^2= 38.11, df=9,70 p<0.0001) (Figure 1.4).

Caged stem bioassay

I found a total of nine eggs on one of the ten stem sections of green ash that had been caged and two eggs on two of the ten caged portions of white ash stems. No eggs were found on walnut or tree lilac stems (Table 1.3). Egg density did not differ significantly among species (χ^2=3.72, df=3,36, p=0.29) (Table 1.3).

A total of 49 galleries were excavated on four green ash stems and a single gallery was excavated on one white ash stem. No galleries or feeding attempts were found on the other six green ash, nine white ash, or any of the walnut or tree lilac stems (Table 1.3). Green ash stems had significantly greater gallery densities than black walnut and tree lilac stems, but did not differ significantly from white ash stems (χ^2=10.08, df=3,36, p=0.0179) (Table 1.3). Three of the four green ash stems that had eggs and galleries also had rough, flaky bark. The remaining ash stem with galleries had smooth bark. The six green ash without eggs or galleries and all of the white ash stems had smooth bark.

DISCUSSION

The first goal of these bioassays was to determine if *A. planipennis* would oviposit on non-ash species in a no-choice situation. Results from the cut branch bioassays showed that *A. planipennis* will oviposit on species other than ash, even on the plastic boxes. It is not surprising, therefore, that egg density or beetle fecundity did not vary among species in these bioassays.

My ability to find eggs was often dependent upon certain bark characteristics, especially when egg density was low. For example, privet, tree lilac, and hackberry 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 had rough bark and finding eggs at low density was challenging.

Beetle fecundity was almost two times greater in 2003 than in 2004. This may have been due to several factors. Wild beetles were used in 2003 while lab reared beetles were used in 2004. Although it was impossible to determine age and mating status of the wild beetles, they still produced more eggs than the lab reared beetles. Interestingly, while I found more eggs in 2003 than 2004, the 2003 eggs yielded fewer galleries than in 2004. *Agrilus planipennis* fecundity decreases as females age (D.L. Miller, pers. comm.) and females used in 2003 may have been older than females used in 2004. The eggs I found on branch sections and on the plastic boxes all appeared solid and intact. I was able to distinguish between reddish-brown viable eggs and non-viable eggs which remain white. Less than 3% of the eggs I found were non-viable.

Fewer eggs than expected were laid on the caged stems in 2004 and beetles in this study were often observed trying to escape their cages. Previous studies have also

reported oviposition problems with caged buprestids. Barter (1957) reported *A. anxius* expended so much energy trying to escape cages, that they spent little time doing anything else, resulting in decreased longevity of beetles in field cages. Nash et al. (1951) also encountered difficulties in obtaining borer attacks in cages and "eggs laid were negligible and did not develop." Akers and Nielson (1990) similarly reported low fecundity and reduced longevity with caged *A. anxius*.

Most eggs were laid on the green ash stems with rough, flaky bark where they could be hidden in cracks and under bark flakes. Only one of the two eggs laid on smooth bark white ash produced a larval gallery and that egg was positioned at the site of an old, healed-over branch junction where there were a few punctate creases in the bark. Barter (1957) reported that *A. anxius* 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. Additional research needs to be done to further examine the influence of bark texture or tactile stimuli on *A. planipennis* oviposition.

The second goal of these bioassays was to determine whether early instar *A. planipennis* larvae would develop in non-ash species. In nearly all cases, pseudo-replication was avoided by collecting branch samples from multiple trees of each species used in the bioassays. I was unable to collect from multiple trees of Japanese tree lilac, but in related studies of this species, I found no evidence of intra-specific variation. The majority of larvae I recorded on ash were second instars when branches were peeled and all larvae were dead or dying due to desiccation of the small branch sections. Four larvae reached the fourth instar on four black ash branches and two fourth instar larvae were

found on one blue ash branch. Black and blue ash branches may have retained moisture better than green or white ash, prolonging survival of the larvae.

Twenty-five percent of the galleries on privet branches had larvae that developed to the second or third instar, suggesting that privet may be a suitable host. Previous studies of adult *A. planipennis* feeding found that male longevity was 20 days on privet (*Ligustrum*) and 13 days on swamp privet (*Forestiera*) (Haack et al. 2004). Female longevity in theses studies was not reported. In two-choice and multiple-choice tests, *A. planipennis* fed readily on privet foliage in the presence of ash foliage (Haack and Petrice 2005). Other adult *A. planipennis* feeding trials found no significant difference in the mortality rates of beetles feeding on green ash or privet foliage over a nine day bioassay (p=0.59), but beetles consumed more green ash than privet foliage (p<0.01) (Pell, Anulewicz, and McCullough 2005, unpubl. data). Additional research is needed to further assess whether *A. planipennis* larvae can complete development on live privet.

Neonate larvae attempted to feed on black walnut, American elm, and hackberry, but invariably failed to develop. These species, along with hickory that had no feeding attempts, appear to be unsuitable hosts. While it is possible that females may oviposit on hosts that are actually unsuitable for larval development (Thompson 1988), the artificial design of a no-choice experiment must be taken into consideration. These results show that, given no other choice, females will oviposit on non-host species. As reported in Chapter Two, however, I also observed *A. planipennis* ovipositing on non-ash species in the field.

Foliage may also influence host selection by adult beetles who must feed on leaves throughout their life span (Thompson 1988). In my studies, only green ash foliage

was available to females. If *A. planipennis* host selection is influenced by the species of foliage available for feeding due to conditioning, there would have been a stronger preference for the green ash branches than was actually observed.

Asian literature includes reports of *A. planipennis* attacks in Japan and Korea on *Juglans*, *Ulmus*, and *Pterocarya* (Akiyama and Ohmomo 1997, Sugiura 1999), but attacks in North America have only been observed on *Fraxinus* species. The North American population of *A. planipennis* may have experienced a genetic bottleneck because it is likely that only a small number of beetles were introduced into North America. The small founding population probably represented only a fraction of the parent population's genetic variation. As a result of this founder effect, North American populations may lack the genetic breadth of their parent populations (e.g., the genotypes associated with development on other tree genera) and this may limit their host range to *Fraxinus*. Other examples of successful invasions after genetic bottlenecks include the Argentine ant (*Linepithema humile*) (Suarez et al. 1999) and the imported fire ant (*Solenopsis invicta*) (Ross et al. 1993). Genetic changes in the introduced ant populations resulted in changes in behavior (Suarez et al. 1999) and social organization of colonies (Ross et al. 1993).

Since *A. planipennis* host range is limited to *Fraxinus* in China (Chinese Academy of Science 1986, Yu 1992), it could be that the original source of the North American population was from China. Future studies of *A. planipennis* host range should include Asian tree species, originally listed as hosts in the literature, to evaluate development of North American *A. planipennis* populations on these species.

The taxonomy of *A. planipennis* is complex and this complicates the delineation of its specific host range. *Agrilus planipennis* is known in many parts of the world and in the literature as *A. marcopoli* (Yu 1992), *A. planipennis ulmi* (Akiyama and Ohmomo 2000, Sugiura 1999) and *A. feretrius* (Jendek 1994) each with individual and overlapping host species. Several examples of species subpopulation variations in host range can be found in the literature. Parry and Goyer (2004) found that the forest tent caterpillar, *Malacasoma disstria*, originally thought to be an extreme generalist, is actually a composite of regionally adapted populations. Similarly, Sunnucks et al. (1997) found that the aphid pest species *Therioaphis trifolii* is a generalist in Western Palaearctic region on alfalfa, clovers, and related legumes, but displays a particular form in Australia that feeds almost exclusively on alfalfa. Fumanal et al. (2004) found that host conditioning can influence the host range of the collar gall weevil *Ceutorhynchus assimilis.* Host specificity tests in lab and field experiments showed heterogeneity in the host range of the weevils reared from different host plants as determined by larval development. Genetic analysis of *A. planipennis* is underway to compare populations of *A. planipennis* in the U.S. with populations in Asia, including China where the host range is limited to *Fraxinus,* and Japan and Korea where hosts include *Juglans*, *Ulmus*, and *Pterocarya* (Bray et al. 2005).

Table 1.1. Mean (±SE) diameter, length, and surface area for branch sections used in no-choice bioassays in 2003 and 2004.

Species	n	Diameter (cm)	Length (cm)	Surface area (cm^2)
2003 Bioassays				
Green ash	7	8.3 ± 0.2	16.7 ± 0.3	256.6 ± 12.0
White ash	7	8.4 ± 0.1	17.3 ± 0.3	247.0 ± 16.8
Black ash	4	6.6 ± 0.3	17.3 ± 0.5	232.0 ± 13.0
Privet	5	4.8 ± 0.4	17.0 ± 0.3	176.0 ± 13.5
American elm	5	7.8 ± 0.1	17.2 ± 0.4	243.2 ± 21.3
Black walnut	4	8.0 ± 0.1	17.0 ± 0.1	251.0 ± 22.4
Hickory	5	9.2 ± 0.1	15.2 ±0.6	251.4 ± 11.5
Hackberry	5	8.9 ± 0.2	16.4 ± 0.6	258.8 ± 6.0
2004 Bioassays				
Green ash	8	9.0 ± 0.6	16.8 ± 0.3	409.4 ± 25.4
White ash	8	8.8 ± 0.5	16.8 ± 0.3	408.5 ± 17.3
Black ash	8	7.7 ± 0.2	16.6 ± 0.3	351.5 ± 9.2
Blue ash	8	7.2 ± 0.3	16.5 ± 0.3	316.5 ± 20.8
Privet	8	6.4 ± 0.3	16.9 ± 0.2	319.0 ± 14.7
Japanese tree lilac	8	4.6 ± 0.3	16.9 ± 0.4	224.8 ± 16.1
American elm	8	9.3 ± 0.2	15.4 ± 1.1	408.0 ± 31.3
Black walnut	8	9.5 ± 0.1	15.7± 0.6	392.5 ± 22.6
Hickory	8	9.7 ± 0.1	17.0 ± 0.3	475.5 ± 8.4
Hackberry	8	9.3 ± 0.2	16.6 ± 0.3	405.6 ± 22.7

Table 1.2. Total number of *Agrilus planipennis* eggs and galleries, number of eggs and galleries on branch sections, and mean (±SE) number of eggs/beetle/day in no-choice bioassays in 2003 and 2004.

	No. branch sections	Total no. eggs on bark	No. branch sections with eggs	Mean no. eggs/beetle/day	Total galleries	No. branch sections with galleries	Total larval feeding attempts	No. branch sections with larval feeding attempts	Mean % area with galleries
2003									
Green ash	7	128	6	0.6 ± 0.29	44	3	0	0	14.3 ± 6.94
White ash	7	148	6	1.1 ± 0.39	72	4	0	0	9.1 ± 3.63
Black ash	4	93	4	0.7 ± 0.27	37	3	0	0	50 ± 20.10
Privet	5	37	3	0.2 ± 0.14	22	4	0	0	7.2 ± 4.28
American elm	5	21	3	0.2 ± 0.07	0	0	3	1	0
Black walnut	4	30	1	0.2 ± 0.18	0	0	11	2	0
Hickory	5	31	3	0.3 ± 0.17	0	0	0	0	0
Hackberry	5	37	3	0.2 ± 0.17	0	0	5	1	0
2004									
Green ash	8	93	6	0.4 ± 0.19 **ab**	110	6	0	0	30.6 ± 12.37 **a**
White ash	8	100	5	0.4 ± 0.14 **ab**	105	5	0	0	2.6 ± 1.61 **ab**
Black ash	8	88	7	0.4 ± 0.10 **a**	83	7	0	0	12.0 ± 7.19 **a**
Blue ash	8	112	5	0.4 ± 0.19 **abc**	95	6	0	0	23.3 ± 12.78 **ab**
Privet	8	91	8	0.4 ± 0.08 **a**	77	6	0	0	4.7 ± 2.20 **ab**
Japanese tree lilac	8	28	4	0.1 ± 0.05 **bcd**	0	0	30	4	**0 ab**
American elm	8	19	2	0.1 ± 0.06**d**	0	0	8	1	**0 ab**
Black walnut	8	5	3	0 ± 0.01 **d**	0	0	26	3	**0 ab**
Hickory	8	11	3	0.1 ± 0.07 **cd**	0	0	0	0	**0 b**
Hackberry	8	30	5	0.1 ± 0.05 **abcd**	0	0	1	1	0 ± 0.01 **ab**

Within columns, means followed by the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p < 0.05$).

Table 1.3. Mean (±SE) tree diameter at breast height (DBH), height, and number of *Agrilus planipennis* eggs/m^2 and galleries/m^2 for four species of trees used in no-choice caged stem bioassays in 2004.

	Green ash	**White ash**	**Tree lilac**	**Black walnut**
DBH (cm)	6.0 ± 0.2	6.3 ± 0.2	4.6 ± 0.1	6.4 ± 0.2
Height (m)	4.3 ± 0.1	4.3 ± 0.1	2.5 ± 0.1	5.1 ± 0.2
Total no. of eggs on bark	9	2	0	0
Mean no. of eggs/m^2	6.2 ± 6.2	1.5 ± 1.0	0	0
Total no. of galleries	49	1	0	0
Mean no. of galleries/m^2	34.8 ± 24.9 **a**	0.8 ± 0.8 **ab**	0 **b**	0 **b**

Within rows, means followed by the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p < 0.05$).

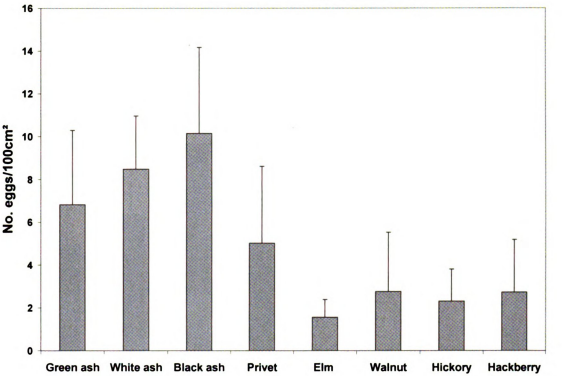

Figure 1.1. Mean (±SE) number of *Agrilus planipennis* eggs per 100 cm^2 on three known host species (ash) and five potential alternate host species in the 2003 no-choice cut branch bioassay. n=4 for black ash and walnut, n=5 for privet, elm, hickory and hackberry, n=7 for green and white ash.

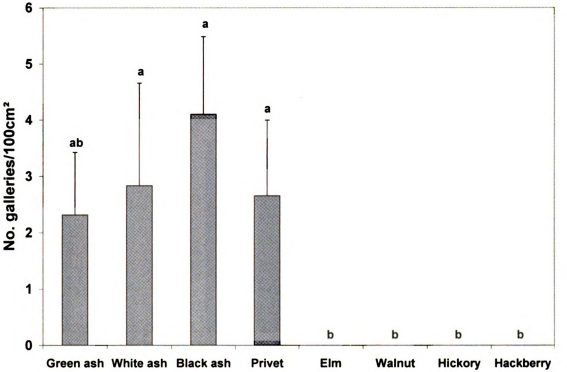

Figure 1.2. Mean (±SE) number of *Agrilus planipennis* galleries per 100 cm² on three known host species (ash) and five potential alternate host species in the 2003 no-choice cut branch bioassay. n=4 for black ash and walnut, n=5 for privet, elm, hickory and hackberry, n=7 for green ash and white ash. Means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

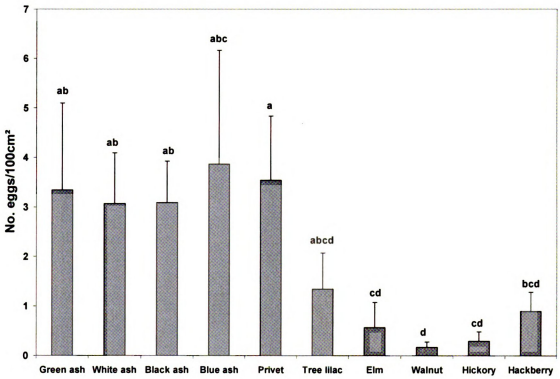

Figure 1.3. Mean (±SE) number of *Agrilus planipennis* eggs per 100 cm² on four known host species (ash) and six potential alternate host species in the 2004 no-choice cut branch bioassay. n=8 branches per species. Means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

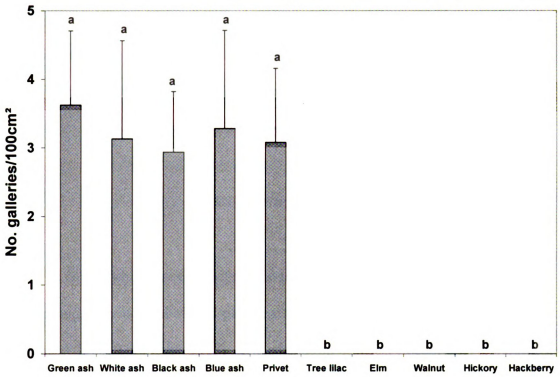

Figure 1.4. Mean (±SE) number of *Agrilus planipennis* galleries per 100 cm² on four known host species (ash) and six potential alternate host species in the 2004 no-choice cut branch bioassay. n=8 branches per species. Means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

CHAPTER TWO

Oviposition Preference and Larval Development of the Emerald Ash Borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in Multiple-Choice Field Experiments

INTRODUCTION

The relationship between oviposition preference and host suitability is critical in understanding the evolution of host-plant interactions in herbivorous insects (Futuyma and Peterson 1985, Thompson 1988). The adaptation of insect populations to their host plant involves two major classes of characters: a) behaviors that influence the choice of a plant for feeding or oviposition and b) physiological traits that affect growth and reproduction on a particular host (Via 1986). Presumably, adult females presented with a variety of potential hosts will select the host species that bestows the greatest fitness to their offspring. In some insect populations, however, host selection by females results in varying degrees of performance by offspring. Thompson (1988) concluded that adult host preference does not always correlate with larval performance because some females oviposit on hosts unsuitable for larval development.

Because the vast majority of wood-boring larvae are legless and cannot disperse to alternate or more suitable hosts, oviposition choices by adult females are critical (Hanks et al. 1993). Wood-borers typically rely on olfaction to locate potential hosts that are often patchily distributed within forested areas (Haack and Slansky 1986). After

oviposition, survival of offspring is driven by host suitability rather than host preference, since larvae must feed and develop on the host chosen by the female (Hanks 1999).

Emerald ash borer (*Agrilus planipennis* Fairmaire), an Asian buprestid discovered in June 2002, is established in much of southeastern Michigan and Windsor, Ontario (Cappaert et al. 2005c). This phloem-feeding species is native to northeastern China, Korea, Japan, Mongolia, the Russian Far East, and Taiwan (Yu 1992) and was likely introduced into North America through solid wood packing material (Cappaert et al. 2005c).

The life cycle of *A. planipennis* in North America is generally completed in one year, although some individuals may require two years to complete development (Siegert et al. 2005). Females can lay 30-60 eggs from late June to late July. Eggs are laid on or just under the bark surface and inside bark cracks and crevices (Chinese Academy of Science 1986, Yu 1992). Larvae feed under the bark in the cambium and phloem from July to October, eventually scoring the outer sapwood. Larval galleries disrupt vascular tissue, eventually girdling and killing the host plant. Larvae pass through four instars (Cappaert et al. 2005c) and most overwinter as pre-pupal larvae in the first cm of outer sapwood or in the bark of thick-barked trees (Cappaert et al. 2005a).

Although limited reports suggest that *A. planipennis* is not a major pest in Asia (Akiyama and Ohmomo 2000), in North America, *A. planipennis* is aggressively attacking healthy, as well as stressed, green ash (*Fraxinus pennsylvanica* Marsh.), white ash (*F. americana* L.), black ash (*F. nigra* Marsh.), and blue ash (*F. quadrangulata* Michx.). As of March 2006, an estimated 12-15 million ash trees in southeastern Michigan were dead or dying.

In its native range, *A. planipennis* reportedly attacks other species including *Ulmus davidiana* var. *japonica* Planch., *Juglans mandshurica* var. *sieboldiana* and var. *sachalinensis*, and *Pterocarya rhoifolia* Sieb. & Zucc. (Akiyama and Ohmomo 1997, Sugiura 1999). North American members of these genera, including American elm (*U. americana* L.), black walnut (*J. nigra* L.), and hickory (*Carya* sp.), are common in landscapes and wooded areas in North America. Other members of the ash family (Oleaceae), including Japanese tree lilac (*Syringa reticulata* Bl.), are also commonly used in landscape settings.

Attacks on these genera have not been observed in North America, but there is concern about potential host switching as *A. planipennis* population densities increase and ash trees die in the infested area. If *A. planipennis* can attack additional species, environmental and economic impacts on forest resources could increase dramatically in North America. Given the extensive damage that *A. planipennis* has already caused to the *Fraxinus* resource in southeastern Michigan, there is an urgent need to evaluate the susceptibility of other commonly planted landscape and forest trees that may serve as alternate hosts to *A. planipennis*.

I monitored adult *A. planipennis* landing rates, oviposition preference, and early larval instar development on North American congeners of potential alternate hosts, including American elm, black walnut, Japanese tree lilac, hickory, and hackberry (*Celtis occidentalis*). In three multiple choice studies, I placed freshly cut logs and nursery trees of ash and potential alternate host species at multiple sites in the core area of *A. planipennis* infestation.

MATERIALS AND METHODS

Logs attached to t-posts

Logs of six different species, including green ash (*Fraxinus pennsylvanica*), white ash (*F. americana*), black walnut (*Juglans nigra*), American elm (*Ulmus americana*), shagbark hickory (*Carya ovata*), and hackberry (*Celtis occidentalis*), a member of the elm family, were harvested from eight to ten trees of each species at Michigan State University's W.K. Kellogg Forest, Kalamazoo Co., MI on 16 June 2003. As of 2006, ash trees at the Kellogg Forest were not infested by *A. planipennis*. Cut ends of the logs were waxed on 17 June 2003 to prevent desiccation. Logs were approx. 60 cm long and diameter ranged from 7.3 to 18.2 cm with an average of 13.1 (± 0.2 SE) cm (Table 2.1). We pounded t-posts, 2 m tall, into the ground, approximately 10 m apart at four sites in the core area of *A. planipennis* infestation (locations given below). One log was attached vertically to the top of each t-post with 14 gauge baling wire, positioning half of the log above the top of the post. Logs were set out in a randomized complete block design with 4-8 blocks at each site (see below). There were a total of 24 logs per species, with the exception of white ash that only had 23 logs (24 blocks, 143 logs total). Black plastic drain pipe, approx. 12 cm in diameter and 60 cm long, was included in half of the blocks as a "control" to see if *A. planipennis* adults would land on non-wood material. The drain pipes were attached vertically to the t-posts, giving a dark silhouette similar to the logs. Seventy-five percent of the logs of each species were randomly selected to be wrapped with clear, plastic, stretch wrap and covered with Tanglefoot® (109 logs total). All 16 of the drain pipes were wrapped. The top half of the logs was covered with wrap, leaving

the bark on the bottom half of the logs exposed for oviposition. Logs and drain pipes remained on posts from 17 June to 14 August 2003.

Study sites included Bicentennial Park in Livonia, Wayne Co., MI which had a high *A. planipennis* population density in 2003. There were many heavily infested ash trees in woodlots within and immediately surrounding the park. Six blocks were set up at this site along the border of an infested woodlot, surrounding open fields. Kensington Metropark, Oakland Co., MI was selected for its moderate *A. planipennis* population density. At this site, six blocks were set up at the north end of the park near a wooded area where infestation levels in individual trees ranged from low to very high. Two blocks were set up in two parallel lines, one block along the edge and one 4 m inside the woodlot. The remaining four blocks were placed in an open field flanked on two sides by wooded areas. The third site, Western Golf Course and Country Club in Redford, Wayne Co., MI, was expected to have a low *A. planipennis* population density because of the extensive removal of infested ash trees in the fall and winter of 2002. Approximately 20 lightly to moderately infested ash trees remained on the course, along with seemingly healthy, untreated ash trees. Additional ash trees with *A. planipennis* occurred in residential areas surrounding the course. Eight blocks were set up in a linear fashion in unmowed areas of the course, near live, presumably infested ash trees. A fourth site on Eckles Road, a disposal site for infested ash material in Plymouth Township, Wayne Co., MI, received large quantities of infested ash logs and wood chips from the surrounding area. Infested logs were stockpiled until they could be chipped, providing a source of newly emerged adult beetles during much of the summer. There were no other ash trees

on the site or in the immediate surroundings which were comprised of warehouses and vacant buildings. Four blocks were arranged linearly around the perimeter of the site.

Logs were checked weekly from 20 June to 8 August and adult beetles were counted and removed from the Tanglefoot® to assess landing rates. Tanglefoot® bands were removed on 7 and 8 August. Logs from all sites were retrieved on 14 August 2003 and stored in an unheated shed until dissected.

Logs were returned to the laboratory for inspection and dissection between 5 November 2003 and 16 March 2004. Total bark surface area was calculated from the log length and circumference at the middle of each log (Table 2.1). The number of eggs found in 5 min of inspection was recorded. The total number of eggs laid on each species of log was considered an expression of host preference. Bark was carefully peeled down to the wood using a drawknife. Number and stage of larvae was recorded and standardized by m^2 of log surface area.

Logs attached to ash trees

2003 study: Ten black walnut and ten American elm logs were harvested from three to four trees of each species at Hudson Mills Golf Course, Washtenaw Co., MI on 9 June 2003 and stored outdoors. Because these logs came from an infested area, several logs of walnut and elm were peeled preliminarily to confirm that they were uninfested. Ten green ash logs were harvested three trees at Kellogg Forest on 23 June 2003. Logs were cut to approx. 60 cm long and diameter ranged from 9.2 to 14.6 cm with an average of 11.9 (± 0.3 SE) cm (Table 2.2). Both ends of all logs were waxed on 24 June 2003.

Three logs, one of each species, were attached to the stem of a large, infested green ash tree, 5-7 m aboveground (one group per tree). Logs were attached vertically

with plastic cable ties, in random order, one on top of the other, rotating around the circumference of the stem. Five green ash trees were selected at Bicentennial Park. On three of the trees (approx. 25 cm DBH and 17 m height) growing 5-10 m inside the edge of a woodlot, I placed logs below the canopy. Logs were attached to the stem at mid-canopy of two other trees (approx. 20 cm DBH and 12 m height) growing in the open on the edge of a recreation field, next to a paved parking area. Logs were also placed on the stem, just below the canopy, of five green ash trees with average DBH of 25 cm and height of 20 m, inside a wooded area at Kensington Metropark. Both sites were selected because of the high number of infested ash trees in the area, determined by canopy dieback, woodpecker attacks along the stems of the trees, and presence of adult beetles. Logs remained in place from 24 June to 14 August 2003, then were retrieved and stored outdoors in an unheated shed until dissected.

Logs were inspected and dissected between 26 August and 4 September 2003. Total bark surface area was calculated from the length and circumference of each log (Table 2.2). Inspection and peeling methods were as described earlier.

2004 study: Eighteen green ash, nine white ash, nine black walnut, and nine American elm logs were harvested from three to six trees of each species at Kellogg Forest and nine blue ash logs were harvested from three trees at an infested woodlot in Superior Township on 15 June 2004. A subset of blue ash logs were peeled prior to the start of the study and found to be uninfested. Logs (54 total) were approx. 60 cm long with a diameter range from 9.3 to 16.7 cm, averaging 13.0 (± 0.3 SE) cm and waxed on both ends on 16 June 2004 (Table 2.2).

Two sites with moderately high levels of *A. planipennis* densities were selected in the city of Ann Arbor, Washtenaw Co., MI: Parker Mills and Marshall Parks. Both parks were heavily wooded with moderate densities of white ash trees. At each park, nine white ash trees (18 trees total, approx. 15 m in height, DBH ranging from 10 to 20 cm, averaging 17.0 (±0.6 SE) cm) were selected. Trees had low to moderate infestation, as determined by canopy dieback and presence of woodpecker attacks and *A. planipennis* exit holes. All trees were inside a wooded area.

Groups of three logs were attached to live trees in an incomplete block design. Each group consisted of a green ash log (control), a second ash log (white or blue ash), and a non-ash log (elm or walnut). The three logs were attached, using plastic cable ties, to the stem of a large, infested white ash tree, 5-7 m aboveground (one group per tree). Green ash logs were always placed in the middle position of the group along the stem and the two other logs alternated between the top and bottom positions. Logs remained in place from 17 June to 11 August 2004, then were retrieved and stored outdoors until dissected. Logs were inspected and dissected between 17 September and 10 December 2004. Inspection and peeling methods were as described earlier.

2005 study: In 2005, we attached both green and white ash logs to both green and white ash trees. Forty green ash, 40 white ash, and 13 black walnut logs were harvested from four to 15 trees of each species at Kellogg Forest and 14 black ash logs from four trees at the Nan Weston Nature Preserve at Sharon Hollow, an apparently uninfested site in Washtenaw Co., MI on 13 June 2005. Thirteen blue ash logs were harvested from four trees at the Strait Creek Nature Preserve, another uninfested site in Adams Co., OH on 10

June 2005. Logs (120 total) were approx. 60 cm long, ranging from 48 to 66 cm in diameter, averaging 12.5 (±0.2 SE) cm and waxed on both ends (Table 2.2).

Ten infested ash trees at each of four sites with moderately high densities of *A. planipennis* were selected in the core *A. planipennis* infestation area in southeast MI (40 trees total). Two sites were composed of street trees growing in the open and two sites had trees growing in woodlots. Two other sites had green ash trees, one with green ash street trees and the other with green ash woodlot trees. The design was similar for the two white ash sites; one had white ash street trees and the other had white ash woodlot trees.

Ten green ash trees growing along the edge of a wooded area were selected at Kensington Metropark. Ten open grown green ash street trees located in the Wellington neighborhood of Ann Arbor were also chosen. Ten white ash street trees were chosen in a second Ann Arbor neighborhood, Windemere, where white ash was the predominant street tree species. Lastly, ten white ash trees growing in the Dicken Woods woodlot in Ann Arbor were also chosen for the study. DBH ranged from 15 to 70 cm, with an average of 31.0 (±1.9 SE) cm and heights ranged from 12 to 18 m.

Groups of three logs were attached to trees using an incomplete block design. Each group consisted of a green ash log (control), a white ash log (control), and a third log (black ash, blue ash, or black walnut). On 15 and 16 June 2005, logs were placed in random order along the stem of the trees and attached using plastic cable ties. Logs remained in place until 17 August 2005 when they were retrieved and stored outdoors until they were debarked between 30 September and 1 December 2005. Logs were not

inspected for eggs, but were measured and peeled down to the wood using methods described earlier.

Nursery trees

2004 study: On 12 May 2004, green ash, white ash, Japanese tree lilac and black walnut balled-in-burlap trees were delivered from Poplar Farms Nursery, Waterman, IL and planted at Matthaei Botanical Gardens, Washtenaw Co., MI. Ten blocks of trees were set up in a randomized complete block design (40 trees total). Trees were planted in an open field, slightly above ground, 3 m apart, in five rows of eight trees. The exposed root balls were covered with wood mulch and drip line irrigation was installed for frequent watering. Trees had an average DBH of 5.8 (±0.1 SE) cm (Table 2.3).

Screen cages were constructed around each tree to confine lab reared beetles around the lower 1 m of the stem for no-choice bioassays described in Chapter One. The un-caged portions of the trees, however, remained exposed to high density *A. planipennis* populations in the area. Trees were in place throughout the 2004 *A. planipennis* flight season and were harvested late in the fall after larvae had finished feeding. Four of the ten blocks of trees were randomly selected for harvesting on 1 and 2 November 2004. Trees were cut just above the cage, returned to the lab, and dissected between 1 November and 10 December 2004. The remaining six blocks were cut and dissected in March 2005. In the laboratory, the main stem and branches greater than 0.5 cm in diameter were carefully peeled down to the wood. Number and stage of the larvae and the diameter of the stem or branch the larvae was found on was recorded. The average area sampled for each tree was 0.55 (±0.06 SE) m^2 per tree (Table 2.3).

For all trees, relative smoothness or roughness of the bark was estimated. Three categories of bark roughness were designated. Low bark roughness indicated very few to no cracks or crevices in the bark (smooth bark). Medium bark roughness described bark with a moderate abundance of cracks and crevices for oviposition. High bark roughness indicated a high abundance of cracks and crevices. Branch junctions in trees can also have cracks and crevices, even on smooth bark trees. Therefore, the number of galleries originating within 5 cm of a branch junction was also recorded.

2005 study: The study was repeated in 2005 using the same four species purchased from the same nursery in 2004. Root balls from the previous study were removed and new trees were planted in the existing holes on 13 May 2005. Exposed root balls were covered with wood mulch and drip line irrigation was re-installed. Trees had an average DBH of 7.1 (±0.2 SE) cm (Table 2.3).

Screen cages were again constructed for no-choice tests as described earlier. Un-caged portions of the trees were harvested and debarked two blocks at a time in December 2005 and January 2006. Peeling methods and estimates of bark roughness were as described earlier. Total surface area sampled per tree (Table 2.3) and number of galleries originating within 5 cm of a branch junction was similarly recorded.

American elm survey

The objective of this survey was to determine if *A. planipennis* adult females would oviposit on elm trees adjacent to or in close proximity to infested ash trees. Bicentennial Park was chosen for sampling because of the high density *A. planipennis* population present in 2003, rapidly decreasing availability of suitable host material (live ash trees), and the high number of elm and green ash trees growing together.

On 19 December 2003, nine American elm trees, each growing within 5 m of 28 highly infested green ash trees were selected for sampling. Three of the nine elms had trunks that were physically in contact with the trunks of infested ash trees. Diameter at breast height was recorded for each ash and elm tree. Distance from each ash to the nearest elm was recorded. Distance of elms from the nearest ash ranged from 0 to 4.6 m, with an average of 2.8 (±0.3 SE) m. Average DBH for green ash and elm was 25.8 (±1.4 SE) cm and 15.6 (±1.3 SE) cm, respectively.

Percentage of phloem girdled by *A. planipennis* larvae was visually estimated by removing an approx. 30 × 30 cm window of bark at 1.5 m above ground on each ash tree. Twenty-five of the 28 ash trees had apparently died in 2003. Canopy dieback and heavy *A. planipennis* density on the remaining three trees suggested that these trees would be dead within a year.

The nine elm trees were felled (6 to 8 cm aboveground), and carefully debarked with drawknives to look for *A. planipennis* galleries. Four of the elms were completely peeled and 50-80% of the bark on the trunk and branches of the other five trees was removed in long, vertical strips from top to bottom. A total of 28.4 m^2 of elm surface area was sampled, averaging 3.2 (±0.5 SE) m^2 per tree.

Statistical analysis

All variables were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Landing rates and gallery density for logs attached to ash trees in 2005 were normalized by log (x+1) and square-root (x+1) transformations, respectively (Ott and Longnecker 2001). Gallery density for logs attached to ash trees was analyzed using the MIXED procedure for mixed models in SAS statistical software

(SAS Institute, Inc. 1989) with site type (street tree or woodlot tree), tree species (green or white ash), and log species as fixed effects and tree number as a random effect. Differences among treatment means were tested as unplanned comparisons and tests were applied only when overall analysis of variance (ANOVA) was significant ($p<0.05$). Two and three-way ANOVAs were performed to determine significant effects and interactions among site location, tree type, and species factors using SAS statistical software (SAS Institute, Inc. 1989). When significant, Fisher's protected least significant difference (LSD) test was used to determine which species differed within the appropriate marginal comparisons (Ott and Longnecker 2001).

Other variables, including eggs/m^2 and galleries/m^2 were not normalized by transformations. For non-normal variables, Friedman's two-way nonparametric ANOVA was used to determine significant interactions between site location and species factors (SAS Institute, Inc. 1989). When significant, nonparametric Wilcoxon Rank Sum and Kruskal-Wallis tests were used within each site location to determine if egg and gallery densities differed among species (Conover 1971, Kruskal and Wallis 1952). When the Kruskall-Wallis test was significant, nonparametric multiple comparisons ($p<0.05$) were used to identify differences among species (Zar 1984, Conover 1971). The Wilcoxon Ranked Sum test was also used to evaluate bark roughness as an effect on gallery density. All analyses were conducted at the $p<0.05$ level of significance.

RESULTS

Logs attached to t-posts

A total of 602 *A. planipennis* adults were collected from the 109 logs and 16 pipes with Tanglefoot®, ranging from 0 to 48 with an average of 4.8 (±0.7 SE) beetles per log. Thirty-four percent of the beetles were caught on the six blocks of logs at Bicentennial, 33% on the four blocks at Eckles, and 21% on the six blocks at Kensington. Only 12% of the beetles were captured on the eight blocks at Western, which was significantly fewer than the number captured at the other three sites (F=21.28, df=3,121, p<0.0001). Black walnut logs captured the most beetles; 133, followed by white ash and green ash, which accounted for 39% of all beetles (Table 2.4). Significantly more *A. planipennis* adults landed on ash, elm, walnut, and hickory logs than on drain pipes, which accounted for only 3% of adults (F=3.04, df=6,118, p=0.0091) (Table 2.4). Number of adults collected from hackberry logs did not differ significantly from the other logs or the drain pipes (Table 2.4). The interaction between site location and species was not significant (F=0.92, df=18, p=0.56).

Green and white ash logs combined had four times as many eggs as the non-ash species combined (Table 2.4). Seventy-eight percent of the 256 eggs I found were on ash logs. Fifty-five percent the ash logs had eggs compared to 23% of the non-ash logs (Table 2.4). Twelve of the 23 eggs found on walnut were on a single log at Western. No eggs were found on the drain pipes. The number of eggs found in 5 min of searching ranged from 0 to 32 with an average of 1.8 (± 0.3 SE) eggs per log.

Site location and log species significantly affected egg density (χ^2=12.69, df=3,139, p=0.0054 and χ^2=31.18, df=5,137, p<0.0001, respectively). Logs at

Kensington had significantly higher egg densities than logs at Western, but egg densities on logs at Bicentennial and Eckles were not significantly different from egg densities on logs at Kensington or Western. White ash logs had significantly more eggs/m^2 than elm, walnut, and hickory logs (Table 2.4). There was a significant interaction between site and species factors (F=2.0, df=15, p=0.0208), but this interaction was related to very low egg densities on logs at Eckles and Western and higher densities at Bicentennial and Kensington. Therefore, marginal comparisons among species within sites are not presented.

A total of 432 *A. planipennis* larval galleries were found on 19 of the 47 green and white ash logs (Table 2.4). The number of galleries per ash log ranged from 0 to 75 with an average of 9.2 (±2.7 SE). Green and white ash logs at Bicentennial had the highest gallery density with approx. 110 galleries/m^2, followed by Kensington with approx. 50 galleries/m^2. Galleries were only found on white ash logs at Eckles and Western.

Four walnut logs had discernable *A. planipennis* feeding attempts made by first instar larvae (Table 2.4). Forty-seven of the 51 larval feeding attempts on walnut were on a single log. All feeding attempts on walnut, however, were small, less than 2 cm long, and consistently resulted in larval death (Table 2.4). Elm, hickory, and hackberry logs had no feeding attempts (Table 2.4). Feeding attempts were distinguished from normal galleries for purpose of analysis and logs with feeding attempts were assigned a gallery density of zero.

Site and log species significantly affected gallery density (χ^2=11.64, df=3,139, p=0.0087 and χ^2=45.77, df=5,137, p<0.0001, respectively). There were significantly

more galleries/m^2 on logs at Bicentennial and Kensington than at Western, but gallery densities on logs at Eckles did not differ significantly from the other sites. The ash logs had significantly more galleries/m^2 than the non-ash logs (Table 2.4). The interaction was significant (F=4.79, df=15, p<0.0001) probably because of the extremely low gallery densities on logs at Eckles and Western and higher densities at Bicentennial and Kensington. Of the 72 logs inspected from Eckles and Western, only 39 galleries were found on 3 white ash logs. Therefore, marginal comparisons among species within sites are not presented.

Logs attached to ash trees

2003 study: The number of *A. planipennis* eggs found in 5 min of searching ranged from 0 to 35 per log, with an average of 3.8 (±1.4 SE) eggs per log. Site location did not significantly affect egg density (S=227.0, df=1,28, p=0.83), but effects of log species were significant (χ^2=15.45, df=2,27 p=0.0004). *Agrilus planipennis* females laid significantly more eggs/m^2 on green ash logs than on walnut and elm logs (Table 2.5). The interaction between site and species factors was not significant (F=1.57, df=2, p=0.23).

Overall, the mean number of galleries per green ash log was 40.6 (±10.1 SE). Only three feeding attempts, each less than 1 cm long, were identified on one of the ten black walnut logs. These feeding attempts were not included in the analysis and logs with feeding attempts were assigned a gallery density of zero. No galleries were found on any of the elm logs (Table 2.5). Gallery density varied significantly among species (χ^2=27.49 df=2,27, p<0.0001), but not among sites (S=240.0, df=1,28, p=0.72). Green ash

logs had significantly more *A. planipennis* galleries/m² than walnut and elm (Table 2.5). The interaction between site and species factors was insignificant (F=0.0, df=2, p=1.0).

2004 study: The number of eggs found in 5 min of searching ranged from 0 to 13 with an average of 1.5 (± 0.4 SE) eggs per log. Egg densities of white ash logs were almost five times that of logs of other species, including green ash and blue ash (Table 2.5). Only one egg was found on walnut and no eggs were found on elm. Egg density varied significantly by species (χ^2=23.79, df=4,49, p<0.0001), but not by site (S=658.5, df=1,52, p=0.11). Egg density did not differ significantly among the three ash species, however, white ash logs had significantly more eggs/m² than elm and walnut logs (Table 2.5). The interaction between site and species factors was not significant (F=1.22, df=4, p=0.32).

A total of 389 galleries were found on the three ash species. Gallery density varied significantly among sites (S=618.0, df=1,52, p=0.0231). On average, there were almost five times more galleries/m² on the logs at Parker Mills than the logs at Marshall Park. Gallery density also varied significantly by species (χ^2=35.61, df=4,49, p<0.001). White ash logs had significantly more galleries/m² than the other species with more than three times more galleries than the ash other species (Table 2.5). Only one small feeding attempt was found on a walnut log and it was not included in the analysis. No feeding attempts were found on elm (Table 2.5). The interaction between site and species was not significant (F=0.32, df=4, p=0.87).

2005 study: *Agrilus planipennis* larvae excavated 800 and 621 galleries on 36 green ash logs and 35 white ash logs, respectively (Table 2.5). I found 148 small larval feeding attempts were made on seven of the 12 black walnut logs and those logs were

assigned a gallery density value of zero. Two main effects, site type (street tree vs. woodlot trees) and tree species (green ash vs. white ash), were marginally insignificant (F=3.97, df=1,61, p=0.0509 and F=3.66, df=1,61, p=0.0604, respectively). Gallery density varied significantly among species (F=16.23, df=4,61, p<0.0001). Green ash logs had significantly more galleries/m^2 than black ash, blue ash, and black walnut logs, but gallery density in white ash logs did not differ significantly from the other ash logs (Table 2.5). None of the two-way interactions were significant: site type and log species (F=2.28, df=4,61, p=0.07), tree species and site type (F=0.78, df=1,61, p=0.38), and tree species and log species (F=0.43, df=4,61, p=0.79).

A significant three-way interaction occurred for tree species, site type, and log species (F=3.33, df=4,61, p=0.0156). Therefore, means were separated and analyzed within each site type and tree type. At the Wellington site with green ash street trees, there were significantly more galleries/m^2 in the green ash, white ash, and black ash logs than on the walnut logs (Figure 2.1a). At Kensington, where logs were attached to green ash woodlot trees, green ash and white ash logs had significantly more galleries/m^2 than blue ash, black ash, and walnut logs (Figure 2.1b). Green and blue ash logs also had significantly more galleries/m^2 than white ash, black ash, and walnut logs at Windemere, were logs were attached to white ash street trees (Figure 2.1c). At Dicken Woods, logs were in white ash woodlot trees and there were significantly more galleries/m^2 on green ash, white ash, and black ash logs than on the walnut logs (Figure 2.1d).

Nursery trees

2004 study: Similar numbers of galleries were found on the upper portion of the green and white ash trees in the nursery at the Matthaei Botanical Gardens (Table 2.3).

Sixty-eight percent of the galleries found on green ash were on the four trees with high bark roughness, while 32% of the galleries were on green ash with smooth bark. A single larval feeding attempt was found on one Japanese tree lilac and that feeding attempt was not included in the statistical analyses. No feeding attempts were found on black walnut. An average of 146.9 (±46.3 SE) galleries/m^2 was calculated for green ash trees (Table 2.3). Galleries density did not differ significantly between green and white ash trees, but the ash trees had significantly more galleries/m^2 than tree lilac and walnut (overall χ^2=33.82, df=3,36, p<0.0001) (Table 2.3).

On smooth-barked trees, rough bark texture can still be found around branch junctions. Ten white ash and six green ash trees had smooth bark on the stem and 63% and 71% of galleries, respectively, originated within 5 cm of a branch junction on those smooth-barked trees. Four green ash trees had high bark roughness along the main stem and had 53% galleries within 5 cm of a branch junction. Japanese tree lilac had medium bark roughness and the one small larval feeding attempt was found within 5 cm of a branch junction. Black walnut also had high bark roughness, but I did not observe larval feeding. Ash stems with rough bark and ash stems with smooth bark had an average of 458 (±51 SE) and 137 (±13 SE) galleries/m^2, respectively. Rough bark ash stems had significantly higher gallery densities than the ash stems with smooth bark (S=74.0, df=1,18, p<0.0001). The difference in the percentage of galleries originating within 5 cm of a branch junction between smooth bark and rough bark green ash trees was not significant (χ^2=2.93, df=1,8, p=0.09) (Table 2.3).

2005 study: Green ash trees had 365 more galleries than white ash trees. Over 70% of the galleries on green ash trees were on the five trees with rough bark. Two small

larval feeding attempts were found on one Japanese tree lilac and were not included in the analysis. No feeding attempts were found on black walnut. There were significantly more galleries on green ash and white ash trees than on tree lilac and walnut (χ^2=33.46, df=3,36, p<0.0001) (Table 2.3).

Ten white ash and five green ash trees had smooth bark. On these trees, 75% and 93% of galleries, respectively, originated within 5 cm of a branch junction. Five green ash trees had high bark roughness along the main stem and had 74% of the galleries originating within 5 cm of a branch junction. Japanese tree lilac had medium bark roughness and the two small larval feeding attempts were both within 5 cm of a branch junction. Black walnut also had high bark roughness, but no feeding attempts were found. Ash stems with rough bark and ash stems with smooth bark had an average of 255 (±26 SE) and 163 (±21 SE) galleries/m^2, respectively. Rough bark ash stems had significantly higher gallery densities than the ash stems with smooth bark (S=80.0, df=1,18, p<0.0146). The smooth bark green ash trees had significantly more galleries originate within 5 cm of a branch junction than the rough bark green ash trees (χ^2=6.32, df=1,8, p=0.0119).

American elm survey

Each of the nine elm trees that were peeled appeared healthy and contained no apparent insect infestation or pathogens of any kind. On two of the elm trees I peeled, at least 200 cm^2 of bark on the trunk was directly contacting bark on the trunk of ash trees that had been completely girdled by *A. planipennis* larvae. In this situation, adults could have readily laid eggs on elm bark and larvae would have been able to feed from ash into elm with little exposure.

DISCUSSION

The primary goal of these studies was to examine how *A. planipennis* adult females would respond in the field when presented with a choice between known hosts and potential alternate hosts for oviposition. Logs and trees were dissected to evaluate suitability of the host species for larval development. Monitoring *A. planipennis* landing rates on the logs on t-posts showed that, in the field, adults will often land on species other than ash. *Agrilus planipennis* adults distinguished between the cut logs, in general, and black plastic drain pipe. Although not measured in this study, volatiles associated with cut logs presumably affect the ability of females to distinguish between the logs and the plastic pipe (Dunn et al. 1986a, 1986b, Kimmerer and Kozlowski 1982).

My ability to find *A. planipennis* eggs varied among species and depended largely on bark texture. Bark of some species, like hackberry, lacked flaky layers of periderm and eggs were apparent because they were on the surface. Other species, including ash, required meticulous examination and careful removal of the flaky, outer layers of bark to reveal hidden eggs. On ash, egg densities were likely underestimated and were considerably lower than gallery densities for this reason. Therefore, estimates of egg density should not be considered an accurate measure of host preference.

As predicted, population densities of *A. planipennis* varied considerably among sites, ranging from a low-level infestation at Western, a moderate-level at Kensington, and high-level infestations at Bicentennial and Eckles. Thirty-three percent of adults captured in this study came from the logs at the Eckles disposal yard, but these logs had the lowest egg and gallery densities of all four sites. Low egg and gallery densities at

Eckles were probably caused by the lack of ash foliage in the surrounding area. Females need to feed for at least two weeks before mating and ovipositing (Bauer et al. 2004). Beetles that emerged from material at this site may have landed on the logs, but no oviposition would have occurred because they needed to fly off site to find live ash trees for feeding. Similarly, several other species of *Agrilus* are known to require foliage for maturation feeding (Carlson and Knight 1969).

At Kensington, the two logs wrapped with Tanglefoot® in one block placed 4 m inside the woodlot only caught three adults throughout the duration of the flight season compared to 86 adults caught on the other 11 logs in sunlight at that site. This suggests that beetles preferred for sunny, open areas over shaded areas. Other studies have found that traps or trap trees located in sunny, open conditions were more attractive than those within closed canopy woodlots (Francese et al. 2006, McCullough et al. 2006, Poland et al. 2005). Many *Agrilus* sp. and other buprestids show a similar tendency to select hosts which are exposed to sunlight (Barter 1957, Carlson and Knight 1969).

By attaching logs to the trunks of infested ash trees in the second study, artificial conditions were created to facilitate females ovipositing within the ash trees to oviposit on logs of other species. In this setting, logs of potential alternate host species were surrounded by ash volatiles emitted from foliage, wood, and bark. In 2003, I attached green ash logs to green ash trees and found very high gallery densities in green ash logs. In 2004, green and white ash logs were both attached to white ash trees and I found high gallery densities in the white ash logs, but considerably lower densities in the green ash logs. Though highly debated, Hopkins' host selection principle is based on the observations that show many adult insects demonstrate a preference for the host species

on which they themselves developed as larvae (Barron 2001). Since the majority of the *A. planipennis* adults probably emerged from the surrounding white ash trees and there were significantly more galleries on the white ash logs, I considered the possibility of host conditioning influencing results in 2004. Therefore, I repeated the study for a third year in 2005, and attached both green and white ash logs to green and white ash trees. If a conditioning effect had occurred, I would have expected higher gallery densities in the green ash logs that were attached to green ash trees and lower gallery densities in green ash logs attached to white ash trees. Similarly, I expected the higher gallery density on white ash logs to occur in sites with white ash trees.

My results, however, were not consistent with a conditioning effect. At three of the four sites in 2005, green ash logs had more galleries/m^2 than white ash logs and differences were only significant at Windemere, where the logs were in white ash street trees. In both 2004 and 2005, there were more galleries in white ash logs than green ash logs only in forested sites dominated by white ash. In the 2005 site with open-grown white ash street trees, green ash logs had more galleries than white ash logs.

As in the t-post study, exposure to sun or shade probably played an important role in host selection by *A. planipennis* females. In 2003, two of the ten trees used in my study were open-grown, while the other eight trees were in closed canopy woodlots. In 2004, all 18 trees were in closed canopy woodlots and, in 2005, I initially selected open-grown and shaded trees. The Kensington trees were along the edge of woodlots, while trees at Dicken Woods were within a closed canopy. Logs on trees in the shade, overall, had roughly 20% fewer galleries/m^2 than those in the sun.

While eggs were often laid on non-ash species, larvae attempted to feed only on black walnut logs, though always unsuccessfully. In both the t-post and logs attached to ash tree studies, the only non-ash species to sustain any larval feeding was black walnut. These feeding attempts on black walnut consisted of very thin galleries that were usually less than a few centimeters long. A few were 10-20 cm long, but there was never any evidence of larval development through successive instars before feeding stopped. The highest number of feeding attempts was on black walnut logs attached to ash trees. Here, these non-host logs would have been engulfed in ash volatiles and females may have been more likely to mistakenly oviposit on these logs than on logs on t-posts. I also noted that larval feeding attempts were only found on black walnut logs that were attached to open-grown ash street trees and not on logs attached to trees in woodlots. This trend is most likely explained by greater beetle activity at sunny sites than shaded sites.

Gallery densities were higher, overall, on the logs attached to ash trees than on the logs on t-posts, probably due to the fact that females spend time feeding on leaves and hence lay more eggs when foliage is available. In addition, higher concentrations of leaf and bark volatiles would have been associated with the logs in the trees than compared to logs on the t-posts. These volatiles were, presumably, an important stimulus for *A. planipennis* oviposition, and were either greatly reduced or completely lacking around the cut logs on t-posts or chemically different when produced by the cut logs. Future studies should focus on determining variations among the chemical profiles of volatiles produced by ash species under varying conditions, including cut logs.

Bicentennial Park had a woodlot with heavily infested green ash trees with a large number of American elm trees growing in close proximity. Several of these elms were in physical contact with the infested ash. I hypothesized that if *A. planipennis* could utilize American elm as a host, either by intentionally or accidentally ovipositing on elms in close contact with ash, I would find evidence of this at this site. More than 28 m^2 of elm phloem surface area was inspected and no sign of *A. planipennis* attack was found. This, along with results from the no-choice bioassays and field studies, suggests that American elm is not a suitable host for *A. planipennis*.

The study with nursery trees provided beetles with live trees with intact vascular systems and foliage rather than cut logs. Moisture loss in cut logs can influence both host selection and larval development (Haack and Slansky 1986, Hanks et al. 1999), but would not have been a factor in the nursery tree study. I hypothesized that larval feeding attempts would not be found on live black walnut trees because living trees have natural defense mechanisms in place that are no longer fully intact or functional in cut logs (Hanks1999, 1993). There were no *A. planipennis* galleries or feeding attempts found on any of the black walnut trees in 2004 or 2005. A total of three small, unsuccessful feeding attempts were made on two of the Japanese tree lilac nursery trees. These consisted of narrow galleries approx. 1 mm wide and less than 3 cm long and there was no evidence to suggest that larvae could successfully complete development on this species.

In 2004 and 2005, green ash nursery trees had higher gallery densities than the white ash trees, but differences were not statistically significant. There appeared to be only a weak preference for green ash over white ash in these studies. Field surveys of

green and white ash trees showed that green ash trees consistently had more canopy dieback and higher densities of *A. planipennis* exit holes and woodpecker attacks than white ash trees growing in the same area (see Chapter 3). Interestingly, there were twice as many galleries found on the ash nursery trees in 2005 than in 2004. This was probably due to the decrease in the number of suitable ash trees, as most ash trees in the surrounding area succumbed to *A. planipennis* by 2004. In 2005, the 20 ash nursery trees were probably the only live host trees in a 1.5 km radius. The incident of higher attack densities may be indicative of what could happen in the core area of *A. planipennis* infestation. As *A. planipennis* population densities increase and suitable ash host population densities decrease, small pockets of ash that had initially escaped infestation in previous years, may be attacked at high rates.

One factor that could be driving *A. planipennis* preference for green ash trees over white ash trees in this size class could be bark texture. Other studies on *Agrilus* species have also reported a preference for rough-barked portions of trees for oviposition (Barter 1957, Loerch and Cameron 1984). Rough bark provides more oviposition sites where females can hide eggs and where eggs are more likely to escape predation by other insects. On smooth-barked trees, branch junctions provide bumps and rough patches in the bark where eggs can be hidden. If there was a positive relationship between bark roughness and oviposition preference, I would have expected more galleries to originate near branch junctions on smooth-barked trees than on rough-barked trees. This occurred in 2005; significantly more galleries originated near branch junctions on smooth-barked green ash than on rough-barked green ash trees. In 2004, there were also more galleries

near branch junctions on smooth-barked than rough-barked green ash, but the difference was marginally insignificant (p=0.09), probably due to the small sample size.

In both years, galleries on green ash trees with rough bark were so dense that most larvae ran out of phloem and died before reaching the fourth instar. I have observed these high attack rates on other ash trees in the field. In some cases, trees have few or no *A. planipennis* exit holes on the trunk, but are completely infested by larvae that died from intra-specific competition for the limited phloem resource. Additional studies are needed to further examine differences in ash phloem quality and bark characteristics and how these factors affect host suitability and preference.

In each of my studies, *A. planipennis* appeared to prefer ash species over the non-ash species and there appeared to be a preference for green ash over the other ash species. In addition to green and white ash trees, I have observed full development and emergence of *A. planipennis* from black and blue ash trees in the field, so there is no question that these species are suitable hosts. What is unclear is the order of preference among all four species, whether this preference is consistent, and how host preference is affected by *A. planipennis* density and host tree exposure to sun. While females occasionally oviposited on black walnut and Japanese tree lilac, these species do not appear to be suitable hosts for larval development.

Table 2.1. Mean (±SE) diameter, length, and surface area for logs of six species attached to t-posts in *Agrilus planipennis*- infested areas in 2003.

Species	n	Diameter (cm)	Length (cm)	Surface area (m^2)
Green ash	24	12.0 ± 0.4	62.0 ± 0.2	0.23 ± 0.01
White ash	23	13.0 ± 0.5	61.1 ± 0.2	0.24 ± 0.01
American elm	24	13.2 ± 0.4	61.4 ± 0.2	0.25 ± 0.01
Black walnut	24	14.1 ± 0.5	59.8 ± 0.9	0.26 ± 0.01
Hickory	24	13.5 ± 0.4	60.7 ± 0.2	0.26 ± 0.01
Hackberry	24	13.0 ± 0.4	61.5 ± 0.3	0.25 ± 0.01

Table 2.2. Mean (±SE) diameter, length, and surface area for logs of five species attached to *Agrilus planipennis*- infested ash trees in 2003, 2004, and 2005.

Species	n	Diameter (cm)	Length (cm)	Surface area (m^2)
2003 Logs attached to green ash trees				
Green ash	10	10.8 ± 0.2	62.0 ± 0.5	0.21 ± 0.01
American elm	10	12.2 ± 0.6	59.1 ± 1.7	0.22 ± 0.01
Black walnut	10	12.6 ± 0.6	57.2 ± 1.4	0.23 ± 0.01
2004 Logs attached to white ash trees[1]				
Green ash	18	13.6 ± 0.5	60.3 ± 0.5	0.23 ± 0.01
White ash	9	12.4 ± 0.8	60.2 ± 0.1	0.21 ± 0.01
Blue ash	9	11.4 ± 0.4	60.3 ± 0.8	0.20 ± 0.01
American elm	9	12.3 ± 0.5	60.7 ± 0.5	0.20 ± 0.01
Black walnut	9	14.4 ± 0.6	60.0 ± 0.2	0.23 ± 0.01
2005 Logs attached to green and white ash trees[1]				
Green ash	39	11.8 ± 0.3	61.4 ± 0.2	0.21 ± 0.01
White ash	39	13.0 ± 0.3	61.3 ± 0.3	0.24 ± 0.01
Black ash	14	11.8 ± 0.3	58.5 ± 1.3	0.21 ± 0.01
Blue ash	12	12.5 ± 0.6	58.4 ± 1.2	0.22 ± 0.01
Black walnut	12	14.3 ± 0.5	60.9 ± 0.6	0.24 ± 0.01

[1]Incomplete block design.

Table 2.3. Mean (±SE) tree diameter at breast height (DBH), surface area sampled, and total and mean (±SE) number of *Agrilus planipennis* galleries per m^2 for four species of trees used in multiple-choice nursery tree studies in 2004 and 2005.

	No. trees	DBH (cm)	Mean surface area sampled (m^2)	Total galleries	Mean no. galleries/m^2	Total feeding attempts	Mean no. feeding attempts/m^2
2004 Nursery trees							
Green ash	10	6.0 ± 0.2	0.46 ± 0.09	535	145.7 ± 46.6 **a**	0	0
White ash	10	6.3 ± 0.2	0.73 ± 0.07	522	76.5 ± 6.7 **a**	0	0
Japanese tree lilac	10	4.6 ± 0.1	0.26 ± 0.02	0	0 **b**	1	0.4 ± 0.4
Black walnut	10	6.4 ± 0.2	0.56 ± 0.03	0	0 **b**	0	0
2005 Nursery trees							
Green ash	10	8.0 ± 0.2	1.98 ± 0.27	1258	58.9 ± 10.0 **a**	0	0
White ash	10	7.5 ± 0.2	1.71 ± 0.09	893	51.6 ± 2.7 **a**	0	0
Japanese tree lilac	10	5.3 ± 0.1	0.31 ± 0.03	0	0 **b**	2	0.5 ± 0.5
Black walnut	10	7.7 ± 0.2	0.61 ± 0.03	0	0 **b**	0	0

Within columns, means followed by the same letter are not significantly different (Kruskal-Wallis test and nonparametric multip comparisons procedure; $p < 0.05$).

Table 2.4. Total and mean (±SE) number of *Agrilus planipennis* adults, eggs per m^2, and galleries per m^2 for logs and drain pipes attached to t-posts at four field sites in *Agrilus planipennis*- infested areas in 2003.

	Green ash	White ash	American elm	Black walnut	Hickory	Hackberry	"Control" pipe
Adult landing rates							
No. of logs	19	18	18	18	18	18	16
No. of logs with adults	16	14	16	15	11	12	7
Total no. of adults	112	121	86	133	83	49	25
Mean no. of adults	5.9 ± 1.3 **a**	6.7 ± 2.5 **a**	4.8 ± 1.3 **a**	7.4 ± 2.9 **a**	4.6 ± 1.3 **a**	2.7 ± 0.7 **ab**	1.1 ± 0.5 **b**
Eggs							
No. of logs	24	23	24	24	24	24	
No. of logs with eggs	12	14	5	3	2	12	
Total no. of eggs	79	120	8	23	3	23	
Mean no. of eggs/m^2	14.1 ± 3.9 **ab**	21.7 ± 6.5**a**	1.2 ± 0.5 **bc**	1 ± 0.6 **bc**	0.5 ± 0.4 **c**	3.7 ± 0.9 **abc**	
Larval galleries							
No. of logs with galleries or feeding attempts	8	11	0	4	0	0	
Total no. of galleries	217	215	0	0	0	0	
Mean no. of galleries/m^2	39.6 ± 17.1 **a**	36.5 ± 14.8 **a**	0 **b**	0 **b**	0 **b**	0 **b**	
Total no. of feeding attempts	0	0	0	51	0	0	
Mean no of feeding attempts/m^2	0	0	0	7.6 ± 7.0	0	0	

Within rows, means followed by the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparisons procedure; $p < 0.05$).

Table 2.5. Mean (±SE) number of *Agrilus planipennis* eggs per m^2 and galleries per m^2 for logs attached to *Agrilus planipennis*-infested ash trees in 2003, 2004, and 2005.

	Green ash	White ash	Black ash	Blue ash	American elm	Black walnut
2003 Logs attached to green ash trees						
No. of logs	10				10	10
No. of logs with eggs	9				5	1
Total no. of eggs	99				12	3
Mean no. of eggs/m^2	47.1 ± 15.8 **a**				4.9 ± 2.4 **b**	1.5 ± 1.5 **b**
No. logs with galleries or feeding attempts	10				0	1
Total no. of galleries	406				0	0
Mean no. of galleries/m^2	195.5 ± 49.5 **a**				0 **b**	0 **b**
Total no. of feeding attempts	0				0	3
Mean no. of feeding attempts/m^2	0				0	0
2004 Logs attached to white ash trees						
No. of logs	18	9		9	9	9
No. of logs with eggs	10	8		4	0	1
Total no. of eggs	19	49		10	0	1
Mean no. of eggs/m^2	4.9 ± 1.3 **ab**	28.1 ± 6.8 **a**		5.2 ± 2.7 **ab**	0 **b**	0.6 ± 0.6 **b**
No. logs with galleries or feeding attempts	14	9		6	0	0
Total no. of galleries	75	266		28	0	0
Mean no. of galleries/m^2	17.0 ± 3.3 **b**	143.6 ± 33.5 **a**		14.0 ± 5.0 **bc**	0 **c**	0 **c**
Total no. of feeding attempts	0	0		0	0	1
Mean no. of feeding attempts/m^2	0	0		0	0	0
2005 Logs attached to green and white ash trees						
No. of logs	39	39	14	12		12
No. logs with galleries or feeding attempts	36	35	14	10		7
Total no. of galleries	800	621	122	115		0
Mean no. of galleries/m^2	97.4 ± 13.3 **a**	70.0 ± 8.9 **ab**	39.6 ± 7.2 **b**	42.2 ± 16.6 **b**		0 **c**
Total no. of feeding attempts	0	0	0	0		148
Mean no. of feeding attempts/m^2	0	0	0	0		48.4 ± 19.8

Within rows, means followed by the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparisons procedure; $p < 0.05$).

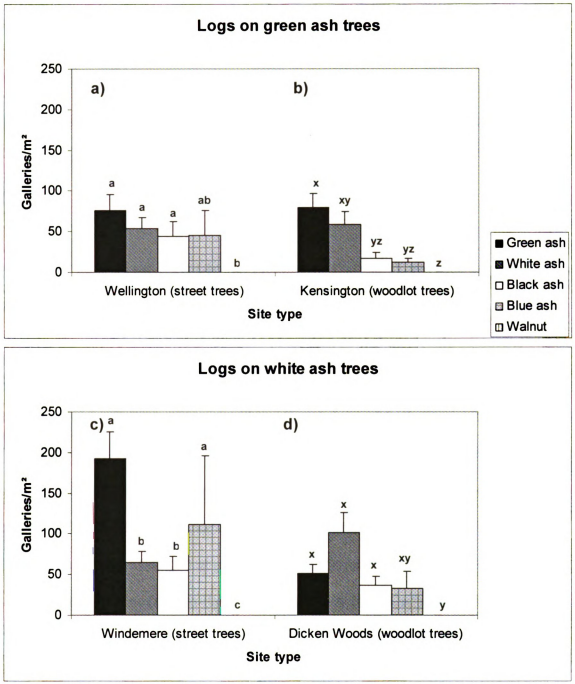

Figure 2.1. Mean (± SE) number of *Agrilus planipennis* galleries per m^2 on logs of five species attached to *Agrilus planipennis*-infested **a)** green ash street trees, **b)** green ash woodlot trees, **c)** white ash street trees and **d)** white ash woodlot trees in 2005. Within site and tree types, means with the same letter are not significantly different (Fisher's protected LSD test; $p<0.05$).

CHAPTER 3

Field Surveys of Emerald Ash Borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) Density and Canopy Dieback in Three North American Ash Species

INTRODUCTION

The emerald ash borer (*Agrilus planipennis* Fairmaire), an Asian buprestid discovered in June 2002, is established in much of southeast Michigan and Windsor, Ontario, Canada (Cappaert et al. 2005c). More than 35 distinct, localized infestations have been found since 2003 in other areas of Michigan, Ohio, and Indiana. *Agrilus planipennis* is native to northeastern China, Korea, Japan, Mongolia, the Russian Far East, and Taiwan (Yu 1992). This phloem-feeding species was likely introduced into North America through solid wood packing material (Cappaert et al. 2005c), such as crating, pallets, or dunnage made from ash wood containing bark strips.

In southeast Michigan, *A. planipennis* adult emergence begins as early as mid- to late May or early June (Brown-Rytlewski and Wilson 2005, Cappaert et al. 2005c). Adult beetles live for 3-6 weeks (Cappaert et al. 2005c). Upon emergence, beetles feed on ash foliage for 5-7 days before mating and females feed for another 5-7 days before oviposition begins (Bauer et al. 2004, Lyons et al. 2004). Adult activity peaks from late June to early July (Cappaert et al. 2005c). Most eggs are laid in July and early August. Eggs hatch in approx. two weeks (Lyons et al. 2004). Larvae feed on phloem and outer sapwood for several weeks and complete their feeding in autumn (McCullough and

Katovich 2004). Most *A. planipennis* overwinter as pre-pupal larvae in shallow chambers excavated in the outer sapwood or in the bark of thick-barked trees. Woodpecker predation of pre-pupal larvae occurs mostly during winter and early spring and can range from 9-95% (Cappaert et al. 2005b). Pupation begins in late April or May. Newly eclosed adults may remain in pupal chambers for 1-2 weeks, before emerging head-first, leaving a D-shaped exit hole 3-4 mm in diameter (McCullough and Katovich 2004).

In North America, *A. planipennis* is aggressively attacking healthy, as well as stressed, ash trees (*Fraxinus* sp.). As of early 2006, an estimated 12-15 million ash trees southeast Michigan were dead or dying. However, limited reports suggest that *A. planipennis* is not a major pest in Asia (Akiyama and Ohmomo 2000).

Agrilus planipennis has the potential to spread throughout North America via natural dispersal by adult beetles and artificial spread caused by humans moving infested ash material. *Fraxinus* species are widely distributed across the eastern U.S. and portions of southeastern Canada, occurring in many different forest ecosystems (Harlow et al. 1991). There are at least 16 native species of *Fraxinus* in the U.S. (Harlow et al. 1991), four of which are found in Michigan: green ash (*F. pennsylvanica* Marsh.), white ash (*F. americana* L.), black ash (*F. nigra* Marsh.), and blue ash (*F. quadrangulata* Michx.) (Barnes and Wagner 1981). More than 802 million ash trees occur on timberlands in Michigan alone [USDA Forest Inventory and Analysis (FIA) database 2005]. Ash trees are also one of the more widely planted trees in many urban areas of the U.S. (Giedraitis and Kielbaso 1982, Ottman and Kielbaso 1976).

In natural forests, *A. planipennis* could substantially alter biodiversity and stand dynamics (MacFarlane and Meyer 2005). Green ash is the most widely distributed of the

North American ashes, both naturally in forested areas and as a street tree (Harlow et al. 1991). White ash is the most abundant native ash species and is a component of more than 24 forest cover types (Harlow et al. 1991). White ash is also highly valued as a street tree for its form and fall color. Blue ash occurs on dry, limestone uplands. Southeast Michigan is the northern edge of the range for blue ash and this species is relatively rare in Michigan in comparison with other ash species. Blue and white ash will often occur together on sites with better soils. Black ash, typically found in northern regions, grows in swamps, peat bogs, stream and riverbanks (Harlow et al. 1991).

Given the extensive damage that *A. planipennis* has already caused to the *Fraxinus* resource in southeast Michigan, an important question is whether *A. planipennis* preferentially feeds or oviposits on certain North American species of *Fraxinus*. Understanding the preference hierarchy and the underlying mechanisms of *A. planipennis* attraction among the *Fraxinus* species will increase our ability to accurately assess stand susceptibility to this pest based on species composition. It is also important for developing survey, detection, and management options and predicting spread and impacts of *A. planipennis* throughout North America.

To assess host preference, I quantified the density of *A. planipennis* exit holes and woodpecker attacks at four sites where green and white ash street trees were planted together and at two woodlots where white and blue ash trees co-occurred. I also monitored canopy dieback and changes in exit hole and woodpecker attack densities over time.

MATERIALS AND METHODS

Study sites

Study sites were selected based on four main criteria. Sites had to have two species of ash growing in close proximity, with similar range in DBH (diameter at breast height). Trees within each site had to be untreated, growing under similar conditions, and were assumed to be exposed to similar levels of *A. planipennis* pressure. Permission to survey trees was acquired from the appropriate parties.

Green vs. white ash street trees: In 2003, I sampled green and white ash street trees at two sites. The first site, Butzel Elementary and Middle School, was in Detroit, Wayne Co., MI. There were eight, small green ash and eight white ash trees growing around the perimeter of the school yard. The second site on Larned Boulevard, also in Detroit, had eleven green ash trees located on the east side of the street and eleven white ash trees in the grassy median. In 2004, a third site was added to the survey. The Windemere neighborhood in Ann Arbor, Washtenaw Co., MI, had an abundance of green and white ash street trees growing in the grass strip between the sidewalk and the street. Seven green ash and seven white ash trees were selected for surveying at this site. A fourth site, added in 2005, had clusters of green and white ash trees within a five mile radius of the Lakeside Terraces condominium complex in Sterling Heights, Macomb Co., MI. Eight green ash and nine white ash trees growing within 30 m of one another were selected for survey. Trees ranged in DBH from 4 to 38 cm (Table 3.1).

White vs. blue ash woodlot trees: In 2004, I identified two woodlots with *A. planipennis* populations that had both white and blue ash trees growing in close proximity. In Superior Township, Washtenaw Co., MI, DBH of white and blue ash trees

ranged from 16.5 to 32 cm and trees were growing 6-24 m apart (Table 3.1). In a woodlot in Plymouth Township, Wayne Co., MI, DBH of trees ranged from 11 to 35 cm and trees were growing 3-7 m apart (Table 3.1). Both woodlots were surveyed in 2004, and Superior Twp. was surveyed again in 2005 (Table 3.1).

Sampling and data collection

Green vs. white ash street trees: For each tree, I made visual estimates of canopy dieback in late summer and quantified the number of *A. planipennis* exit holes and woodpecker attacks on larvae in the fall, after adult emergence was complete. Woodpecker attacks occur primarily in winter and early spring when pre-pupae are present (Cappaert et al. 2005b, c). Fall surveys were cumulative and included *A. planipennis* that had developed and emerged or pre-pupae that had been preyed upon by woodpeckers through that year's survey.

Trees with DBH greater than 5 cm were surveyed around the entire trunk or branch circumference in four areas: at 1.5 and 3 m aboveground, at the base of the lowest first order branch, and 1 m out on that branch. On each tree, an area of approx. 0.26 m^2 was inspected at 1.5 m above ground and 0.13 m^2 at the other three sample areas [average total area of 0.65 (±0.01 SE) m^2 per tree]. Areas above 1.5 m were accessed using a ladder. On 11 trees at Butzel with a DBH less than 5 cm, I examined the entire stem and all the branches. On average, I inspected 0.52 (±0.03 SE) m^2 on each of the small trees. Percentage of the canopy that was dead, number of *A. planipennis* exit holes, and number of woodpecker attacks on *A. planipennis* pre-pupal larvae was recorded for each area inspected on each tree. Mean *A. planipennis* density per tree was calculated by

combining the total number of *A. planipennis* exit holes and woodpecker attacks and dividing it by the total area sampled in the tree.

Survey methods were modified in 2004 because related research showed that first attacks by *A. planipennis* typically occur in the upper canopy (Cappaert et al. 2005a). Therefore, methods for subsequent surveys were adjusted to sample higher in the trees. Trees with DBH greater than 5 cm were inspected by tree climbers who surveyed four to six intervals between 1.5 and 8.5 m aboveground on each tree. An average area of 1.04 (±0.07 SE) m^2 was inspected on each tree. Small trees (<5 cm) were completely surveyed as before. With the exception of the sample area at 1.5 m aboveground, sample areas were randomly selected and may or may not have overlapped with areas sampled the previous year.

In 2003, 19 green ash and 19 white ash (38 trees total) at Butzel and Larned were surveyed for canopy dieback on 12 August and *A. planipennis* exit holes and woodpecker attacks on 12 August and 11 October. In 2004, canopy dieback estimates for 26 green ash and 26 white ash (52 trees total) were made at Butzel, Larned, and Windemere on 13 September. During July 2004, seven dead trees were felled and removed from the survey sites by the respective municipalities: four green ash trees at Larned and two green ash and one white ash tree at Butzel. Because canopy dieback on these trees was greater than 90%, dieback was recorded for these trees as 100% in the subsequent year's survey. Data for exit holes and woodpecker attacks on these trees were collected on 6 August 2004 at Butzel and Larned and on 9 August at Windemere.

Seven additional dead trees were removed in 2005: one green ash and two white ash trees at Butzel and two green ash and two white ash trees at Windemere. The two

white ash trees at Windemere were cut down after dieback estimates were made, but before exit hole and woodpecker attack data could be collected. Dieback for these five trees was recorded as 100% in 2005. Dieback estimates were made on 16 September 2005 at all sites, except Lakeside, where leaves began falling before I could estimate dieback. Exit holes and woodpecker attacks on 25 green ash and 30 white ash (55 trees total) were counted on 12 October at Butzel and Windemere, 19 October at Lakeside, and 19 and 21 October 2005 at Larned. Data collected from 25 green ash and 26 white ash trees (51 trees total) at Larned and Butzel in 2003 and at Windemere in 2004 were used to assess the relationship between canopy dieback and *A. planipennis* density.

White vs. blue ash woodlot trees: On 2 March 2004, two white ash and three blue ash trees were surveyed by climbers in the Superior Twp. woodlot. Seven to 11 intervals on the main stem and branches between 1.5 and 15 m aboveground were examined on each tree and *A. planipennis* exit holes and woodpecker attacks were recorded for each area. An average of 2.9 (±0.22 SE) m^2 per tree was inspected. On 28 May 2004, before the onset of *A. planipennis* adult emergence, two additional white ash and two blue ash trees were felled. Four to eight intervals between 1.5 and 15 m aboveground were inspected on each tree. An average of 0.8 (±0.09 SE) m^2 per tree was examined. Trees that were felled were debarked and the percentage of phloem surface area covered by *A. planipennis* galleries was estimated. Means were calculated by combining the total number of exit holes and woodpecker attacks and dividing it by the total area sampled in the tree. No significant difference was found between *A. planipennis* density data gathered by climbing trees or by felling them (S=26.0, df=1,7,

p=0.09). Because of the difficulty of accurately estimating canopy dieback on closed-canopy woodlot trees, dieback estimates were not recorded.

On 20 January 2005, five white ash and five blue ash trees in the Plymouth Twp. woodlot were felled. Five to 16 trunk and branch sections between 1.5 and 17 m aboveground were removed from the trees and taken to the laboratory for inspection. Average area sampled per tree was 1.4 (±0.21 SE) m^2. Number of *A. planipennis* exit holes, woodpecker attacks, and percentage of phloem surface area covered by galleries were recorded.

On 21 November and 2 December 2005, another four white ash and four blue ash trees in the Superior Twp. woodlot were felled and inspected using methods from the 2004 Plymouth Twp. survey. Five to eight sections were taken from each tree between 1.5 and 21.5 m aboveground, with an average sample area of 1.1 (±0.10 SE) m^2 per tree.

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Data were not normally distributed after transformations. Therefore, the nonparametric Wilcoxon Rank Sum test (Ott and Longnecker 2001) was used to determine if the mean number of *A. planipennis* exit holes and woodpecker attacks density, percentage of phloem surface area covered by galleries, and percentage of canopy dieback differed significantly between species at each site.

Data from Larned and Butzel in 2003 and Windemere in 2004 was pooled and tested for normality. Data were normally distributed and simple linear regression was used to determine if canopy dieback was a significant predictor of *A. planipennis* exit hole and woodpecker attack densities. Two outliers were identified in the data set and

were excluded. All analyses were conducted at the $p<0.05$ level of significance, using SAS statistical software (SAS Institute, Inc. 1989).

RESULTS

Green vs. white ash street trees

At Butzel, canopy dieback of green ash trees was, on average, 13-20% higher than canopy dieback of white ash trees during the three year period, but differences were not significant in 2003 (S=83.5, df=1,14, p=0.0532), 2004 (S=79.0, df=1,14, p=0.13), or 2005 (S=73.0, df=1,14, p=0.31) (Figure 3.1a). Average canopy dieback in these small green ash and white ash trees increased 32% and 25%, respectively, from 2003 to 2004, and 13% and 18%, respectively, from 2004 to 2005. (Figure 3.1a). In 2004, average canopy dieback, excluding the three dead trees that were removed, was 43% (±10.2 SE) for green ash and 30% (±18.2 SE) for white ash. In 2005, canopy dieback (excluding the dead trees) was 53% (±11.4 SE) for green ash and 29% (±18.5 SE) for white ash.

Green ash trees at Butzel had an average of 17-39 more exit holes and woodpecker attacks per m^2 than white ash trees throughout the study, but differences were not significant in 2003 (S=80.0, df=1,14, p=0.11), 2004 (S=47.0, df=1,11, p=0.27), or 2005 (S=33.0, df=1,8, p=0.31) (Figure 3.1b). Exit hole and woodpecker attack densities in green ash more than tripled from 2003 to 2004 and increased five fold in white ash (Figure 3.1b). Densities remained relatively consistent from 2004 to 2005 (Figure 3.1b). Average exit hole density ranged from 21-78 exit holes/m^2 in green ash and 9-43 exit holes/m^2 in white ash from 2003 to 2005. Woodpecker attacks were found on both branches and trunks of green and white ash trees. Average woodpecker attack density ranged from 4-13 woodpecker attacks/m^2 in green ash and 0-10 in white ash over the three year period. On average, by 2005, woodpeckers had preyed upon 21% of the *A. planipennis* in these trees.

At Larned, green ash trees had significantly more canopy dieback than white ash trees in 2003 (S=182.0, df=1,20, p<0.0001), 2004 (S=187.0, df=1,20, p<0.0001), and 2005 (S=181.5, df=1,20, p<0.0001) (Figure 3.2a). Dieback increased approx. 25% each year for both species and green ash trees averaged 49-69% more dieback than white ash trees (Figure 3.2a). In 2004, six of the seven green ash trees remaining on site had 100% canopy dieback and only 10% of the canopy was alive on the other. By 2005, all of the green ash trees in the survey were dead.

Green ash trees at Larned had 1.5 to four times as many *A. planipennis* exit holes and woodpecker attacks per m^2 as white ash trees throughout the study. Exit hole and woodpecker attack densities were significantly greater in green ash than in white ash in 2003 and 2004 (S=169.0, df=1,20, p=0.0019 and S=101.0, df=1,16, p<0.0004, respectively), but differences were marginally insignificant in 2005 (S=85.0, df=1,16, p=0.0521) (Figure 3.2b). Exit hole and woodpecker attack densities in green ash increased by 45% from 2003 to 2004 and by 41% from 2004 to 2005. Densities in white ash decreased slightly from 2003 to 2004, but almost tripled from 2004 to 2005 (Figure 3.2b). Average exit hole density ranged from 50-114 exit holes/m^2 in green ash and 6-14 exits holes/m^2 in white ash from 2003 to 2005. Average woodpecker attack density ranged from 2-10 woodpecker attacks/m^2 in green ash and 4-35 woodpecks/m^2 in white ash. By 2005, all trees surveyed had woodpecks and, on average, woodpeckers had preyed upon 34% of the *A. planipennis* in these trees.

At Windemere, green ash trees had significantly more canopy dieback than white ash trees in both 2004 (S=77.0, df=1,12, p<0.0003) and 2005 (S=77.0, df=1,12, p<0.0003) (Figure 3.3a). Green ash was trees were at or near 100% dieback both years,

while average dieback in white ash trees increased over 45% from 2004 to 2005. In 2005, two green ash trees were removed in mid-summer and all of the five remaining green ash trees were dead by September.

In 2004, green ash trees at Windemere had significantly more *A. planipennis* exit holes and woodpecker attacks per m^2 than white ash trees (S=66.0, df=1,12, p=0.0487) (Figure 3.3b). In 2005, however, white ash trees had significantly greater exit hole and woodpeck densities than the dead or dying green ash (S=17.0, df=1,8, p=0.0159). One white ash tree in 2005 with a recorded density of 229 exit holes and woodpecker attacks per m^2 was considered an outlier and removed from the data set. The data were reanalyzed and white ash trees still had significantly more exits holes and woodpecker attacks per m^2 than green ash trees (S=28.0, df=1,7, p=0.0317) (Figure 3.3b). Average exit hole density ranged from 44-76 exit holes/m^2 on green ash and 32-93 exit holes/m^2 on white ash from 2003 to 2005. Average woodpecker attack density ranged from 6-14 woodpecker attacks/m^2 on green ash and 13-46 woodpecker attacks/m^2 on white ash over the three-year period. By 2005, all trees had woodpecker attacks and, on average, woodpeckers had preyed upon 27 % of the *A. planipennis* in these trees.

Green ash trees at Lakeside had significantly more exit holes and woodpecks per m^2 than white ash trees, with an average of 22.8 (±6.86 SE) for green ash and 5.9 (±1.02 SE) for white ash (S=94.0, df=1,15, p=0.0180). On green ash trees, 56% of the average total density was comprised of exit holes and 44% was comprised of woodpecker attacks. On white ash trees, exit holes made up 29% of the average total density and woodpecker attacks made up 71% of *A. planipennis* density.

Overall, using the Butzel, Larned, and Windemere data, canopy dieback increased linearly as the density of *A. planipennis* exit holes and woodpecker attacks increased. A positive and significant linear relationship existed between these two variables for green ash, for white ash, and the two species combined (Figure 3.4). In general, if dieback was less than 30%, less than 30 exit holes and woodpecker attacks per m^2 were found. If dieback was less than 50%, the density of exit holes and woodpecker attacks was between 30 and 60 per m^2 (Figure 3.4). Dead trees were found with densities as low as 37 to 50 exit holes and woodpecker attacks per m^2. Overall, on trees with more than 90% dieback, the density of exit holes and woodpecker attacks ranged from 60 to 155 per m^2 (Figure 3.4).

White vs. blue ash woodlot trees

The white ash trees sampled in these studies were heavily infested and declining or dying. Blue ash trees of similar size and growing in the same site conditions had much lower exit hole and woodpecker attack densities than white ash. In 2004, white ash trees in the Plymouth Twp. woodlot had significantly more *A. planipennis* exit holes and woodpecker attacks per m^2 than blue ash trees, with an average of 80.1 (±20.65 SE) for white ash and 11.5 (±7.83 SE) for blue ash (S=16.0, df=1,8, p=0.0079). Exit holes made up 70% of the average total *A. planipennis* density in white ash trees and woodpecker attacks comprised the remaining 30%. On blue ash trees, exit holes accounted for 61% of the average total density and woodpecker attacks made up the other 39%. Exit holes and woodpecker attacks were found on branches and trunks of both species. Percentage of phloem surface area covered by galleries ranged from 0 to 100% in white ash, with an average of 39% (±10.9 SE) and from 0 to 40% in blue ash, with an average of 8% (±5.7

SE). Significantly more phloem surface area was covered by galleries in white ash than in blue ash (S=18.0, df=1,8, p=0.0278).

Similarly, in the Superior Twp. woodlot, white ash trees had significantly more exit holes and woodpecker attacks per m^2 than blue ash in 2004 (S=30.0, df=1,7, p=0.0079) and 2005 (S=10, df=1,6, p=0.0143) (Figure 3.5). There was a significant increase in the density of exit holes and woodpecker attacks from 2004 to 2005 for the white ash trees in the Superior Twp. woodlot (S=26.0, df=1,6, p=0.0143) (Figure 3.5), but not for the blue ash trees (S=22.0, df=1,7, p=0.36) (Figure 3.5). For the two trees of each species that were felled and debarked in 2004, the percentage of phloem surface area covered by galleries ranged from 25 to 100% in white ash, with an average of 67% (±5.9 SE) and from 0 to 40% in blue ash, with an average of 15% (±7.6 SE). In 2005, percentage of phloem covered by galleries ranged from 0 to 100% in white ash, with an average of 73% (±8.1 SE) and from 0 to 30% in blue ash, with an average of 7% (±5.0 SE). The percentage of phloem covered by galleries did not differ significantly between species in 2004 (S=7.0, df=1,2, p=0.17), but were significant in 2005 (S=10.0, df=1,6, p=0.0143). Throughout all surveys, a large portion of the galleries found on blue ash trees had callous wood growing around the edges of the galleries. Little to no callous wood was observed on white ash trees.

At all sites surveyed, more than 86% of the green, white and blue ash trees had at least one woodpecker attack. The percentage of *A. planipennis* larvae killed by woodpeckers on urban street trees ranged from 0 to 100%, with an average of 51% (±6.5 SE). This predation rate was similar for woodlot trees, where woodpecker attacks killed 0 to 100% of *A. planipennis* larvae, with an average mortality of 48% (±6.6 SE).

DISCUSSION

At all sites, in all years, green ash trees showed a greater percentage of canopy dieback related to *A. planipennis* infestation than white ash trees. As expected, dieback in both species increased from year to year as a new generation of *A. planipennis* larvae began to feed under the bark each year and populations continued to build. The larvae progressively consumed more phloem each year, further limiting translocation of water and nutrients throughout the tree. While clearly high densities of phloem-borers are associated with canopy dieback (Ball and Simmons 1980, Haack and Slansky 1986, Miller et al. 1991, others), canopy dieback is merely a symptom of infestation and not a direct measure of larval density. The data that I collected from these trees represents the cumulative density of *A. planipennis* larvae that successfully developed and emerged or were preyed upon by woodpeckers as pre-pupal larvae. It does not reflect total larval density which would include live and dead larvae remaining in the tree. In the field, I debarked several heavily infested trees that had few or no exit holes or woodpecker attacks at eye level, yet 100% of the phloem was consumed by larvae. Further examination showed that virtually all of the larvae had died by the second or third instar because of intra-specific competition for phloem.

Some tree species may be more "tolerant" of phloem-boring insects and sustain less canopy dieback than other species with similar infestation levels. Only a small portion of the sapwood actively conducts water: the outermost one or two growth rings in ring-porous hardwoods (e.g., ash, chestnut, and oak), and several of the outermost rings in diffuse-porous hardwoods (e.g., aspen, birch, and maple) and conifers (Kozlowski and

Pallardy 1997, Kozlowski and Winget 1963). These patterns of water conduction explain in part why ring-porous hardwoods are generally more vulnerable to wilt diseases (e.g., chestnut blight, Dutch elm disease, and oak wilt) (Kozlowski and Pallardy 1997) and girdling insects (e.g., *Agrilus bilineatus* on oaks) (Haack and Benjamin 1982), than diffuse-porous hardwoods or conifers. The nearly identical linear models I found for green and white ash imply a similar relationship between canopy dieback and exit hole and woodpecker attack density. This is intuitive as the more vascular tissue destroyed by larval galleries, the less foliage and root area that can be supported. The model for green ash indicated a negative y-intercept because it's plausible that some canopy dieback occurs in the absence of *A. planipennis* exit holes or woodpecker attacks. Smitley et al. (2006) also reported a significant relationship (R^2=0.59) between canopy dieback and *A. planipennis* exit hole density in ash trees they surveyed at 33 sites in southeast Michigan. However, they only surveyed the trunks of the trees and, therefore, had a lower density of exit holes relative to canopy dieback than reflected in my data. For example, in Smitley et al.'s model, trees with less than 30% dieback had almost no exit holes, while in my model, trees with 30% or less dieback had less than 30 exit holes and woodpecker attacks per m^2. They did not indicate whether they surveyed green ash, white ash, or both.

One of the key questions these studies addressed is whether or not *A. planipennis* exhibits a host preference hierarchy among the predominant ash species native to Michigan. Results suggest that, when growing together, *A. planipennis* preferred green ash street trees to white ash street trees in the core area of infestation in southeast Michigan. McCullough et al. (2005) reported similar differences in canopy dieback and larval densities for green and white ash control (untreated) trees monitored in a three-year

insecticide study. At a residential site in Washtenaw Co., they recorded over 60% dieback on green ash trees and less than 10% dieback on white ash trees.

Results over this three year period also illustrate the progression of the infestation, which increased in density each year in both species studied. *Agrilus planipennis* densities in white ash increased as available phloem in green ash declined. Throughout the study, the only exception to this trend was at Windemere, in 2005, where white ash trees actually had greater exit hole and woodpecker attack densities than green ash. Unlike the other sites that had fairly equal numbers of each species in the surrounding area, Windemere was unique in that its street trees were predominantly white ash with an occasional green ash tree mixed in with the white ash trees. Singer (1983) points out that insects may not use their most highly preferred host if they encounter another acceptable host first.

Differences between species were not significant in any year at Butzel for canopy dieback or exit hole and woodpecker attack density. This was probably due to the smaller tree size at this site. The majority of the trees at this site were less than 5 cm DBH and *A. planipennis* may not show a preference between species for trees in small size classes because of the limited phloem available in small trees. In addition, there was a small decrease (four exit holes and woodpecker attacks per m^2) in exit hole and woodpecker attack densities in the white ash from 2003 to 2004 at Larned and a rather large decrease (40 exit holes and woodpecker attacks per m^2) in the green ash from 2004 to 2005 at Windemere. The removal of dead trees likely accounted for the apparent decrease in the overall mean *A. planipennis* densities at Windemere.

Agrilus planipennis also clearly preferred white ash trees over blue ash trees in the two woodlots I surveyed. A large portion of the white ash trees in both woodlots had already been killed by *A. planipennis* in 2002 and 2003. Densities calculated from the dead trees I sampled at all residential and woodlot sites, therefore, suggest a maximum number of *A. planipennis* per m^2 that a single tree can successfully host was approx. 107 (±10.8 SE) for green ash and 109 (±10.6 SE) for white ash.

It is interesting that a large portion of the galleries on blue ash trees had callous wood around the edges or covering a substantial proportion of the galleries. It was also evident that most larvae would have taken two years to develop on these trees. Cappaert et al. (2005a) reported that *A. planipennis* could have a two-year life cycle in lightly to moderately infested trees. Future studies are needed to address potential differences among ash species in their phloem chemistry and their initial resistance to *A. planipennis* larval feeding.

Previous studies have shown that woodpeckers can play an important role regulating populations of wood-boring beetle larvae (Akers and Nielsen 1990b, Anderson 1944, Barter 1957, Fayt et al. 2005, Nash et al. 1951). An overall average of 33% of the *A. planipennis* were preyed upon by woodpeckers in trees I surveyed. Anderson (1944) reported that 10% of *A. anxius* borer larvae were preyed upon by woodpeckers overwinter, while Akers and Nielsen (1990b) found that almost 30% of the total holes on European white birch resulted from woodpecker predation of *A. anxius* larvae. Two other studies by Barter (1957) and Nash et al. (1951) found predation rates on *A. anxius* larvae ranged from very little to up to 50%. Studies on other natural enemies of *A. planipennis* in Michigan reported less than 2% of larvae were infected with pathogenic

fungi, and less than 1% of larvae and 0.5% of eggs were parasitized by parasitoids (Bauer et al. 2006). In its native range in China, *A. planipennis* larval parasitism rates by hymenopteran parasitoids were less than 7% (Liu et al. 2003). Woodpeckers are currently the best natural enemy of *A. planipennis* in North America (Cappaert et al. 2005b, Fuester and Schaefer 2006, Marshall et al. 2006, Strazana 2006). More research needs to be conducted on woodpecker abundance, behavior, and habitat use and their effects on *A. planipennis*.

These studies were observational and used exit hole and woodpecker attack densities to measure *A. planipennis* host preference. They serve as a stepping stone to initiate additional studies to identify mechanisms that underlie this apparent preference. Attack of woody plants by phloem-feeding Coleoptera, especially Buprestidae and Scolytidae, is closely associated with the condition of the host tree (Anderson 1944, Larsson et al. 1983, Dunn et al. 1986a, b, 1987, 1990d, Waring and Pitman 1980). Trees weakened by drought (Mattson and Haack 1987), disease (Dunn et al. 1990c, Potter and Hartman 1993), lightening strikes (Hodges and Pickard 1971), defoliation (Houston 1981, Wright et al. 1984), phloem and xylem girdling (Dunn et al. 1986a, Haack and Benjamin 1982) or stand competition (Hard 1985) are most likely to be attacked and killed by phloem-feeding borers. While *A. planipennis* is capable of attacking healthy trees, it appears to prefer stressed trees (McCullough et al. 2005, 2006). The first year I surveyed Larned, there was one white ash tree that had 75% canopy dieback while the other white ash trees had little to none. While I could not find physical evidence of stress in the form of mechanical damage to the trunk, obvious soil compaction, etc., it was apparent that this tree was more attractive to *A. planipennis* than the other white ash.

While stress is important in host selection of many phloem and wood-boring insects, it is unlikely that stress alone is driving this differential attack on ash species because it appears to be so widespread. Other factors, such as differences in foliar chemistry among the ash species, could be influencing *A. planipennis* host preference. Other trees species, such as birch (*Betula* sp.) (Laitinen et al. 2005, Taipale et al. 1994) pine (*Pinus* sp.) (Hanover 1966), and willow (*Salix* sp.) (Orians et al. 1996) exhibit inter- and intraspecific variation in the types and concentrations of secondary compounds found in leaves and bark. Female *A. planipennis* feed on foliage for at least 14 days before they oviposit and the species they feed upon may influence the species they select for oviposition.

Bark texture could be another factor driving host preference. Larval densities on rough-barked ash trees were significantly higher than on smooth-barked ash trees of the same species (see Chapter Two). Other studies on *Agrilus* species have also reported a preference for rough-barked portions of trees for oviposition (Barter 1957, Loerch and Cameron 1984). Many varieties of green ash tend to have rough bark before and after they reach maturity, while most varieties of white ash have smooth bark before they reach maturity (pers. obs.). Blue ash trees tend to have rough bark (pers. obs.), however, so bark roughness should not be considered a sole factor influencing host selection.

A combination of many factors may affect *A. planipennis* host preference and should not be considered mutually exclusive. Controlled studies need to be designed to increase our understanding of host selection behavior. More work is needed to investigate differences in ash species in relation to factors such as foliar chemistry and nutrition, phenolic concentrations and other chemicals in the bark and phloem, bark

texture, and stress-inducing variables such as drought, girdling, disease, and transplanting. Future studies should also be designed to evaluate the relative preference of other ash species, including black ash.

These surveys of *A. planipennis* host preference provide baseline data that can be used to more thoroughly assess the susceptibility of both urban and forest trees based on species composition and to increase efficiency in developing survey, detection, and management options. For example, in areas where mixes of ash species occur, efforts should focus on surveying the preferred species (i.e., green ash) when trying to detect newly established or low density populations of *A. planipennis*. These data also support regulations in other states that promote using green or white ash (versus the less preferred blue ash) for trap trees to detect new infestations.

Table 3.1. Number of trees (n) and mean (± SE) diameter at breast height (DBH) for green, white, and blue ash trees at survey sites in *Agrilus planipennis*- infested areas.

	Green Ash[1]		White Ash[1]		
Site	**n**	**DBH (cm)**	**n**	**DBH (cm)**	**Survey year(s)**
Butzel	8	8.1 ± 1.2	8	4.2 ± 0.2	2003, 2004, 2005
Larned	11	18.0 ± 0.5	11	23.3 ± 0.7	2003, 2004, 2005
Windemere	7	27.7 ± 2.5	7	23.3 ± 1.3	2004, 2005
Lakeside	8	32.0 ± 5.3	9	33.0 ± 4.7	2005
	Blue Ash[2]		**White Ash[2]**		
Superior Twp.	5	20.1 ± 3.9	4	20.6 ± 2.4	2004
Plymouth Twp.	5	22.2 ± 3.7	5	24.2 ± 4.6	2004
Superior Twp.	4	21.2 ± 2.4	4	23.4 ± 4.0	2005

[1]The same trees were re-surveyed each year.

[2]Trees were felled for sampling and new trees were selected each year.

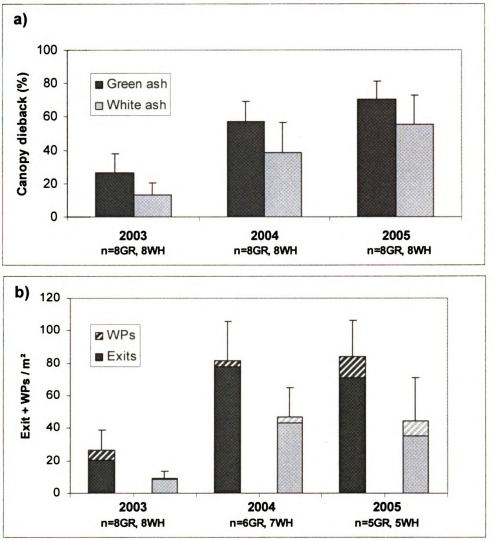

Figure 3.1 a) Mean (± SE) percent canopy dieback and **b)** mean (± SE) number of *Agrilus planipennis* exits holes (exits) and woodpecker attacks (WPs) per m^2 for the small green (GR) and white (WH) ash trees at Butzel in 2003, 2004, and 2005. Three dead green ash and three dead white ash trees with 100% dieback were removed in 2004 and 2005. Means within years were not significantly different between species (Wilcoxon Rank Sum test; $p<0.05$).

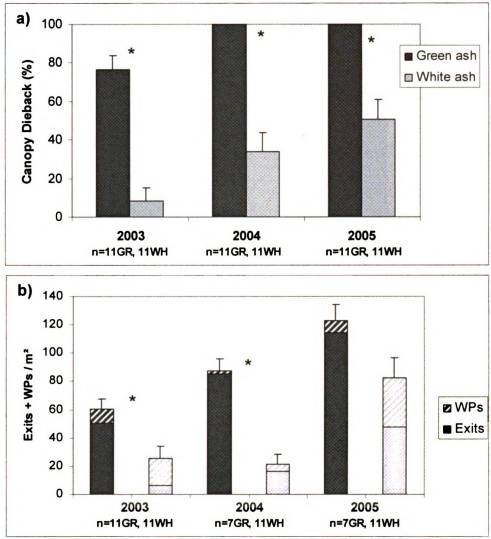

Figure 3.2 a) Mean (± SE) percent canopy dieback and **b)** mean (± SE) number of *Agrilus planipennis* exits holes (exits) and woodpecker attacks (WPs) per m^2 for green (GR) and white (WH) ash trees at Larned in 2003, 2004, and 2005. Four dead green ash trees with 100% dieback were removed in 2004. * Indicates significant differences between green and white ash trees (Wilcoxon Rank Sum test; $p<0.05$).

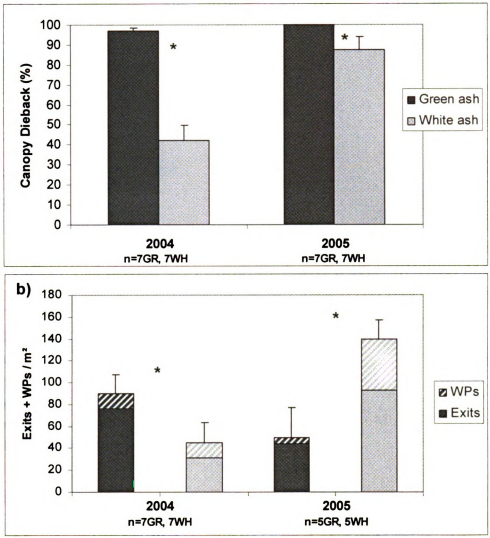

Figure 3.3 a) Mean (± SE) percent canopy dieback and **b)** mean (± SE) number of *Agrilus planipennis* exits holes (exits) and woodpecker attacks (WPs) per m^2 for green (GR) and white (WH) ash trees at Windemere in 2004 and 2005. Two dead green ash trees with 100% dieback were removed in 2005. One outlier for exits and woodpecks per m^2 was removed from the 2005 white ash tree data set. * Indicates significant differences between green and white ash trees (Wilcoxon Rank Sum test; $p<0.05$).

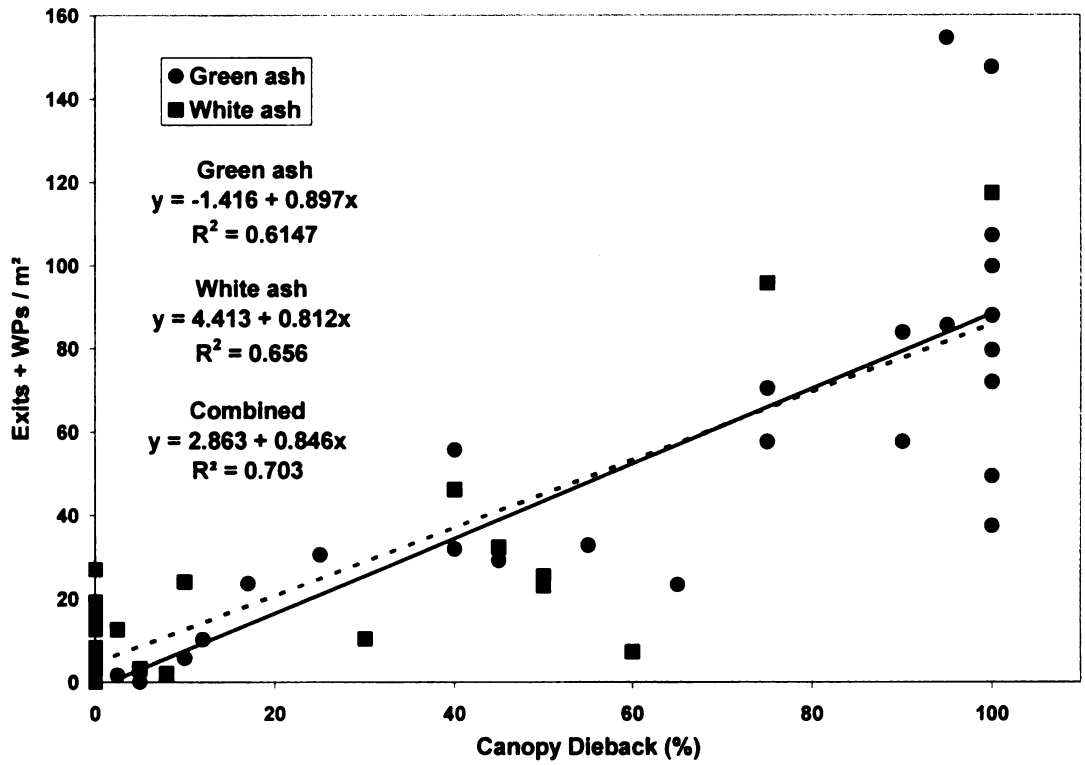

Figure 3.4. Percent canopy dieback and number of *Agrilus planipennis* exit holes (exits) and woodpecker attacks (WPs) per m^2 for 25 green ash and 24 white ash trees (49 trees total) at three sites in the *Agrilus planipennis*-infested areas.

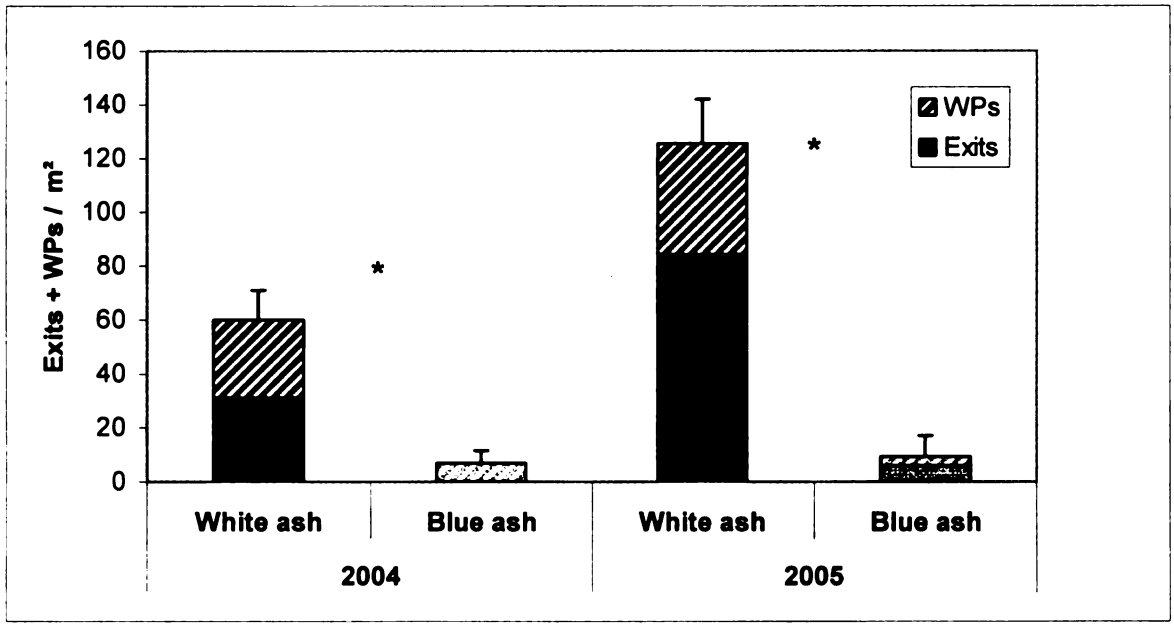

Figure 3.5. Mean (± SE) number of *Agrilus planipennis* exit holes (exits) and woodpecker attacks (WPs) per m^2 for white and blue ash trees at Superior Twp in 2004 and 2005. * Indicates significant differences between white and blue ash trees (Wilcoxon Rank Sum test; $p<0.05$).

APPENDICES

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2006-05

Title of thesis or dissertation (or other research projects):

HOST RANGE AND PREFERENCE OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE), IN NORTH AMERICA

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Andrea Christine Anulewicz

Date 13 July 2006

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Agrilus planipennis Fairmaire	MICHIGAN: Washtenaw Co. Matthaei Botanical Gardens City of Ann Arbor Emerged from green and white ash stem and logs. 21-Jun-04 10-Feb-06 det. A.C. Anulewicz					7	8		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Andrea Christine Anulewicz

Date 13-Jul-06

Voucher № 2006-05
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

APPENDIX 2

Leaf-Feeding Preference of *Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae) on Four North American Ash Species

This appendix contains the methods and results of a study designed to evaluate *Agrilus planipennis* adult leaf-feeding preference among four North American species of ash using two-choice bioassays. I felt that this study didn't quite fit in with the contents of the other three chapters of this thesis and lacked substantial, definitive, or conclusive results, but still warranted reporting in some format.

2004 bioassays: Foliage from four different species of ash was collected on each of three set up days of the experiment: 22 July, 29 July, and 5 August 2004. White and black ash leaves were collected from trees at Elm Park in Livonia, Wayne Co., MI. Blue ash leaves were collected from a private woodlot in Superior Township, Washtenaw Co., MI and green ash leaves from Matthaei Botanical Gardens in Ann Arbor, Washtenaw Co., MI. All trees were untreated, in the core area of *A. planipennis* infestation, and showed varying degrees of foliar feeding.

Leaves randomly selected for the study were photocopied before the start of each bioassay to capture, in an image, the amount of leaf area present. Two leaves of a single species were paired and stems submerged in a vial of water to prevent desiccation. Each vial contained three cotton wicks to stabilize the leaves and provide drinking water for the beetles. Two vials, each with a different leaf species, were placed in opposite ends of clear, plastic boxes (31 x 23 x 10 cm). Boxes were kept on a lab bench at 24-27°C.

Adult beetles were reared from bolts of infested ash trees collected in the core area of *A. planipennis* infestation. Rearing logs were kept in cold storage at 1-2°C with a minimum of 80% humidity until needed. Six newly emerged, unfed beetles were released from the center of each box and allowed to feed undisturbed for five days. Six pair-wise combinations of species were used: 1) green and white ash, 2) green and black ash, 3) green and blue ash, 4) white and black ash, 5) white and blue ash, and 6) black and blue ash. Bioassays were initiated on 22 July, 29 July, and 5 August 2004 as beetles emerged from their logs. Each bioassay contained three to four replicates (12 replicates total). Fresh foliage was collected on each day that the bioassays were initiated.

At the end of each five-day bioassay, leaves were removed from their boxes and photocopied a second time. Before and after feeding images of leaves were scanned and analyzed using WinFolia 2004a Leaf Analysis Software by Regent Instruments, Inc. The amount of leaf area consumed was estimated by calculating the difference in leaf area before and after the bioassay. Because there were two leaves of each species in the box, total leaf area consumed for each species was calculated by combining the leaf area consumed on each of the two leaves in the box, generating one value for each pair of leaves in the box. The proportion of total leaflets fed upon was also recorded. Three classes of feeding behavior were designated: 'marginal', 'to the vein', and 'through the vein'. 'Marginal' feeding described any feeding that took place along the margin of the leaflet, less than halfway to the main vein of the leaflet. 'To the vein' feeding described any feeding that occurred more than halfway to the main vein of the leaflet and 'through the vein' feeding was any feeding that included the main vein of the leaflet. The proportion of leaflets within each feeding class was calculated by combining the total

number of leaflets on the pair of leaves in each box that exhibited the feeding class of interest and dividing it by the total number of leaflets in the pair of leaves.

Data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Data were not normal and could not be normalized with transformations. Friedman's nonparametric two-way analysis of variance (ANOVA) was used, therefore, to determine significant interactions between species and the pair-wise combination factors (SAS Institute, Inc. 1989). The nonparametric Kruskal-Wallis test was then used to determine if total leaf area consumed and proportion of leaflets fed upon within each feeding category differed among species (Kruskal and Wallis 1952). When significant, nonparametric multiple comparisons ($p<0.05$) were used to identify differences among species (Zar 1984, Conover 1971).

Differences in leaf area consumed among species were analyzed without the pair-wise combination factor because there was not a significant interaction between the two effects (F=0.42, df=3, p=0.74). Average leaf area consumed was significantly greater on green ash, white ash, and black ash than on blue ash (χ^2= 25.35, df=3,140, p<0.0001) (Figure A2.1).

The average proportion of leaflets that had one or more types of feeding was 0.71 (±0.03 SE) for green ash, 0.69 (±0.03 SE) for black ash, 0.65 (±0.03 SE) for blue ash, and 0.63 (±0.03 SE) for white ash. Overall, the proportion of leaflets feeding in general did not differ significant among species (χ^2= 2.40, df=3,140, p=0.49). Within the 'marginal' feeding class, blue ash had a significantly greater proportion of leaflets with 'marginal' feeding than green or white ash (χ^2= 19.69, df=3,140, p=0.0002) (Figure A2.2). Green and black ash had significantly greater proportions of leaflets with feeding more than half

way to the main vein than blue ash (χ^2= 50.93, df=3,140, p<0.0001) (Figure A2.2). Blue ash also had a significantly lower proportion of leaflets with feeding through the main vein than green, white, and black ash (χ^2= 27.29, df=3,140, p<0.0001) (Figure A2.2). 'Marginal' feeding was the most common type of feeding and 'through vein' was the least common.

2005 bioassays: This study was repeated again in 2005 using the same methods as in 2004. Leaves from the lower canopy exposed to direct sunlight of four different species of ash were collected on each of five set up days of the experiment: 28 June, 30 July, and 1 August 2005. Green and white ash leaves were collected from trees at Elm Park. Blue ash leaves were collected from a tree in Plymouth Township, Wayne Co., MI, and black ash leaves from Hudson Mills Metropark, Washtenaw Co., MI. All trees were untreated, in the core area of *A. planipennis* infestation, and showed varying degrees of foliar feeding.

Leaves were photocopied before the start of each bioassay. This time, only one leaf of each species was selected and stems were submerged in a vial of water to prevent desiccation. Adult beetles were reared from infested ash logs using the same methods as in 2004. Six newly emerged, unfed beetles were released from the center of each box and allowed to feed undisturbed for five days. The same six possible pair-wise combinations of species were used. Bioassays were initiated on 28 June, 18 July, and 1 August 2005 as beetles emerged from their logs. Each bioassay contained four to seven replicates (15 replicates total). Fresh foliage was collected on each day that bioassays were initiated.

At the end of each five-day bioassay, leaves were removed from their boxes and photocopied again. Before and after feeding images of leaves were scanned and analyzed

using WinFolia Leaf Analysis Software. The total amount of leaf area consumed was estimated by calculating the difference in leaf area before and after the bioassay. The proportion of leaflets within each of the three feeding classes described in 2004 was calculated. Data were analyzed using the same methods as in 2004.

Similar to the 2004 analysis, there was not a significant interaction between species and pair-wise combination, therefore, differences in leaf area consumed among species were analyzed independently of pair-wise combination (F=0.42, df=3, p=0.74). Significantly more leaf area was consumed on white ash than on green or blue ash (χ^2= 38.75, df=3,146, p<0.0001) (Figure A2.3).

The average proportion of leaflets that had one or more types of feeding was 1.0 (±0 SE) for black and blue ash, 0.96 (±0.02 SE) for white ash, and 0.93 (±0.02 SE) for green ash. Overall, black and blue ash had significantly greater proportions of leaflets with feeding in general than green ash (χ^2= 30.44, df=3,176, p<0.0001). However, it should be noted that for the 2005 bioassay, the majority of the black and blue ash leaves were made up of only two to three leaflets, while green and white ash leaves had five to seven. Similar to results in 2004, 'marginal' feeding was the most common type of feeding pattern and 'through vein' feeding was the least common. Within the 'marginal' feeding class, black and blue ash had significantly greater proportions of leaflets with 'marginal' feeding than green ash (χ^2= 30.44, df=3,176, p<0.0001) (Figure A2.4). White and black ash had significantly greater proportions of leaflets with feeding more than half way to the main vein than blue ash (χ^2= 14.41, df=3,176, p=0.0024) (Figure A2.4). Blue ash also had a significantly lower proportion of leaflets with feeding through the main vein than green and white ash (χ^2= 26.62, df=3,176, p<0.0001) (Figure A2.4).

Data from both years suggest blue ash is least preferred for leaf feeding and feeding is mostly marginal on blue ash.

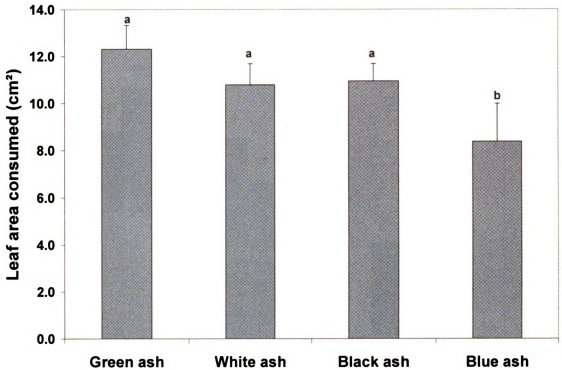

Figure A2.1. Mean (± SE) leaf area consumed for four ash species by *Agrilus planipennis* beetles in five days during a two-choice bioassay in 2004. n=36 replicates of six beetles feeding on one leaf for each species. Means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

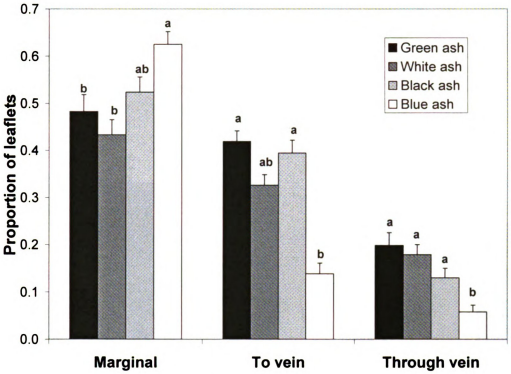

Figure A2.2. Mean (± SE) proportion of leaflets of four species of ash fed on by *Agrilus planipennis* beetles in each of four feeding categories in five days during a two-choice bioassay in 2004. n=36 replicates of six beetles feeding on one leaf for each species. Within feeding types, means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

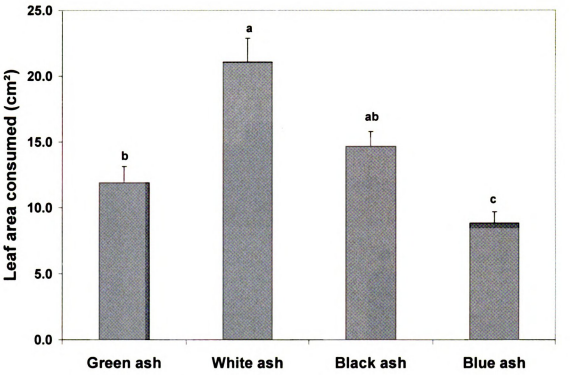

Figure A2.3. Mean (± SE) leaf area consumed for four ash species by *Agrilus planipennis* beetles in five days during a two-choice bioassay in 2005. n=29 replicates of six beetles feeding on one leaf for green ash, n=41 for white ash, n=40 for black ash, n=40 for blue ash. Means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

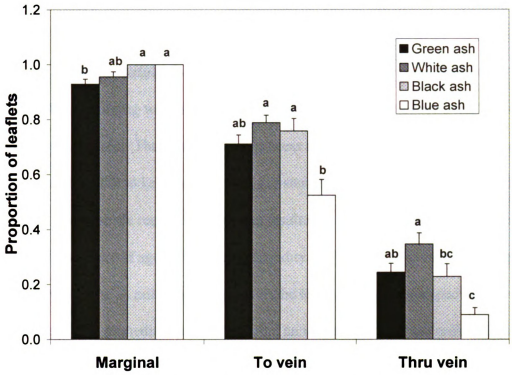

Figure A2.4. Mean (± SE) proportion of leaflets of four species of ash fed on by *Agrilus planipennis* beetles in each of four feeding categories in five days during a two-choice bioassay in 2005. n=45 replicates of six beetles feeding on one leaf for each species. Within feeding types, means with the same letter are not significantly different (Kruskal-Wallis test and nonparametric multiple comparison procedure; $p<0.05$).

APPENDIX 3

Unsuccessful No-Choice and Two-Choice Laboratory Bioassays

When working with new, exotic insects, many difficulties arise that can impede productive research. The insect rearing process can be full of challenges from selecting the appropriate diet to keeping the rearing system free of pathogens. In 2005, there was a problem in our beetle rearing process that resulted in high mortality when beetles reached approx. two weeks of age and reduced fecundity in beetles that lived long enough to be used in bioassays. A nutrient deficiency in the feeding foliage was speculated early in the process and was immediately addressed, but an unidentified and unconfirmed pathogen may have contaminated the system, invalidating any of the 2005 oviposition bioassays. While the results of these studies are unreliable and inconclusive, I feel that the methods are still valid for use in future studies, therefore they are presented in this appendix.

Two-choice cut branch bioassays

2004 bioassays: Green and white ash bolts were harvested from Michigan State Universities W.K. Kellogg Forest, Kalamazoo Co., MI. Black ash bolts were collected from Kensington Metropark, Oakland Co., MI and blue ash bolts from the same private woodlot in Superior Township were blue ash foliage was collected for leaf-feeding bioassays. All bolts were harvested and waxed to slow desiccation on 24 May 2004 and kept in cold storage at 1-2°C with a minimum of 80% humidity.

At the start of each bioassay, branches of each species were cut to approx. 20 cm in length (Table A3.1). All cut surfaces were waxed with paraffin. Two branches, each a different species of ash, and one green ash leaf collected from an untreated, infested

green ash were placed in opposing corners of a screened cage (30 x 30 x 30 cm). Ash leaf stems were submerged in vials of water to slow desiccation. Each vial contained two cotton wicks to provide drinking water for the beetles. Cages were kept outside in a screened-in greenhouse, leaving them exposed to ambient temperatures and wind conditions, but protected from rain.

Adult beetles were reared from bolts of infested ash trees collected in the core area of *A. planipennis* infestation using the same methods described in the leaf-feeding bioassays. Once emerged from rearing logs, similarly aged groups of beetles (approx. 200) were placed in screen cages (60 x 60 x 60 cm) and feed green ash foliage collected from untreated, infested trees. Cages were kept in growth chambers at 24°C, 60% humidity, and 16:8 light:dark photoperiod. Beetles were allowed to feed and mate for two weeks before bioassays began.

The same six pair-wise combinations of ash species used in the leaf-feeding bioassay were used again here. Two bioassays were set up on 1 and 28 June 2004. On each set up day, beetles were sexed and one male/female pair was placed in each box (60 pairs total). Each bioassay had five replicates (10 replicates total). Boxes were checked twice a week and foliage was replaced weekly. Twenty-six of the 60 pairs were observed mating. Adults lived from two to 49 days in the cages. If a female beetle died during the first three days of the bioassay, she was replaced with a similarly aged beetle. The first bioassay was conducted from 1 June to 27 June and the second from 28 June to 16 August. By the third day of the second bioassay, twenty-seven of the 60 beetles in the bioassay had died. It was suspected that the unusually high mortality may have been caused by bleach residue left on the cages during cleaning after the first bioassay. On 1

July, beetles were removed from their cages and placed into Petri dishes. The cages were washed with soapy water, rinsed thoroughly with water, dried and set up again. Dead beetles were replaced with similarly aged beetles. Beetle mortality returned to expected levels.

Upon death of the female beetle, the branches were removed and stored on a lab bench at 24°C. The entire surface of each branch was inspected for eggs, larvae, and galleries 30-45 days after it was removed from its cage. Diameter of each branch was measure and total bark surface area was calculated (Table A3.1). The bark surface of each branch was first inspected with a magnifying lens to locate eggs on the surface, then eggs were removed with forceps to prevent them from being recounted. Forceps or a small knife were then used to chip off bark flakes to revel eggs hidden in bark layers. After 15 min of searching, total number of eggs was recorded. Bark was carefully peeled down to the wood using a small drawknife or chisel. Number and stage of the larvae on each branch was recorded.

A total of 258 eggs were found on 52 of the 120 branches with an average of 2.2 (± 0.4 SE) eggs per branch. This low number of eggs may indicate that branches in this type of setting are not as attractive to females for oviposition. A total of 70 small galleries excavated by first instar larvae were found on 15 of the 120 branches, averaging 0.6 (± 0.3 SE) galleries per branch. This low number of galleries may suggest that cut branches will desiccate quickly and lose the necessary moisture content to support early instar development.

2005 bioassays: Green and white ash branches were collected on 9 May and 13 June 2005 from the same locations as in the 2004 bioassay. Black ash branches were

harvested from the Nan Weston Nature Preserve at Sharon Hollow, Washtenaw Co., MI on 12 May and 13 June 2005. Blue ash branches were also collected from the Nan Weston Nature Preserve on 12 May and the Strait Creek Nature Preserve in Adams Co., OH on 10 June 2005. Cut ends were waxed and branches remained in cold storage at 1-2°C. At the start of each bioassay, branches of each species were cut to approx. 20 cm in length (Table A3.1).

Two branches and one wooden dowel (1.9 cm diam. x 18 cm height) were placed in random order in the left, middle or right positions in a clear, plastic box (31 x 23 x 10 cm). In order to slow branch desiccation, boxes were kept in growth chambers at 24°C, 60% humidity, and 16:8 light:dark photoperiod. Each branch and dowel was wrapped with thin, white, plastic Easy Gardener® Tree Wrap 513. The wrap was cut into 2.5 cm wide strips and wrapped around the branch or dowel leaving 3-4 cm of exposed branch in between the strips of tree wrap. This was done to provide additional crevices for egg laying.

Adult beetles were reared from bolts of infested ash using the same methods as in 2004. Once emerged from rearing logs, similarly aged groups of beetles (approx. 20) were placed in clear, plastic cylinders (10 x 20 cm) and fed foliage collected from infested potted green ash saplings. Beetles were allowed to feed and mate for two weeks before bioassays began. Approx. 35 holes were drilled into the lids and sides of the cylinders to allow for ventilation.

The same six pair-wise combinations of ash species used in the 2004 bioassays were initiated on 16 May and 20 June 2005. On each day that bioassays were inititated, beetles were sexed and one male/female pair was placed in each box (32 pairs total).

Each bioassay had three replicates (6 replicates total). Boxes were checked twice a week and foliage was replaced weekly. Eleven of the 32 pairs were observed mating. Adults lived from three to 28 days in the boxes. If a female beetle died during the first three days of the bioassay, she was replaced with a similarly aged beetle. The first bioassay was conducted from 16 May to 13 June and the second from 20 June to 18 July. Upon death of the female beetle, the branches were removed and stored on a lab bench at 24°C. The entire surface of each branch was inspected for eggs, larvae, and galleries approx. 28 days after it was removed from its cage. Diameter of each branch was measure and total bark surface area was calculated (Table A3.1). Inspection and peeling methods were as described in 2004.

A total of 112 eggs were found on 41 of the 72 branches with an average of 1.6 (± 0.2 SE) eggs per branch. A total of 61 small galleries excavated by first instar larvae were found on 13 of the 72 branches, averaging 1.3 (± 0.4 SE) galleries per branch.

No-choice cut branch bioassays: In 2005, branches of green ash, white ash, black ash, blue ash, privet, and tree lilac, along with two Asian elm species, *Ulmus davidiana* Planch. and *U. japonica* (Sarg. ex Rehder.) Sarg.. These species were previously reported as host for *A. planipennis* in Asia (Akiyama and Ohmomo 1997, Sugiura 1999). Green ash, white ash, and Japanese tree lilac branches were collected from the same locations as in the 2004 bioassays. Green and white ash branches were cut on 9 May and 13 June 2005. Japanese tree lilac branches were cut on 17 May and17 June 2005. Black ash branches were harvested from the Nan Weston Nature Preserve at Sharon Hollow, Washtenaw Co., MI on 12 May and 13 June 2005. Blue ash branches were also collected from the Nan Weston Nature Preserve on 12 May and the Strait

Creek Nature Preserve in Adams Co., OH on 10 June 2005. Both Asian elm species were harvested from the Morton Arboretum Ware Research Field, Dupage Co., IL on 16 May and 27 June 2005 and sent by overnight mail to Michigan. Privet branches were collected from Ann Arbor, Washtenaw Co, MI on 18 May and 21 June 2005. Cut ends of branches were waxed and remained in cold storage at 1-2° C until the start of each bioassay. Eight branches of each species were cut to approx. 17 cm in length (Table B.2). Cut ends were waxed with paraffin.

Boxes were set up and beetles were reared using the same methods as in 2004. Once beetles were emerged from rearing logs, similarly aged groups of beetles (approx. 20) were placed in clear, plastic cylinders (10 x 20 cm) and fed foliage collected from uninfested potted green ash saplings. Beetles were allowed to feed and mate for two weeks before bioassays began. Approximately 35 holes were drilled into the lids and sides of the cylinders to allow for ventilation.

One each day bioassays were initiated, beetles were sexed and one male/female pair was placed in each box (64 pairs total). The two bioassays each contained four replicates of each species and the first ran from 18 May to 10 June and the second from 29 June to 20 July 2005. Boxes were kept in growth chambers at 24°C, 60% humidity, and 16:8 light:dark photoperiod. If a female beetle died during the first three days of the bioassay, she was replaced with a similarly aged beetle. Adults lived from three to 23 days in the boxes. Upon death of the female beetle, the branch was removed from the box and stored in growth chambers under the above conditions.

The entire surface of each branch was inspected for eggs and bark was peeled to assess larval feeding 28-32 days after it was removed from its box. Diameter of each

branch was measured and total bark surface area was calculated (Table A3.2). Bark was carefully peeled down to the wood using a small knife or chisel and diameter of each branch was measured. Number and stage of larvae on each branch section and a visual estimate of the percentage of the cambium/phloem area covered by larval galleries was recorded. Galleries were also standardized 100 cm^2.

I only found 101 eggs on 15 of the 84 branches used in this bioassay, averaging 1.2 (±0.6 SE) eggs per branch. A total of 57 galleries were excavated on eight branches, averaging 0.8 (±0.4 SE) galleries per branch.

No-choice caged stem bioassay: The study was a repeat of the caged stem bioassay reported in Chapter 1 using the same four tree species, green ash, white ash, black walnut, and Japanese tree lilac, purchased from Poplar Farms Nursery in Waterman, IL. Ten blocks of trees were set up in a randomized complete block design (40 trees total). Trees were planted in an open field slightly above ground, 3 m apart, in fives rows of eight trees. The partially exposed root balls were covered with wood mulch and drip line irrigation was installed for frequent watering. Root balls from the 2004 study were removed and new trees were put in the existing holes on 13 May 2005. Trees had an average caliper of 7.1 (±0.2 SE) cm.

Screen cages were constructed using the same methods as in 2004. However, prior to constructing the cages, the stem of each tree was wrapped with thin, white, plastic Easy Gardener® Tree Wrap 513. The wrap was first cut into 2.5 cm wide strips and then wrapped around the stem leaving 4-5 cm of exposed bark in between the strips of tree wrap. This was done to provide additional crevices for egg laying. Instead of using foliage stems for feeding, small potted green ash seedlings were placed in each

cage and were watered twice a week. For detailed methods of cage construction, refer to Chapter 1.

Because of the reduced fecundity and longevity of the beetles used in this bioassay, I felt that no useful data would come from peeling these stems. Therefore, they were left in place for use in future studies.

Table A3.1. Mean (±SE) diameter, length, and surface area for each species used in the 2004 and 2005 two-choice oviposition bioassay. n=15 for each species.

Species	Diameter (cm)	Length (cm)	Surface area (cm^2)
2004 Bioassay n=30 branches per species			
Green ash	7.8 (0.2)	20.3 (0.1)	439.2 (11.6)
White ash	8.1 (0.2)	20.0 (0.1)	468.4 (8.3)
Black ash	7.4 (0.3)	19.9 (0.1)	424.5 (18.8)
Blue ash	8.9 (0.2)	20.1 (0.1)	506.1 (12.8)
2005 Bioassay n=27 branches per species			
Green ash	6.5 (0.2)	17.1 (0.1)	305.4 (7.2)
White ash	7.2 (0.1)	17.0 (0.1)	338.7 (5.1)
Black ash	6.9 (0.1)	16.8 (0.1)	319.2 (9.4)
Blue ash	6.2 (0.1)	16.9 (0.1)	287.5 (4.9)

Table A3.2. Mean (±SE) diameter, length, and surface area for each species used in the 2005 no-choice cut branch bioassays. n=8 for each species.

Species	Diameter (cm)	Length (cm)	Surface area (cm^2)
Green ash	6.9 (0.4)	16.4 (0.4)	310.0 (18.6)
White ash	7.1 (0.4)	16.5 (0.2)	323.8 (18.7)
Black ash	6.5 (0.2)	16.6 (0.2)	301.4 (9.0)
Blue ash	7.8 (0.1)	16.8 (0.2)	364.3 (3.7)
Privet	4.8 (0.3)	16.9 (0.2)	230.5 (14.7)
Tree lilac	5.7 (0.3)	16.9 (0.2)	268.9 (10.9)
Ulmus davidiana	5.3 (0.3)	17.3 (0.2)	244.1 (11.5)
U. japonica	5.3 (0.4)	17.4 (0.3)	248.9 (19.9)

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