MSU
2
2010

This is to certify that the
thesis entitled

TREE VIGOR AND ITS RELATION TO EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE) ADULT HOST PREFERENCE AND LARVAL DEVELOPMENT ON GREEN AND WHITE ASH TREES

presented by

Chenin Kathleen Limback

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Entomology

Major Professor's Signature

26 August 2010
Date

MSU is an Affirmative Action/Equal Opportunity Employer

LIBRARY
Michigan State
University

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.
MAY BE RECALLED with earlier due date if requested.

DATE DUE	DATE DUE	DATE DUE

5/08 K:/Proj/Acc&Pres/CIRC/DateDue.indd

TREE VIGOR AND ITS RELATION TO EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE) ADULT HOST PREFERENCE AND LARVAL DEVELOPMENT ON GREEN AND WHITE ASH TREES

By

Chenin Kathleen Limback

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Entomology

2010

ABSTRACT

TREE VIGOR AND ITS RELATION TO EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE) ADULT HOST PREFERENCE AND LARVAL DEVELOPMENT ON GREEN AND WHITE ASH TREES

By

Chenin Kathleen Limback

Host preference of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) was compared between green and white ash trees subjected to four treatments (girdling, fertilization, fertilization + Agri-Fos fungicide, untreated controls) in 2008 and 2009. Girdling reduced foliar chlorophyll content, nitrogen concentration, and photosynthesis rates and increased foliar carbohydrates. Effects of fertilization were minimal. Significantly more *A. planipennis* adults were captured on sticky bands on girdled trees than on other trees. The highest numbers of larvae per m^2 were found under the bark of girdled trees and green ash trees. Larval mortality caused by intraspecific competition was higher in girdled trees than other trees. Most larvae under the bark of girdled trees would have developed in one year. Approximately 30% of larvae on non-girdled green ash trees would have developed in one year in 2009. In contrast, nearly all larvae on white ash trees which were not girdled would have required two years for development. Non-girdled white ash trees were generally not attractive to *A. planipennis* adults and had the lowest larval densities. When girdled, however, white ash trees were more attractive than green ash trees which were not girdled.

Adult *A. planipennis* foliar feeding preferences were evaluated in 2009 on green ash seedlings which were girdled, fertilized, or left as untreated controls. Foliage from girdled seedlings had lower nitrogen concentrations and lower photosynthesis rates than foliage from trees of other treatments. Adult *A. planipennis* consumed more leaf area on girdled seedlings than on fertilized or untreated seedlings in no-choice bioassays. In an outdoor choice assay, adults appeared to preferentially feed on fertilized over untreated seedlings.

DEDICATION

This thesis is dedicated to the memory of my two great-grandmothers, Lillian Bishop and Jessie James. These two women, each in their own way, set early examples for me about what it means to be an intelligent, independent woman. They were wonderful role models and human beings and always remain fondly in my memory.

ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor, Dr. Deborah G. McCullough, for her guidance and support. None of this would have been possible without her encouragement and dedication to my project and my career. She was always available to answer any questions or give advice, while at the same time encouraging me to become an independent and competent researcher. Along with Deb I would like to thank my committee members, Dr. Therese M. Poland and Dr. Bert M. Cregg, for their assistance in developing my project and their willingness to help any time I needed advice. I would also like to thank my undergraduate biology professor, Dr. James B. Martin at Adrian College, for introducing me to the exciting field of entomology and inspiring me to become a scientist.

I would have been unable to complete my research if not for the help of all of the undergraduate workers who were willing to assist me in my many projects. I would like to thank Joanna Bloese, Tara Dell, James Wieferich, Jacob Bournay, Emily Pastula, Erin Burkett, Keali Chambers, Ben Schmitt, Alison Barc, and Andrew Krieger. I would also like to thank the lab technicians Andrea Anulewicz, David Cappaert, Bob MacDonald, Jacob Baker, and Nick Gooch, as well as post-doctorates Nathan Siegert and Rodrigo Mercader. Their assistance in plantation set-up and maintenance, insecticide treatment, and help with any research questions I may have had was invaluable. Advice and support from other graduate students in the lab including Andrew Tluczek, Sara Tanis, and Stephen Burr have also been indispensable.

I thank Yigen Chen, Monica Hufnagel, Debbie Miller, Tina Ciaramitaro, and the rest of the workers and post-doctorates from the USDA Forest Service Northern Research Station for their help with projects, including volatile collections and nutrient analyses. I also thank Amanda Taylor from Bert Cregg's lab for her help with chlorophyll and photosynthesis testing.

I would like to thank Paul Bloese, Randy Klevickas, and the rest of the staff of the Tree Research Center for the use of their facilities and for their advice and support with regards to maintaining my plants under optimal conditions.

Finally, I thank my parents, Glenn and Nancy Limback, and my sister Kristin Limback for their support and understanding throughout my graduate school experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Density and Development of Emerald Ash Borer (*Agrilus planipennis* Fairmaire) Larvae on Stressed and Vigorous Green and White Ash Trees.

Introduction

The invasive emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) was first discovered in Michigan and Windsor, Ontario, Canada in 2002 and has become a devastating pest of ash (*Fraxinus* spp.) in the eastern United States (Poland and McCullough 2006). This phloem feeding beetle is native to Asia, including eastern Russia, northeastern China, Japan, Korea, Taiwan, and Mongolia (Jendek 1994, Haack et al. 2002). An estimated 7 million ash trees were already dead or dying in Michigan when *A. planipennis* was identified in 2002 (Poland and McCullough 2006). It has now killed more than 40 million ash trees in southeast Michigan alone (www.emeraldashborer.info 2010). Additional populations have been found in at least 14 other states and Quebec (www.emeraldashborer.info 2010). There are more than 800 million ash trees in Michigan forests (USDA–FS FIA 2006, Liu and Bauer 2006). Ash is also a popularly planted street tree in urban areas. Death of these trees caused by *A. planipennis* could result in significant economic losses (Kovas et al. 2010). There is concern that this invasive pest, if not controlled, could spread throughout North America, largely eliminating the ash resource.

A. planipennis adults feed on leaves of ash trees, but do not harm the tree (Cappaert et al. 2005b). Damage is caused by larvae feeding in the cambial region. Galleries in the phloem disrupt nutrient flow and may also score the outer sapwood, affecting water conduction (Cappaert et al. 2005b). Adult beetle emergence begins in late

spring and continues through early summer. Adult female *A. planipennis* require 5-7 d of feeding before mating, and 5-7 d more before beginning oviposition (Bauer et al. 2004, Lyons et al. 2004). Beetles continue to feed and oviposit throughout their 3-6 wk lifespan (Bauer et al. 2004, Cappaert et al. 2005b). Females deposit their eggs in bark crevices or under flaps of bark (Bauer et al. 2004). After eclosion, most larvae complete four instars, overwinter as prepupae, then pupate and emerge in late spring (Cappaert et al. 2005b, Bauer et al. 2004). However, some *A. planipennis* overwinter as early instars and require a second year of development (2-yr larvae) (Cappaert et al. 2005a). Higher proportions of 2-yr larvae occur on healthy trees that are lightly infested than on stressed trees, such as girdled trees (Cappaert et al. 2005a, Tluczek 2009).

Five native ash tree species in the eastern United States are threatened by *A. planipennis*: green ash (*Fraxinus pennsylvanica* Marsh), white ash (*Fraxinus americana* L.), blue ash (*Fraxinus quadrangulata* Michx.), black ash (*Fraxinus nigra* Marsh), and pumpkin ash (*Fraxinus profunda* Michx.) (Cappaert et al. 2005b, Poland and McCullough 2006). A non-native ash species, European ash (*Fraxinus excelsior* L.) is occasionally planted as a street tree and may also be threatened by *A. planipennis*. Previous research indicated that, when growing together, *A. planipennis* preferentially colonized green ash trees over white ash trees, and green ash trees succumbed to attack first (Anulewicz et al. 2007a). The same study also revealed that white ash was colonized by *A. planipennis* over blue ash. Blue ash trees may produce callous tissue over galleries, a possible defense mechanism (Anulewicz et al. 2007a).

Like other *Agrilus* spp., *A. planipennis* prefer to oviposit on stressed trees (McCullough et al. 2009a, 2009b). Native *Agrilus* spp. including the bronze birch borer,

Agrilus anxius Gory, and the two-lined chestnut borer, *Agrilus bilineatus* (Weber), are secondary pests that feed on stressed and dying trees (Anderson 1944, Haack and Benjamin 1982, Dunn et al. 1986). Likewise, *A. planipennis* is a secondary pest throughout its native range, presumably because it shares an evolutionary history with its host trees, which have greater defenses against it than do North American ash species (Yu 1992; Akiyama and Ohmomo 2000; Gould et al. 2005; Herms et al. 2005; Schaefer 2005; Williams et al. 2005, 2006, Eyles et al. 2007).

Although *A. planipennis* will attack and kill healthy trees in the United States, adult beetles display a stronger attraction to stressed trees, such as girdled trees (Cappaert et al. 2005b, Tluczek 2009). Girdled trees have higher larval densities than trees stressed by herbicide, wounding, or exposure to the stress elicitor methyl jasmonate (McCullough et al. 2009a, 2009b; Tluczek 2009). Higher proportions of 1-yr larvae on stressed trees suggest that stressed trees have less resistance to *A. planipennis* than vigorous trees, while larvae in more vigorous trees go through a second year of feeding, possibly to compensate for the trees' defenses.

Although much research has focused on *A. planipennis* and other buprestids' attraction to stressed and dying trees, few studies have examined whether cultural practices to improve tree vigor will have an opposite effect on *A. planipennis* host preference or development. One way of increasing tree vigor may be through fertilization to increase nutrient availability. Another option to increase tree vigor is the application of a phosphite fungicide. Fungicides such as Agri-Fos® (Agrichem, Liquid Fertiliser Pty. Ltd., Loganholme, Queensland, Australia) combined with the bark-penetrating surfactant Pentra-bark™ (Quest Products Corp., Louisburg, KA, USA) induced defense

mechanisms in some hardwood trees (Garbelotto et al. 2007). Defenses induced from phosphate fungicides can include cell wall thickening as well as enhanced lignification and secondary metabolite production (Guest and Grant 1991, Garbelotto et al. 2007). Applying this treatment to the trunks of coast live oak (*Quercus agrifolia* Née) trees decreased the growth of lesions attributed to the fungal pathogen *Phytophthora ramorum*, which causes sudden oak death (Garbelotto et al. 2007). I hypothesized that application of Agri-Fos + Pentra-bark to green ash and white ash trees could induce a defensive response to *A. planipennis* larval feeding through callous tissue production. Callous tissue defense responses occur in birch (*Betula* spp.) trees as a defense against the bronze birch borer (Barter 1957).

Volatile emissions attract some phytophagous insects to their host plants and these volatiles can be induced when plants are stressed (Bernays and Chapman 1994; Dicke 2000, Rodriguez-Saona et al. 2006, de Groot et al. 2008). *A. planipennis* displayed attraction to volatiles emitted from ash foliage damaged by beetle feeding damage or the stress elicitor methyl-jasmonate. Linalool elicited the strongest antennal responses in females and the compound (Z)-3-hexen-1-ol resulted in the strongest antennal responses in males (Rodriguez-Saona et al. 2006). Further studies revealed *A. planipennis* attraction to lures with (Z)-3-hexenol, particularly in males (de Groot et al. 2008). Two compounds, Manuka oil and Phoebe oil, extracted from the New Zealand tea tree (*Leptospermum scoparium* J.R. and G.) and the Brazilian walnut (*Phoebe porosa* Mez.) trees, respectively, contain high amounts of certain ash bark volatiles, and were attractive to *A. planipennis* (Crook et al. 2008). Phloem volatiles may be partially responsible for the strong attraction of *A. planipennis* to girdled trees.

I examined effects of stress and vigor treatments, including girdling, fertilization, and fertilization + Agri-Fos, on adult *A. planipennis* attraction and foliar feeding preferences and larval density, survival and development in white ash and green ash trees. I hypothesized that (1) total larval density would be highest in green ash trees and girdled trees, and lowest in white ash trees and trees treated with fertilizer or fertilizer + Agri-Fos; (2) larval mortality rate would be highest and development fastest in girdled trees; (3) girdling trees would decrease foliar nutrient concentration and photosynthesis rates while increasing volatile productions from foliage and phloem; (4) fertilization and enhanced fertilization treatments would increase foliar nutrient concentration and photosynthesis rates; (5) vigorous trees would be less attractive to adult *A. planipennis,* and (6) larvae feeding on trees treated with fertilizer or fertilizer plus Agri-Fos would have lower survival or would be more likely to require a second year for development.

Study objectives to test these hypotheses were to (1) assess effects of girdling, fertilization and enhanced fertilization on the foliar chlorophyll, photosynthesis rates, and nutrient concentration of green and white ash trees; (2) compare the effects of the three treatments on volatiles produced by green and white ash foliage and phloem; and (3) determine whether *A. planipennis* larval density, survival, or development rate differed between green and white ash or among treatments.

Materials and Methods

Study Sites

Forty *Fraxinus americana* 'Autumn Purple' and forty *Fraxinus pennsylvanica* 'Patmore' were planted in April 2007 at Michigan State University's Tree Research

Center (East Lansing, MI). Diameter at breast height (dbh) averaged (±SE) 5.24 ± 0.07 cm for green ash and 5.65 ± 0.17 cm for white ash at time of planting. Trees were obtained from Poplar Farms Nursery (Waterman, IL) and arrived balled and burlapped in mid-April 2007. Planting occurred from 21 April to 23 April 2007. Green and white ash trees were planted in randomized blocks. Installation of a drip irrigation system was completed on 12 June 2007. Irrigation ran approximately 7 h/d, 2 d/wk at 3.8 liter/h from each of two emitters per tree.

The plantation was regularly mowed and weeded. In 2007, glyphosate (Round-up herbicide, 41% active ingredient (ai) per 3.8 liter water, Monsanto Technology, L.L.C., St. Louis, MO, USA) was applied every 2-3 wk to minimize competing herbaceous vegetation. Foliage on the ash trees was sprayed to runoff with cyfluthrin (Tempo, Do It Yourself Pest Control, Atlanta, GA, USA) to protect trees from colonization by local *A. planipennis* populations. Sprays were applied with a CO_2 backpack sprayer on 5 June 2007 (11.8 ml ai per 378.5 liter of water at 206.8 kpa).

Plantation Study

In 2007, we assigned the 80 trees to 10 blocks. Each block consisted of a two row planting of four green ash and four white ash trees. Within each block, a green ash and a white ash were randomly assigned to one of four treatments: fertilization, Agri-Fos, fertilization + Agri-Fos, and untreated control. Treatments were applied on 6 July 2007 under sunny conditions. Applications of Agri-Fos plus the surfactant Pentra-bark were mixed as 1.4 liter Agri-Fos + 0.1 liter Pentra-bark + 2.4 liter deionized water per 3.9 liter spray. The lower 1.5 m of each trunk was sprayed with a 7.6 liter garden sprayer at a rate

of 24.4 ml ai (mono- and di-potassium salts of phosphorous acid) per tree (approximately 15.81 ml phosphorous acid). Harrell's Pro-Blend with Micronutrients custom-mixed 19-5-10, granular, controlled-release fertilizer (Harrell's, Inc., Sylacauga, AL, USA) was applied around the base of the trees to dripline at a rate of 40 g N per tree (approximately 599 kg per ha).

In 2008, the fertilization and fertilization + Agri-Fos treatments were reapplied to the same trees as in 2007 using the same methods. Trees that were treated with Agri-Fos plus Pentra-bark in 2007, however, were left untreated. Fertilization and Agri-Fos treatments were applied on 15 May 2008 under sunny conditions following the same methods and rates as in 2007. Fertilization and fertilization + Agri-Fos trees had been exposed to two years of treatment at the time of this study. The plantation was subsequently divided in half, with five blocks (40 trees) in each half.

The westernmost five blocks were set aside for study in 2009. Foliage on these trees was sprayed with cyfluthrin via the same methods as in 2007 to protect them from colonization by local *A. planipennis* populations in 2008. Tree wrap (Jobe's Tree Care Products, Easy Gardener Products, Inc., Waco, TX, USA) and screening secured with zip-ties were also placed around the trunks of these trees to exclude oviposition by local *A. planipennis*.

In the remaining (easternmost) five blocks, trees which had been treated with Agri-Fos alone in 2007 were girdled by removing the phloem and cambium from the circumference of the trunk. A 20 cm wide band of bark and phloem was removed 1 m aboveground on the trunk of each assigned tree with drawknives on 28 May 2008. These five easternmost blocks were not sprayed with cyfluthrin or wrapped, permitting

colonization by wild *A. planipennis* during the summer. These trees were felled in autumn to quantify larval density.

In April 2009, tree wrap and screening were removed from the remaining trees. The fertilization and fertilization + Agri-Fos treatments were reapplied in mid-May 2009 under sunny conditions to the same trees as in 2007 and 2008 via the same methods and rates. Fertilization and fertilization + Agri-Fos treatments were thus applied for three consecutive years by summer 2009. Trees which had been previously treated with Agri-Fos plus Pentra-bark in 2007 and left untreated in 2008 were girdled on 21 May 2009, following the same methods as in 2008.

Treatment Analyses

Effects of the four treatments on foliar nutrient concentration, chlorophyll content, and photosynthesis and transpiration rates were quantified in green and white ash trees in 2008 and 2009. To assess foliar nutrients, one leaf from the northern aspect of the lower canopy of each tree was removed on 16 July 2008, 10 July 2009, and 12 August 2009. Leaves were flash frozen in liquid nitrogen and stored at -20 °C until processing. If leaves were so small that one leaf would not provide enough leaf tissue for analysis, two opposite leaves on the same shoot (assumed to be the same age) were selected. Leaf tissue was finely ground in liquid nitrogen and approximately 50 mg was extracted for analysis. Protein (mg/g fresh weight) was determined via Bradford protein assay and amino acid concentration (μmol/g fresh weight) was determined colorimetrically via cadmium-ninhydrin procedure (Doi et al. 1981, Kytö 1996, Fisher et al. 2001, Bi et al. 2003, Chen et al. 2009). Total non-structural carbohydrates (mg/g fresh weight),

calculated as the sum of glucose and starch, were determined using the glucose (HK) assay kit (Sigma-Aldrich, St. Louis, MO, USA) and methods from Jones (1979). Starch was estimated as glucose equivalents (Marquis et al. 1997, Chen et al. 2009).

From 12-14 August 2009, phloem samples were collected for nutrient analysis. Four cores (10 mm diam, appx 2 mm depth) of outer bark, phloem, and cambium were obtained from the north aspect of each tree using cork borers. Two samples above 1.3 m (above the girdle on girdled trees) and two below 1 m (below the girdle on girdled trees) were collected. Cambium and outer bark was scraped from each sample so only phloem remained. Samples were flash frozen in liquid nitrogen and stored at -20 °C until processing. The two cores above 1.3 meters were combined for analysis as well as the two cores below 1 m. Samples were ground in a mill and approximately 50 mg extracted for analysis. Phloem samples were tested for protein, amino acids, total non-structural carbohydrate, and protein:carbohydrate ratio using the same methods applied for foliage analyses.

On 16 July 2008 and 28 August 2009, one leaf from the lower northern aspect of the canopy of each plantation tree was removed for total nitrogen determination. If leaves were so small that one leaf would not provide enough leaf tissue for analysis, two opposite leaves on the same shoot (assumed to be the same age) were selected to obtain ≥1 g leaf material after oven drying. Leaves were oven dried at 65.5°C for 72 hr in a model 30 GC lab oven (Quincy Lab, Inc., Chicago, IL, USA). Samples were sent for total nitrogen analysis via micro-Kjeldahl digestion procedure at Michigan State University's Soil and Plant Nutrient Laboratory.

Foliar chlorophyll content was analyzed weekly in 2008 and 2009 using a Minolta SPAD 502 Chlorophyll Meter (Spectrum Technologies, Inc., Plainifield, IL, USA). Four readings were taken per tree (one on each cardinal aspect of the lower canopy) and averaged each week. The Li-Cor LI-6400 (Li-Cor Biosciences, Lincoln, NE, USA) system was used to measure photosynthesis and transpiration rates on 24 trees on 30 August 2008. The trees were randomly selected from four of the five blocks to represent at least two of each species by treatment combination. In 2009, photosynthesis and transpiration rates were measured on 15 June and 3 August. Photosynthesis rates were measured via foliage gas exchange rates (A_{max}) (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) with a fluorescent leaf chamber. Transpiration rates were measured as mmol $H_2O \cdot m^{-2} \cdot s^{-1}$. Rates were assessed on a leaf from the northern lower canopy and a leaf from the southern lower canopy of all selected trees in both years. Leaves from each aspect were analyzed independently and not averaged per tree. Trees were analyzed with the Li-Cor in mid-afternoon in sunny conditions in both years. Quantum flux was set at 1500 μmols/m^2/s for use as a light source and machine temperature was set at that of the average daily temperature in °C. Flow was set to 500 μms and the mixer set at 400 μms CO_2R.

Adult Feeding Bioassays

In 2009, I conducted three no-choice bioassays with adult *A. planipennis* beetles. Bioassays began on 23 June, 29 June, and 28 July 2009. The 2009 bioassays and caged adult studies discussed below required the use of laboratory-reared *A. planipennis* adults. Beetles were reared from logs which were harvested from naturally invested ash trees

near Lansing, MI in fall 2008 and maintained in cold storage at 3.9°C. After removing logs from cold storage, they were placed in 76.2 cm long, 15.2-30.5 cm diam cardboard tubes (Saginaw Tube Co., Saginaw, MI, USA) with plastic and in a rearing room maintained at 26.7° C. Adult beetle emergence from logs followed in approximately 21 days. Emerging adults were collected daily and immediately transferred to bioassay material for no-choice bioassays. Two leaves opposite each other on the same whorl of a lower canopy branch of each tree were collected and scanned in a flatbed scanner to determine leaf area using WinFOLIA software (Regent Instruments, Inc.; Quebec, Qc, Canada). After scanning, the petiole of each leaf was cut on a slant to provide a fresh surface area for water uptake. Leaves were inserted into water-filled microcentrifuge tubes to maintain moisture and placed individually in 150 mm diam Petri dishes. Two newly-emerged male *A. planipennis* were placed in a dish with one of the leaves from each tree, and two newly-emerged female beetles were placed in a dish with the second leaf. Beetles were allowed to feed for three days. Petri dishes were checked daily to record beetle mortality. After feeding, leaves were re-scanned and total leaf area consumed was determined by comparing original leaf area to the remaining area. Leaf area consumed was divided by total "beetle days" (sum of the number of days, to 0.5 d, that each beetle survived) to obtain a value for total leaf area fed per beetle per day. To account for differences in specific leaf weight between species, areas fed on white and green ash were converted to weight of foliage consumed values using specific leaf weights determined by Chen and Poland (2010). Fresh frass was collected from each dish and weighed to the nearest mg to estimate feeding efficiency on the leaves. A frass

weight: foliage consumed weight ratio was analyzed for species variables to compare *A. planipennis* utilization of nutrients on green and white ash foliage.

Adult A. planipennis *Attraction*

To assess beetle attraction to trees, sticky bands were placed on the trunk and branches of each tree in 2009. On 3 June 2009, the trunk of each plantation tree was wrapped with 20 cm wide bands of plastic wrap from 1.3 m to 1.5 m aboveground. Bands were covered with Tangletrap Insect Trap Coating (Gempler's; GHC Specialty Brands, LLC; Madison, WI, USA). On 6-7 June 2009, two branches (aspects random) in the mid to lower canopy of each tree were also wrapped with 16 cm long bands of plastic wrap, just below a branch with leaves, and coated with Tangletrap. Sticky bands on the trunks and branches were checked weekly for beetle presence through the week of 13 July 2009, after which sticky bands were removed.

Progeny of Caged Adults

In the week of 13 July 2009, a study was initiated to examine larval development on the study trees. After emergence from rearing logs as described above, beetles used in caged adult studies were reared for approximately one week on either leaves from *Fraxinus uhdei* (Wenz.), a tropical, evergreen ash grown in a polyhouse, or from one of the plantation trees. Two plastic 118.3 ml cup cages (Kendall, Tyco Healthcare Group, Mansfield, MA, USA) were attached to each plantation tree with silicone caulking, one on the northern and one on the southern aspect of the trunk between 1.3 and 1.5 m, in the

area where the sticky band was previously located. One male and one female laboratory-reared adult *A. planipennis* were introduced to each cup cage.

Each cup cage included an ash leaf placed in a water-filled microcentrifuge tube for adult feeding. Leaves were replaced as they dried out, approximately every other day. One cup cage per tree had foliage from that tree while the other cage contained leaves from *F. uhdei*. Male and female beetles that died in the first three days were replaced, and female beetles were allowed to oviposit through mid-August. I expected that removal of occasional leaves from the plantation trees to feed the beetles would not cause an excessive amount of stress to the trees, although it is possible that other studies may have been mildly affected.

In mid-September, cup cages were carefully removed and bark in the area immediately underneath and surrounding the cages was peeled using draw-knives. Number and instar of *A. planipennis* larvae were recorded. Larval galleries which began beneath the cages were assumed to have hatched from eggs laid by caged females, while galleries initiated outside the cages were assumed to be wild. Larvae that hatched from eggs laid by caged females were counted and development stage recorded. First and second instars at this time of year were expected to require a second year of development (2-yr larvae), while fourth instars and prepupae were expected to emerge the following summer (1-yr larvae). Because it was possible that some third instar larvae in September may have developed to fourth instars by October, third instar larvae were analyzed separately from other instars. Wild larvae were also recorded and added to the total when trees were felled and peeled in their entirety (see below).

Volatile Production

Foliar volatile emissions were collected from all plantation trees on 9-12 June, 14-16 July, and 11-13 August in 2009. Emissions were collected by enclosing foliage on one branch of each tree in bags (Reynolds® Oven Bags, Reynolds Consumer Products Company, Richmond, VA, USA). Lower branches with foliage which were in close proximity to larger branches were selected, as these would most conveniently lend themselves to volatile collection. Because of this, no consistent aspect was used for all trees. I inserted an activated carbon filter into the opening of each bag along with a Super-Q Volatile collection trap (Analytical Research Systems, Inc.; Gainesville, FL, USA) with 30 mg Alltech Super-Q adsorbent material for volatile collection. These were tightly fixed into the bag on a single branch using twist-ties. The other end of the Super-Q trap was attached to a long tube inserted over a suction pump (Sensidyne, Clearwater, FL) attached to a 9-volt battery. Batteries were suspended from the nearby large branches on each tree. The volatile collection period was 24 hr. Super-Q traps were returned to the lab and volatiles extracted with 150 µL pentane-hexane extraction solvent to which 1.0 ng/µl of heptyl acetate was added as an internal standard with a stream of nitrogen gas. Extracts were topped off with 100 µL hexane to minimize volatization during storage in a -20°C freezer until processing. Processing was conducted by condensing volatiles under a stream of pure gaseous nitrogen, followed with analysis by gas chromatography-mass spectrometry. Two µl of each sample was injected into a Thermo Scientific Trace GC equipped with a DSQ-II MS and a 30 m by 0.25 mm id, 0.25 µm thick TR-1MS column using splitless injections, with the carrier gas being helium. The oven temperature cycled beginning at 1 min at 60°C, then increased to 190°C at 10°C /min, then increased to

300°C at 35°C/min and was subsequently held for 5 min. Volatile compounds were identified by comparing spectral data with spectra from Wiley 275.L and Nist98.L libraries and spectral data from commercially available standards. These were confirmed by retention times of authentic standards, if available. Comparison of peak areas with that of the internal standard and calibration curves generated with synthetic standards were used to quantify compounds. The value of volatile compounds in ng was divided by the dry weight of foliage used for collection to obtain a value for ng/g dry leaf tissue of each compound of interest. All reagents and solvents used for volatile compound analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA).

Phloem volatiles were collected on 13-15 August and 7-9 October 2009. Bags (Reynolds® Oven Bags, Reynolds Consumer Products Company, Richmond, VA, USA) were wrapped around the trunk of each tree from approximately 0.7 m to 1.3 m height (1 m to 1.3 m on girdled trees to avoid epicormic shoots on lower trunk) and secured with duct tape. Bags were loose to allow space inside for air movement. A small hole was cut into each bag and an activated carbon filter and Super-Q Volatile collection trap (Analytical Research Systems, Inc.; Gainesville, FL, USA) were inserted inside. Holes were duct-taped shut around the filter and Super-Q trap. The other end of each Super-Q trap was attached to a long tube inserted over a suction-pump (Sensidyne, Clearwater, FL) attached to a 9-volt battery. The volatile collection period was 24 hr. Super-Q traps were returned to the lab and volatiles extracted and analyzed via the same methods used for the foliar volatiles. Total ng of each compound of interest were standardized by

multiplying values from girdled trees by two to account for the half-length of the collection bag.

Larval Density and Development – Wild A. planipennis

Beginning in September 2008 and again in 2009, plantation trees were felled one block at a time. Felled trees were cut into two sections in 2008 (base of the trunk to 2 m above ground, above 2 m) and three sections in 2009 (base of the trunk to 1 m above ground, 1 m to 2 m, above 2 m). The trunk and all branches greater than 3 cm diam were debarked using drawknives. Bark thickness at the north and south aspect of the top and bottom of each half of the trunk was also measured. Log diam in both years was measured at 1 m above ground, and these measurements were compared between species and among treatments to assess any differences in tree size. Number, stage, and condition (live, dead) of *A. planipennis* larvae were recorded. First to third instars were expected to require a second summer of development before pupation, and fourth instars and prepupae were expected to emerge the following spring.

Area exposed on each tree was quantified using the formula for calculating surface area of a conical frustum:

$$S = \pi (R_1 + R_2) s$$

S = the surface area of the sides of the frustum not including top and bottom circles

R_1 and R_2 = the radii of the top and bottom circles

s = the length up the side of the frustum.

Area was calculated for each section and branch and summed to estimate total area exposed on each tree (m^2). Number of larvae was divided by the total area exposed to standardize larval density (larvae per m^2 of surface area) for each tree. Total larvae in 2009 were also regressed on the total number of adults captured on sticky bands on the trunks to compare landing rates with egg deposition.

Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots (PROC GLIMMIX, SAS Institute 2001). All parametric statistics were run using SAS 9.1 software (PROC GLIMMIX, SAS Institute 2001). Two-way, three-way, or four-way analysis of variance (ANOVA) were applied to data that followed a normal distribution (SAS Institute 2001). If ANOVA results were significant ($p<0.05$), Tukey's honestly significant difference (HSD) multiple comparison test was used to assess differences among treatments (Tukey 1953, SAS Institute 2001).

Foliar nutrient concentrations, volatile compound productions, chlorophyll values, and larval density and development rates were tested using two-way ANOVA to assess effects of tree species and treatment. Phloem nutrient concentrations were tested using three-way ANOVA to assess effects of tree species, tree treatment, and beetle sex. Photosynthesis and transpiration rates were tested using three-way ANOVA to assess effects of tree species, tree treatment, and leaf aspect. Four-way ANOVA was used to assess effects of tree species, tree treatment, cup cage aspect, and foliage type on total larval densities from caged adults. Regression analysis (PROC REG, SAS Institute 2001)

was used to examine the relationship between adult *A. planipennis* captured on sticky bands and total larvae discovered when peeling logs.

Foliar protein and protein:carbohydrate ratios in 2008 were normalized by log (x+1) transformation. Foliar protein in July 2009 and protein: carbohydrate ratios in both months in 2009 were also normalized by log (x+1) transformation. TNC and protein: TNC ratios in phloem in 2009, mean leaf area fed by adult beetles in no-choice bioassay round 2, larval density from caged adults, wild larval density in 2009, and several volatile compounds in 2009 [(Z)-3-hexenyl acetate, trans-ocimene and tetradecane in June, cis-ocimene and α-cubebene in July, α-cubebene and α-farnesene in August, and 4-methyl-dodecane and borneol acetate for trunks in October] were also normalized by log (x+1) transformation. Photosynthesis values in 2008 were normalized by log (x) transformation. The October 2009 volatile compound tetracosane was normalized by inverse (1/x) transformation. The Kenward and Roger (1997) method was used to approximate denominator degrees of freedom when data were unbalanced (SAS Institute 2001).

If transformations did not normalize variables, nonparametric tests were used. Friedman's two-way or three-way nonparametric ANOVA (SAS Institute 2001) was used to test non-normal data involving potential interactions. When significant, nonparametric multiple comparison tests were applied following methods from Conover (1971) and Zar (1984). If the interaction term in Friedman's test was $p \geq 0.50$ or no potential interactions existed, Wilcoxon Rank Sum tests (Ott and Longnecker 2001) and Kruskal-Wallis tests (Kruskal and Wallis 1952) were used. When significant, multiple comparison tests were applied following the same methods (Conover 1971, Zar 1984). Friedman's three-way nonparametric ANOVA was used to test protein and amino acids in phloem in 2009.

Kruskal-Wallis tests were used to analyze mean leaf weight fed per beetle per day and ratio of frass produced: leaf weight fed in no-choice bioassays, adult beetle capture on sticky bands, larval development rate from caged adults, larval mortality in both years, and larval density in 2008. All analyses were conducted at the $p<0.05$ level of significance.

Results

Tree Diameter

Tree diam in 2008 (measured at 1 m aboveground) 1 m differed between species species (F=112.27; df=1,27.44; $p<0.001$) and among trees in different treatments (F=25.77; df=3,27.47; $p<0.001$) (N=39). Green ash trees were slightly larger (6.1 ± 0.13 cm) than white ash trees (7.1 ± 0.10 cm) and trees which had been girdled that spring (5.9 ± 0.26 cm) were somewhat smaller than trees assigned to the other treatments (untreated: 6.8 ± 0.20 cm, fertilized: 6.8 ± 0.16 cm, fertilizer + Agri-Fos: 6.9 ± 0.16 cm).

In contrast to 2008, tree diam (measured at 1 m aboveground) did not differ between species (p=0.37) or among treatments (p=0.50) in 2009. Mean species diameters were 6.3 ± 0.16 cm for green ash trees and 6.1 ± 0.32 cm for white ash trees. Mean diameters for treatments were 5.8 ± 0.30 cm for trees which had been girdled that spring, 6.1 ± 0.41 cm for untreated trees, 6.5 ± 0.41 cm for fertilized trees, and 6.3 ± 0.32 cm for trees treated with fertilizer + Agri-Fos.

Foliar Nutrients and Photosynthesis - 2008

Nitrogen concentration (ppm NH_4 N) was lowest in foliage from girdled trees (χ^2=11.89, df=3,35, p=0.008) but did not differ between species (p=0.07) (Table 1.1). Protein concentration (mg/g fresh weight) differed among treatments (F=3.11; df=3,28; p=0.042) but the conservative Tukey's HSD test did not identify which treatments differed significantly (Table 1.1). No protein differences were found between species (p=0.69). Foliage from girdled trees had lower total amino acid concentration (µmol/g fresh weight) than foliage from trees of other treatments, where amino acid concentration was almost nine-fold higher (F=5.06; df=3,30; p=0.006), but amino acids did not differ between species (p=0.87) (Table 1.1). Total foliar non-structural carbohydrate concentration (mg/g fresh weight) displayed an opposite trend; foliage from girdled trees had higher levels than foliage from all other treatments (F=10.00; df=3,30; p<0.001). Total non-structural carbohydrates also differed between species, with white ash trees having higher carbohydrate concentrations than green ash (F=9.96; df=1,30; p=0.004) (Table 1.1). The ratio of protein: total non-structural carbohydrates was lower in girdled trees than in untreated or fertilized trees, with trees treated with fertilizer + Agri-Fos being intermediate (F=8.26; df=3,24, p<0.001) (Table 1.1). No clear differences between green ash and white ash were observed for protein:carbohydrate ratios (p=0.17).

Chlorophyll content fluctuated over the course of the summer. Girdled trees had less chlorophyll than trees of other treatments throughout the study (p<0.001 for all dates). White ash foliage had lower chlorophyll levels than green ash foliage on most dates during mid-summer (Fig 1.1).

Photosynthesis rates were affected by interactions between species and treatment (F=6.47; df=3,30.13, p=0.002), treatment and aspect (F=4.78; df=3,29.02; p=0.008) and species by aspect (F=5.03; df=1,29.02, p=0.033) (Fig. 1.2). Foliage from girdled trees of both species had lower photosynthesis rates (approximately 4 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) than foliage from other trees (over 12 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), regardless of aspect or species (Fig. 1.2a, c). For green ash, fertilized trees exhibited lower photosynthesis rates than untreated trees or trees treated with fertilizer + Agri-Fos (Fig. 1.2a). Fertilizer + Agri-Fos treated trees had higher photosynthesis rates in green ash than in white ash (Fig. 1.2a). Foliage from the north aspect on untreated trees exhibited higher photosynthesis rates than foliage from the north aspect of fertilized trees (Fig. 1.2c). Trees treated with fertilizer had higher photosynthesis rates on the south aspect of the crown than on the north aspect (Fig. 1.3c). Within white ash trees, leaves on the south aspect had approximately 2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ higher photosynthesis rates than leaves on the north aspect (Fig. 1.2b). Transpiration rates were significantly affected by treatment (F=71.09; df=3,30.48; p<0.001) and were over twice as high on non-girdled trees as they were on girdled trees (Fig. 1.3). Species (p=0.09) and aspect (p=0.08) did not affect transpiration rates.

Foliar Nutrients and Photosynthesis – 2009

In July 2009, protein concentration was over 4 mg/g (fresh weight) higher in white ash foliage than in green ash foliage (F=9.01; df=1,30; p=0.005) but did not differ among treatments (p=0.60) (Table 1.2). Total non-structural carbohydrates (F=7.89;

df=3,25.95; $p<0.001$) and the protein: carbohydrate ratio ($F=3.93$; df=3,27.23; $p=0.019$) differed among treatments. Foliage from girdled trees had approximately two times higher carbohydrate concentration than trees of all other treatments, but the foliar protein:carbohydrate ratio of these trees was half that of trees treated with fertilizer + Agri-Fos (Table 1.2). Treatment ($p=0.08$) and species ($p=0.64$) had no significant effects on amino acid concentrations. Neither total non-structural carbohydrate concentration ($p=0.98$) nor protein: carbohydrate ratios ($p=0.11$) differed between species in July (Table 1.2)

As in 2008, nitrogen concentration (ppm NH_4 N) in 2009 was lowest in foliage from girdled trees ($F=5.81$, df=3,28, $p=0.003$) but did not differ between species ($p=0.21$) (Table 1.2). In August, treatment and species had no effect on foliar protein (treatment $p=0.59$, species $p=0.33$) or amino acid concentration (treatment $p=0.68$, species $p=0.84$). Treatment affected total non-structural carbohydrate concentration ($F=14.33$; df=3,24.96; $p<0.001$) and protein:carbohydrate ratios ($F=3.53$, df=3,25.27; $p=0.029$), but neither of these were affected by species (total non-structural carbohydrate $p=0.76$, protein: carbohydrate ratio $p=0.60$) (Table 1.2). Similar to July, foliage from girdled trees had carbohydrate levels that were at nearly two times higher than in trees of all other treatments, but foliar protein:carbohydrate ratios were less than half those of other treatments (Table 1.2).

Species ($p=0.85$), treatments ($p=0.98$), and location (above or below the girdle, $p=0.08$) did not affect total protein concentration in phloem of plantation trees in 2009 (Table 1.3). There were also no differences in amino acid concentrations between species ($p=0.84$), between phloem from above or below the girdle ($p=0.15$), or among treatments

(p=0.88) in tree phloem (Table 1.3). Total non-structural carbohydrates in phloem were affected by interactions between treatment and location and between species and location (Table 1.3). Carbohydrate concentration below 1 m in girdled trees was at least three times lower than any other location by treatment combination (F=7.57; df=3,54; p<0.001). Phloem carbohydrate concentration below 1m on white ash trees was lower than that of either species above 1.3 m, with green ash phloem below 1 m intermediate (F=4.07; df=1,54.02; p=0.049). Protein: carbohydrate ratios were highest below 1 m in girdled trees, and different from either fertilized or untreated trees above 1.3 m, and untreated trees below 1 m, with the remaining treatment by location combinations as intermediates (F=4.46; df=3,51.04; p=0.007) (Table 1.3).

Chlorophyll content fluctuated over the course of the summer; the mean content of girdled trees dropped by late August (Fig 1.4). As in 2008, girdled trees had less chlorophyll than trees of other treatments for most of the study; however, no clear differences between species were seen (Fig. 1.4). Photosynthesis rates on 15 June were affected by both treatment (F=27.29; df=3,28; p<0.001) and aspect (F=4.45; df=1,28; p=0.044), but not by species (p=0.45) (Fig 1.5). Foliage from girdled trees had lower photosynthesis rates than foliage from trees of other treatments (Fig. 1.5a). Foliage on the southern aspect of trees had higher photosynthesis rates than foliage from the northern aspect (Fig. 1.5b). No differences between species were observed. Photosynthesis rates on 3 August were affected by treatment (F=30.93; df=3,28.36; p<0.001), but not species (p=0.19) or aspect (p=0.23). Girdled tree foliage again had lower photosynthesis rates than foliage from other treatments (Fig. 1.5c), but no species or aspect effects were observed in August. Overall, photosynthesis rates were six to eight times higher on

untreated trees, fertilized trees, and trees treated with fertilizer + Agri-Fos than on girdled trees in both July and August. Transpiration rates were affected by both treatment (F=18.08; df=3,29.47; p<0.001) and species (F=4.72; df=1,28.29; p=0.038) on 15 June and by treatment (F=24.86; df=3,27.76; p<0.001) on 3 August (Fig. 1.6). Mirroring photosynthesis rates, transpiration rates were lowest in girdled trees and highest in green ash trees (Fig. 1.6). Aspect (p=0.08) on 15 June and aspect (p=0.49) and species (p=0.68) on 3 August did not affect transpiration rates.

Volatile Production - 2009

Foliar volatile compounds varied between species and among treatments. In June, three foliar volatile compounds were more abundant in emissions from green ash foliage than from white ash foliage. These compounds included (Z)-3-hexenyl acetate (F=4.34; df=1,26.29; p=0.047), trans-ocimene (F=14.45; df=1,30; p=0.001), and tetradecane (F=3.37; df=3,26.39; p=0.034). Trans-ocimene was also over two times higher in girdled tree foliage than in foliage from untreated trees, while fertilized trees and trees treated with fertilizer + Agri-Fos were intermediate (F=3.32; df=3,30; p=0.033) (Table 1.4).

In July, two foliar volatile compounds were affected by either species or treatment. Cis-ocimene was higher in green ash foliage than in white ash foliage (F=7.49; df=1,15.91; p=0.015) (Table 1.4). The volatile compound α-cubebene was affected by a species by treatment interaction. Foliage from girdled green ash trees contained at least eight times higher concentration of the compound (8.60 ± 2.31 ng/g dry leaf tissue) than any other species by treatment combinations (untreated green ash: 0.55 ± 0.16 ng/g; fertilized green ash: 0.42 ± 0.13 ng/g; fertilized + Agri-Fos green ash: 0.25 ± 0.16 ng/g;

girdled white ash: 1.27 ± 0.56 ng/g; untreated white ash: 0.13 ± 0.01 ng/g; fertilized white ash: 0.96 ± 0.87 ng/g; fertilized + Agri-Fos white ash: 0.37 ± 0.21 ng/g) (F=7.45; df=3,16.89; p=0.002) (N=27).

The compound α-cubebene was also significant in August foliar volatile analyses. Over twice as much was found in green ash than in white ash (F=5.32; df=1,26; p=0.030) Girdled tree foliage also had higher α-cubebene content than untreated or fertilized tree foliage, with foliage treated with fertilizer + Agri-Fos as an intermediate (F=4.70; df=3,26; p=0.009). The compound α-farnesene was found in much higher quantities in girdled tree foliage than in fertilized or untreated tree foliage. Foliage treated with fertilizer + Agri-Fos was again intermediate (F=6.28; df=3,25; p=0.003) (Table 1.4).

For volatiles collected from tree trunks in August, only nonanal was significant; higher amounts were found in girdled trees than in untreated trees, and fertilized trees and trees treated with fertilizer + Agri-Fos were intermediate (F=3.17; df=3,26; p=0.041) (Table 1.5). For volatiles collected from tree trunks in October, the compound 4-methyl-dodecane was over two times higher in girdled trees than in fertilized trees; untreated trees and trees treated with fertilizer + Agri-Fos were intermediate (F= 3.65; df=3,30; p=0.023). Both species (F=11.81; df=1,4.863; p=0.019) and treatment (F=12.82; df=3,5.141; p=0.008) were significant for the compound borneol acetate. White ash trees had higher borneol acetate content than green ash trees and girdled trees had higher borneol acetate content than untreated trees, fertilized trees, or trees treated with fertilizer + Agri-Fos. Finally, the compound tetracosane was over two times higher in girdled trees than in untreated trees, with fertilized trees and trees treated with fertilizer + Agri-Fos as

intermediates (F=4.98; df=3,28; p=0.007) (Table 1.5).

A. planipennis Leaf Feeding – 2009

All three rounds of adult *A. planipennis* no-choice feeding bioassays in 2009 had similar results (Table 1.6). Treatment did not affect leaf area consumed (Round 1: p=0.41, Round 2: p=0.77, Round 3 p=0.11) or frass production (Round 1: p=0.54, Round 2: p=0.43, Round 3 p=0.17). Values varied among rounds and no treatment was consistently highest or lowest (Table 1.6a,b). However, species effects were significant for both factors. In round 1, leaf area consumed per beetle per day was about 1.5 times higher on white ash foliage than on green ash foliage (F=7.51; df=1,64; p=0.008) and results were similar in round 3 (F=8.96; df=1,56; p=0.004). These differences were not significant in round 2 (p=0.56), although the overall mean was still higher for white ash (Table 1.6a). When species means were converted to leaf weight, however, no differences in adult beetle consumption were detected (Round 1 p=0.14, Round 2 p=0.12, Round 3 p=0.67) (Table 1.6c). In all three rounds, weight of frass produced per beetle per day was 1-2 mg higher when beetles fed on white ash foliage compared with green ash foliage (Round 1: F=11.32; df=1,58; p=0.001; Round 2: F=5.58; df=1,64; p=0.021; Round 3: F=11.98; df=1,64; p=0.001) (Table 1.6b). When the ratio of frass produced to leaf weight consumed was analyzed for species, ratios were consistently highest when beetles fed on white ash foliage (Round 1 χ^2=26.17, df=1,37, p<0.001, Round 2 χ^2=23.47, df=1,40, p<0.001, Round 3 χ^2=14.98, df=1,36, p<0.001) (Table 1.6d). Sex of beetles had no effect on total leaf area fed (Round 1 p=0.18, Round 2 p=0.51, Round 3 p=0.89) or total frass produced (Round 1 p=0.07, Round 2 p=0.76, Round 3 p=0.48).

Beetle Capture – 2009

A. planipennis adult captures on sticky bands on the trunks of trees were affected by treatment (χ^2=23.99, df=3,37, p<0.001) but not species (p=0.07) (Fig 1.7). For both species combined, beetle capture on girdled trees was up to eight times higher than on trees of any of the other three treatments (Fig 1.7). Very few beetles were captured on the sticky bands placed in the canopy, and statistical analysis was not performed on these results. In total, one beetle was captured in the canopy of girdled green ash trees, two in untreated green ash trees, and two in fertilized green ash trees. For white ash trees, four beetles were captured on sticky bands in the canopy of untreated trees, two in fertilized trees, and one in the canopy of trees treated with fertilizer + Agri-Fos . In total, beetles captured on sticky bands in the canopy accounted for approximately 10.4% of beetles caught on all sticky bands.

Larval Density and Development – Progeny of Caged Adults 2009

Larval density in the caged adult study was affected by a three-way interaction between species, treatment, and foliage type (F=3.30; df=3,48; p=0.028). Mean densities of larval progeny from caged beetles were highest on the northern aspect of untreated trees where the caged beetles fed on *Fraxinus uhdei* (5.2 ± 1.6 larvae) and on the northern aspect of girdled trees where the beetles fed on foliage from the tree on which they oviposited (4.0 ± 0.9 larvae). Both of these means were higher than on the northern aspect of untreated trees where beetles fed on foliage from the tree on which they oviposited, and where zero larvae were found.

Development rate of larvae under cup cages was different among treatments (Fig. 1.8). Girdled trees had a higher percentage of fourth instars and prepupal larvae (χ^2=45.15, df=3,20, p<0.001) and third instars (χ^2=14.30, df=3,20, p=0.003) than any of the three other treatments. Conversely, girdled trees had the lowest numbers of first and second instar larvae (χ^2=10.32, df=3,20, p=0.016) (Fig. 1.8). Approximately 50% of all larvae found under cup cages on girdled trees were third instars or higher, whereas on all other treatments, nearly 100% of larvae found were first and second instars and likely would have required two years for development. No significant differences in larval development rates between ash species were observed (first and second instars p=0.91, third instars p=0.14, fourth instars and prepupal larvae p=0.42).

Larval Density and Development - 2008

Larval density was affected by both species (F=22.67; df=1,4; p=0.009) and treatment (F=44.14; df=3,10; p<0.001) (Fig 1.9). For both species combined, larval densities were over twice as high on girdled trees than on trees of other treatments. For all treatments combined, larval densities were higher on green ash trees than on white ash trees. Larval densities on green ash trees were upwards of 300 larvae per m^2. Girdled white ash trees had at least 100 more larvae per m^2 than green ash trees which were not girdled. Very few larvae (< 7/m^2) were found on white ash trees which were not girdled (Fig 1.9).

Larval mortality from intraspecific competition was significantly affected by treatment (χ^2=19.84, df=3,35, p<0.001) but not by species (p=0.21) (Fig. 1.10). A higher

percentage (nearly 40% on green ash trees) of larvae were found dead on girdled trees than on trees of all other treatments (Fig. 1.10). Overall, approximately 70.0% of dead larvae were early (1st-3rd) instars. For development rate of larval progeny of wild adults, approximately twice as many larvae on girdled trees were late instars than on trees of any other treatment (χ^2=11.038; df=3; p=0.012) (Fig. 1.11) Larval developmental rates did not differ between species (p=0.34) (Fig. 1.11).

Larval Density and Development- 2009

Larval density in 2009 was affected by a species by treatment interaction (F=17.57; df=3,28; p<0.001) (Fig. 1.12). Total larvae per m^2 was highest in girdled green ash trees (over 300 larvae per m^2), followed by girdled white ash trees (nearly 200 larvae per m^2). The lowest densities (less than 50 larvae per m^2) were on untreated, fertilizer, and fertilizer + Agri-Fos trees regardless of species, and there were no significant differences among these treatments (Fig 1.12). There was a significant linear relationship between larval density and adult beetles captured on the sticky bands on the trees (p<0.001). The predictor coefficient of 10.1 indicates that on average, roughly ten larvae per m^2 were present for every adult beetle captured (Fig. 1.13).

Larval mortality was affected by treatment (χ^2=18.45; df=3, 37; p=<0.001) but not species (p=0.31) (Fig. 1.14). Mortality caused by intraspecific competition was more than four times higher on girdled trees when compared with trees of the other three treatments (Fig 1.14). Overall, approximately 58.1% of dead larvae were early (1st-3rd) instars. Larval development rate was affected by a species by treatment interaction

(F=4.04; df=3,32; p=0.015) (Fig. 1.15). A higher percentage of larvae were late instars on girdled trees of both species than on other trees (Fig. 1.15). Between 70-90% of all larvae found on girdled green and white ash trees were fourth instars or prepupae, whereas on untreated trees, fertilized trees, or trees treated with fertilizer + Agri-Fos, more than 60% of all larvae had reached only the first, second, or third instar (Fig. 1.15).

Discussion

Overall, there were clear differences in the way that white and green ash trees responded to stress- and vigor-inducing treatments. Consistent with previous observations (Anulewicz et al. 2007a), green ash trees were more attractive to *A. planipennis* than white ash trees, supporting my initial hypothesis. Higher numbers of larvae on green ash trees also appeared to die due to intraspecific competition, but these data were not significant. The reasons for these differences between species may reflect differences in tree vigor or nutrient composition. Differences such as the lower chlorophyll content in white ash foliage in 2008 may be part of the reason why other studies have shown white ash to be less attractive to *A. planipennis* than green ash (Anulewicz et al. 2007a). However, this difference was not present in 2009. Additionally, none of the phloem nutrients we measured differed between ash tree species. The lower photosynthesis rates on the northern aspect of the canopy on only white ash trees in 2008 may reflect leaf architecture. White ash leaves are larger and droopier than green ash leaves, and may thus have less of their surface exposed to sunlight at any given time. White ash leaves were also consumed with less efficiency by *A. planipennis* adults, consistent with observations by Chen and Poland (2010). Specific leaf weight is also lower in white ash (0.0116 ±

0.0004 g/cm^2) than in green ash (0.0165 ± 0.0005 g/cm^2), and white ash foliage has been shown to have lower levels of some nutrients (nitrogen, phosphorous, sulfur) than green ash foliage in other studies (Chen and Poland 2010). The lower specific leaf weight of white ash likely explains why beetles needed to feed on a greater leaf area on foliage from these trees, especially considering that no differences in leaf weight consumed were observed.

Higher volatile production from green ash trees may also help to explain why they are generally more attractive than white ash trees (Anulewicz et al. 2007a). (Z)-3-hexenyl acetate was previously identified as comprising a majority of the volatiles found in green and white ash foliage, along with (Z)-3-hexenol (de Groot et al. 2008). It is an antenally active compound for *A. planipennis*, but antennal responses are not as strong compared with other compounds such as (Z)-3-hexenol (Rodriguez-Saona et al. 2006, de Groot et al. 2008). However, antennal responses do not necessarily mirror beetle attraction in the field. The higher production of (Z)-3-hexenyl acetate in green ash foliage which was observed in June 2009 may be partially responsible for high *A. planipennis* attraction to these trees (Rodriguez-Saona et al. 2006).

The volatile compound α-cubebene was also highest girdled green ash trees in July and in girdled trees in August. This is a bark and foliar volatile found in high concentration in Manuka and Phobe oil, two components of lures used for *A. planipennis* attraction (Crook et al. 2008). The higher production of the essential oil borneol acetate in white ash over green ash foliage in October is the only case in which volatile production was higher in white ash.

It is possible that different cultivars of white and green ash may respond differently to treatments or *A. planipennis* attack than the 'Autumn Purple' and 'Patmore' varieties studied here. In another study, the 'Marshall's Seedless' cultivar of green ash was more resistant to *A. planipennis* than 'Patmore', exhibiting traits more similar to 'Autumn Purple' white ash (Rebek et al. 2008). However, those results provide insight into these particular varieties of green and white ash and their response to *A. planipennis* colonization. A 2003 survey in Iowa, a state which has recently been invaded by *A. planipennis* (www.emeraldashborer.info), identified 'Autumn Purple' and 'Patmore' as the most popularly grown cultivars of white ash and green ash respectively among growers (Iles and Vold 2003). Overall, many dead green ash trees have been found in the wild and in urban environments, regardless of cultivar. It is likely that any true differences between cultivars will be so subtle as to have a minimal effect on overall *A. planipennis* choice between white and green ash trees.

All evidence thus far suggests that green ash trees are more attractive to *A. planipennis* than white ash trees. Indeed, white ash trees only became as attractive as green ash trees when the white ash trees were girdled. Girdling is therefore an efficient stressor of ash trees regardless of species, and appears to effectively eliminate whatever inherent defenses untreated or vigorous white ash trees may possess. Girdled white ash trees even became more attractive to *A. planipennis* than untreated green ash trees, which are normally more attractive than white ash trees (Anulewicz et al. 2007a). This, as well as the high adult beetle captures on sticky bands on girdled trees in 2009, is consistent with my initial hypothesis and mirrors the results of previous studies indicating that girdled ash trees are highly attractive to adult *A. planipennis* (Anulewicz et al. 2007b,

McCullough et al. 2009a, 2009b; Tluczek 2009). The significant positive regression relationship between adult capture rates and larval densities in ash trees suggest that most of the adults landing on these trees are choosing to oviposit on them. In one study that examined 23 sites across four years, *A. planipennis* larval density and adult capture was consistently higher on girdled trees than healthy trees or trees stressed by herbicide or methyl jasmonate (McCullough et al. 2009a). This is likely because *A. planipennis* is a secondary pest in its native Asia and is adapted to attack stressed trees (Yu 1992; Akiyama and Ohmomo 2000; Gould et al. 2005; Herms et al. 2005; Schaefer 2005; Williams et al. 2005, 2006; Eyles et al. 2007).

Also consistent with my hypotheses was the fact that nearly 100% of larvae on girdled trees of either species in 2008 and 2009 were fourth instars or prepupae and would have developed in one year. This faster development rate on girdled trees is consistent with past research (Cappaert et al. 2005b, Tluczek 2009). Additionally, most larvae from caged adults would have developed in 2 years on all trees except girdled trees, where around half would have developed in 1 year. Differences in development rates may result from decreased defenses caused by the girdling.

The extremely high mortality (nearly 40%) on girdled green ash trees was a result of intraspecific competition and supports my hypothesis. Girdled green ash trees had an average of over 300 larvae per m^2 in both years. This is much higher than the average emergence density per m^2 in trees killed by *A. planipennis*, identified by McCullough and Siegert (2007) as 88.9 ± 4.6 adults emerged per m^2 of phloem over a range of size classes for both green and white ash trees. Although larval densities on the trees they evaluated

were much higher in some cases, competition for food led to mortality and thus prevented most of these larvae from developing and emerging (McCullough and Siegert (2007). The high larval mortality on girdled trees in this study lend further support to these conclusions.

Analyses of nutrient concentration, chlorophyll content, and photosynthesis and transpiration rates of foliage in 2008 and 2009 further revealed clear differences among treatments. Girdled trees were consistently different from other trees. They had the lowest photosynthesis rates, transpiration rates, chlorophyll levels, and the lowest levels of nitrogen, protein, and amino acids. Nitrogen is a limiting factor in ecological systems and an important source of energy for herbivores (Mattson 1980). However, foliar nitrogen values were only consistently below reported average summer values for ash trees (%) (Chen and Poland 2010) on girdled trees. Total non-structural carbohydrate levels were higher in girdled trees, but the lower ratio of protein to total non-structural carbohydrates is likely a more important factor in nutritional benefits from host material (Simpson and Raubenheimer 1993, Lee et al. 2002, Bede et al. 2007, Chen et al. 2009). These results together further emphasize that girdling is an effective stressor of host trees, and are consistent with initial hypotheses. In girdled tree phloem, the lower carbohydrate levels and higher protein:carbohydrate ratios below the girdle are consistent with previous research in that carbohydrate concentration accumulates above the girdle (Noel 1970, Roper and Williams 1989, Li et al. 2003, Mostafa and Saleh 2006, Chen and Poland 2009).

Photosynthesis rates varied greatly in 2008 due to interactions between the species, treatment, and aspect variables, but results consistently showed over both years

that girdled trees exhibited very low photosynthesis rates. Water loss via transpiration was also lowest on girdled trees, consistent with low photosynthesis rates as stomatal closure to reduce water loss will also decrease photosynthesis (Chaves et al. 2003). The additional fact that leaves on the south aspect of the canopy seem to display higher photosynthesis rates than those on the north aspect may be due to greater sun exposure on the south side of the plantation. Lower photosynthesis in girdled trees is a result of feedback inhibition (Foyer 1987), as accumulation of sucrose changes tree chemistry, causing a shortage of ATP and ADP available for photosynthesis. This is also consistent with the high carbohydrate concentration of girdled foliage.

Volatile production was also higher in girdled tree foliage and phloem than in trees of other treatments. This is consistent with previous evidence of effects of tree stress and volatiles on *A. planipennis* attraction to stressed trees (Rodriguez-Saona et al. 2006). The volatile compound previously described as an important component in some *A. planipennis* lures, α-cubebene, was highest in foliage of girdled green ash trees just as it was found highest in green ash. This likely has a large impact on the attraction of *A. planipennis* to girdled and green ash trees for oviposition. Other volatile compounds found in high quantities in girdled trees in this study which were proven to be antenally active to *A. planipennis* in previous research include α-farnesene in foliage and nonanal in phloem (Rodriguez-Saona et al. 2006).

In contrast to girdling, the results of fertilization were less dramatic. No consistent differences were seen between trees treated with fertilizer or fertilizer + Agri-Fos and untreated trees in any of the studies. This does not support my initial hypotheses. I expected that fertilized trees or trees treated with fertilizer + Agri-Fos would increase tree

vigor but no clear effects on chlorophyll, photosynthesis, or nutrient concentration were seen. No consistent differences in tree size between treatments were observed over the course of the study, although girdled trees seemed small in 2008 after girdling occurred. Also inconsistent with my hypotheses, these trees showed no indication of being less attractive to *A. planipennis* than untreated trees, nor was there consistently lower survival or slower development rates of larvae feeding on these trees. It is possible that trees may have already had ample nutrients for these first few years, being planted balled and burlap with nursery soil. In this case, any fertilizers applied may have had no effect if nitrogen was stored the leaves via luxury consumption rather than being applied to growth (Chapin et al.1980; Chabot and Hicks 1982).

Callous tissue, which was reportedly induced around lesions caused by the sudden oak death pathogen on other tree species (Garbelotto et al. 2007), was never observed around larval galleries on any trees, including those treated with Fertilizer + Agri-Fos, regardless of species. It is therefore likely that adding Agri-Fos to fertilization treatments has no additional effect on green and white ash tree vigor or resistance to *A. planipennis*. Phosphorous concentration was not examined in foliage so it is possible that not enough got into trees to see an effect. However, previous studies have shown trunk sprays to be effective in applications of Agri-Fos or insecticides (Garbelotto et al. 2007, McCullough et al. 2007, 2008). Blue ash trees have occasionally produced callous tissue around larval galleries (Anulewicz et al. 2007a). Therefore it is possible that a treatment of Agri-Fos may help enhance defenses in that particular species of *Fraxinus*, but that would require further research.

Although it is unlikely that fertilization or enhanced fertilization had any important effects on the ash trees in this study, one interesting point may still be of consideration. Green ash trees had higher larval densities than white ash trees across all treatments in 2008, but there were no differences between green and white ash larval densities for non-girdled treatments in 2009. This may be due to an increase in total beetle population in the area over the previous year. It is also possible that after three years of application of the fertilizer treatments, green ash trees may become less attractive to *A. planipennis*, and mirror the attraction of white ash trees, but support for this was not very conclusive. It is also possible that older trees may respond differently to fertilizer or Agri-Fos treatments than the young trees used in this study. Whereas fertilization did not seem to have much affect alone, it may perhaps be effective in conjunction with applications of insecticides such as imidacloprid or dinotefuron. These insecticides do not cause 100% larval mortality on their own (McCullough et al. 2007, 2008), but adding fertilizer may help to increase their effectiveness, either through a simple increase in tree vigor or to help increase root uptake of the insecticides, as has been shown in some studies (DePew 1971, Wilde et al. 1984, DePew and Hooker 1987).

Overall, results of this study indicate *A. planipennis* prefer green ash over white ash trees and girdled trees over ungirdled trees. Ungirdled green ash trees were colonized at higher rates than ungirdled white ash trees, despite the proximity of trees of both species to each other. Girdling white ash trees increased their attractiveness to *A. planipennis* to the point where they were more attractive than untreated green ash trees, and nearly as attractive as girdled green ash trees. Girdled trees could be useful as sinks to attract *A. planipennis,* and these trees could then be removed or destroyed before beetle

emergence, which could help to protect other trees from this pest. Understanding the effects various treatments have on different ash species can help homeowners, arborists, municipalities, and other resource managers weigh options when it comes to caring for their home or street trees. Further, understanding the mechanisms of host preference or resistance may assist in developing cultivars that are more resistant to *A. planipennis*, or enhancing survey methods for this pest.

Table 1.1: Mean (±SE) nutrient concentration of foliage from green ash and white ash trees in 2008. Significance values for nitrogen were determined nonparametrically. Protein and Protein: TNC ratio significance letters are based on log (x+1) transformed values. Values followed by different letters within a column by species or treatment are significantly different from each other (Kruskal-Wallis test, 2-way ANOVA, $p<0.05$). (N=38, 36, 38, 38, 31 respectively by column). (TNC = Total non-structural carbohydrate).

Species	Treatment	Total Nitrogen (ppm NH_4 N)	Protein (mg/g fresh weight ± SE)	Total Amino Acid (umol/g fresh weight ± SE)	TNC (mg/g fresh weight ± SE)	Protein:TNC (% by weight ± SE)
Green Ash	-	49.2 ± 2.7 a	17.7 ± 1.0 a	7.0 ± 1.3 a	3.8 ± 1.0 b	2.2 ± 1.5 a
White Ash	-	45.0 ± 2.7 a	18.6 ± 1.7 a	7.3 ± 1.5 a	7.2 ± 1.1 a	2.7 ± 0.5 a
-	Girdled	38.5 ± 3.9 b	13.5 ± 1.1 a	1.6 ± 1.1 b	10.4 ± 0.7 a	1.4 ± 0.2 b
-	Untreated	45.4 ± 4.5 ab	19.8 ± 2.8 a	9.2 ± 1.8 a	2.4 ± 0.9 b	9.5 ± 2.6 a
-	Fertilized	52.9 ± 1.2 a	19.5 ± 1.4 a	9.1 ± 2.1 a	5.0 ± 1.4 b	9.7 ± 3.3 a
-	Fert + AF	52.8 ± 2.6 a	19.8 ± 1.8 a	9.1 ± 1.4 a	4.0 ± 1.8 b	4.5 ± 1.1 ab

Table 1.2: Mean (±SE) nutrient concentration of foliage from green ash and white ash trees in 2009. Protein significance letters in August and protein: TNC ratio significance letters in both months are based on log (x+1) transformed values. Values followed by different letters within a column by species or treatment are significantly different from each other (2-way ANOVA, $p<0.05$). (N=39, 38, 38, 39 respectively by column for July, N=40, 40, 40, 37, 37 respectively by column for August). (TNC = Total non-structural carbohydrate.)

Month	Species	Treatment	Total Nitrogen (ppm NH_4 N)	Protein (mg/g fresh weight ± SE)	Total Amino Acid (umol/g fresh weight ± SE)	TNC (mg/g fresh weight ± SE)	Protein:TNC (% by weight ± SE)
Jul	Green Ash	-	-	11.1 ± 1.1 b	3.8 ± 1.0 a	6.2 ± 0.7 a	2.6 ± 0.6 a
Jul	White Ash	-	-	15.8 ± 1.6 a	3.4 ± 1.7 a	6.3 ± 0.7 a	3.1 ± 0.4 a
Jul							
Jul	-	Girdled	-	11.6 ± 3.7 a	1.5 ± 0.5 a	9.4 ± 3.0 a	1.5 ± 0.5 b
Jul	-	Untreated	-	13.6 ± 2.0 a	2.1 ± 1.4 a	5.0 ± 0.9 b	3.9 ± 1.3 ab
Jul	-	Fertilized	-	14.6 ± 2.9 a	7.1 ± 2.3 a	5.8 ± 0.8 b	2.9 ± 0.7 ab
Jul	-	Fert + AF	-	13.8 ± 1.2 a	3.4 ± 1.6 a	4.8 ± 0.4 b	3.2 ± 0.5 a
Aug	Green Ash	-	30.7 ± 1.4 a	9.9 ± 1.5 a	5.2 ± 0.6 a	7.6 ± 0.8 a	2.0 ± 0.4 a
Aug	White Ash	-	28.5 ± 1.5 a	10.9 ± 1.3 a	5.0 ± 0.7 a	7.9 ± 0.7 a	2.2 ± 0.2 a
Aug							
Aug	-	Girdled	23.9 ± 1.5 b	8.9 ± 1.1 a	4.6 ± 0.8 a	11.3 ± 1.1 a	1.1 ± 0.2 b
Aug	-	Untreated	29.2 ± 1.8 ab	9.6 ± 1.8 a	4.5 ± 0.7 a	6.3 ± 1.0 b	2.9 ± 0.8 a
Aug	-	Fertilized	32.6 ± 1.8 a	10.1 ± 1.7 a	5.6 ± 1.0 a	7.1 ± 0.8 b	2.8 ± 0.3 a
Aug	-	Fert + AF	32.8 ± 1.8 a	12.9 ± 2.7 a	5.6 ± 1.1 a	6.2 ± 0.5 b	2.3 ± 0.2 a

Table 1.3: Mean (±SE) nutrient concentration of phloem from green ash and white ash trees in 2009. Protein and amino acid significance values were determined nonparametrically. TNC and protein:TNC ratio significance letters are based on log (x+1) transformed values. Values followed by different letters within a column by species, treatment, or location are significantly different from each other (Friedman's 3-way nonparametric ANOVA, 3-way ANOVA, $p<0.05$). (N=79, 79, 74, 71 respectively by column). (TNC = Total non-structural

Species	Treatment	Location	Protein (mg/g fresh weight ± SE)	Total Amino Acid (umol/g fresh weight ± SE)	TNC (mg/g fresh weight ± SE)	Protein:TNC (% by weight ± SE)
Green Ash	-	-	13.1 ± 0.7 a	3.4 ± 0.3 a	-	-
White Ash	-	-	11.6 ± 0.8 a	4.2 ± 0.5 a	-	-
-	Girdled	-	12.9 ± 1.0 a	4.0 ± 0.8 a	-	-
-	Untreated	-	11.3 ± 1.2 a	3.7 ± 0.5 a	-	-
-	Fertilized	-	12.0 ± 0.7 a	3.8 ± 0.5 a	-	-
-	Fert + AF	-	13.2 ± 1.5 a	3.7 ± 0.4 a	-	-
-	-	> 1.3m	12.1 ± 0.8 a	4.1 ± 0.3 a	-	-
-	-	< 1m	12.7 ± 0.7 a	3.4 ± 0.4 a	-	-
-	Girdled	> 1.3m	-	-	5.6 ± 1.0 a	2.4 ± 0.6 b
-	Untreated	> 1.3m	-	-	4.6 ± 0.9 a	3.8 ± 1.1 b
-	Fertilized	> 1.3m	-	-	6.3 ± 1.8 a	3.7 ± 1.0 b
-	Fert + AF	> 1.3m	-	-	5.0 ± 1.1 a	8.1 ± 5.1 ab
-	Girdled	< 1m	-	-	1.0 ± 0.5 b	16.8 ± 5.7 a
-	Untreated	< 1m	-	-	4.8 ± 1.0 a	6.4 ± 3.2 b
-	Fertilized	< 1m	-	-	4.2 ± 0.9 a	5.6 ± 1.5 ab
-	Fert + AF	< 1m	-	-	3.9 ± 1.0 a	5.1 ± 1.1 ab
Green Ash	-	> 1.3m	-	-	5.2 ± 0.8 a	-
Green Ash	-	< 1m	-	-	4.1 ± 0.7 ab	-
White Ash	-	> 1.3m	-	-	5.6 ± 1.0 a	-
White Ash	-	< 1m	-	-	3.0 ± 0.7 b	-

Table 1.4 Mean (±SE) volatile compound production (ng/g dry leaf mass) from foliage of green ash and white ash trees in 2009. Significance letters are based on log (x+1) transformed values. Values followed by different letters within rows and dates are significantly different from each other (2-way ANOVA, $p<0.05$). (N=39, 39, 39, 27, 34, 33 respectively by compounds within dates).

	Species Differences					
	June		July		August	
Compound (ng/g)	Green Ash	White Ash	Green Ash	White Ash	Green Ash	White Ash
(Z)-3-Hexenyl acetate	29.60 ± 10.19 a	11.91 ± 3.55 b	-	-	-	-
trans-Ocimene	45.23 ± 13.62 a	14.86 ± 6.37 a	-	-	-	-
Tetradecane	18.19 ± 5.17 a	8.62 ± 2.08 b	-	-	-	-
cis-Ocimene	-	-	29.28 ± 5.72 a	24.91 ± 11.24 b	-	-
α-Cubebene	-	-	-	-	0.74 ± 0.20 a	0.27 ± 0.08 b

	Treatment Differences - June			
Compound (ng/g)	Girdled	Untreated	Fertilized	Fert + AF
trans-Ocimene	49.45 ± 16.53 a	16.51 ± 5.65 b	40.30 ± 26.39 ab	17.12 ± 6.68 ab

	Treatment Differences - August			
Compound (ng/g)	Girdled	Untreated	Fertilized	Fert + AF
α-Cubebene	1.14 ± 0.33 a	0.19 ± 0.06 b	0.27 ± 0.11 b	0.51 ± 0.22 ab
α-Farnesene	49.50 ± 35.51 a	2.00 ± 0.92 b	1.90 ± 0.65 b	8.45 ± 5.02 ab

Table 1.5 Mean (±SE) volatile compound production (ng) from bark and phloem of green ash and white ash trees in 2009. Significance letters for 4-methyl dodecane and borneol acetate in October are based on log (x+1) transformed values. Significance letters for tetracosane are based on inverse (1/x) transformed values. Values followed by different letters within rows and dates are significantly different from each other (2-way ANOVA, $p<0.05$). (N=17, 35, 38, 17, 41 respectively by row).

	Species Differences			
	August		October	
Compound (ng)	**Green Ash**	**White Ash**	**Green Ash**	**White Ash**
Borneol acetate	-	-	780.71 ± 665.84 b	9731.12 ± 7642.86 a

	Treatment Differences - August			
Compound (ng)	**Girdled**	**Untreated**	**Fertilized**	**Fert + AF**
Nonanal	472.61 ± 135.19 a	110.30 ± 35.14 b	131.86 ± 33.91 b	219.65 ± 167.62 ab

	Treatment Differences - October			
Compound (ng)	**Girdled**	**Untreated**	**Fertilized**	**Fert + AF**
4-Methyl-dodecane	232.01 ± 102.02 a	81.53 ± 25.59 ab	62.88 ± 22.97 b	68.68 ± 22.89 ab
Borneol acetate	22941.74 ± 15763.59 a	171.20 ± 73.89 b	88.45 ± 37.67 b	220.58 ± 98.65 b
Tetracosane	210.80 ± 41.08 a	88.53 ± 10.52 b	111.51 ± 16.15 ab	139.97 ± 44.26 ab

Table 1.6 Mean (±SE) (a) foliage area consumed (cm^2) per beetle per day, (b) weight of frass produced (mg) per beetle per day, (c) weight of foliage consumed (mg) per beetle per day, and (d) frass weight produced: foliage weight consumed ratio (mg/mg) in no-choice bioassays. Leaf area fed in round 2 significance letters are based on log (x+1) transformed values. Significance values for (c) and (d) were determined nonparametrically. Values followed by different letters within columns by species or treatment are significantly different from each other (3-way ANOVA, Kruskal-Wallis test, $p<0.05$). (N=80, 78, 72, 74, 80, 80, 80, 80, 72, 74, 80, 72 respectively by column alphabetically).

		a) Mean leaf area fed beetle^{-1} day^{-1} (cm^2) ± SE			b) Mean frass produced beetle^{-1} day^{-1} (mg) ± SE		
Species	**Treatment**	**Round 1**	**Round 2**	**Round 3**	**Round 1**	**Round 2**	**Round 3**
Green Ash	-	1.60 ± 0.12 b	0.72 ± 0.07 a	0.83 ± 0.06 b	5.7 ± 0.4 b	3.0 ± 0.3 b	3.2 ± 0.2 b
White Ash	-	2.08 ± 0.13 a	0.77 ± 0.06 a	1.17 ± 0.09 a	7.8 ± 0.5 a	3.9 ± 0.3 a	4.4 ± 0.3 a
-	Girdled	1.67 ± 0.21 a	0.77 ± 0.07 a	0.85 ± 0.08 a	6.4 ± 0.8 a	4.0 ± 0.4 a	3.2 ± 0.3 a
-	Untreated	1.94 ± 0.22 a	0.77 ± 0.11 a	1.20 ± 0.17 a	6.6 ± 0.7 a	3.1 ± 0.4 a	4.2 ± 0.4 a
-	Fertilized	1.71 ± 0.14 a	0.66 ± 0.08 a	1.09 ± 0.13 a	6.3 ± 0.5 a	3.4 ± 0.4 a	3.8 ± 0.4 a
-	Fert + AF	2.02 ± 0.16 a	0.79 ± 0.11 a	0.88 ± 0.06 a	7.4 ± 0.7 a	3.4 ± 0.3 a	3.9 ± 0.4 a

		c) Mean leaf weight fed beetle^{-1} day^{-1} (mg) ± SE			d) Mean ratio of frass weight produced: leaf weight fed beetle^{-1} day^{-1} (mg/mg) ± SE		
Species	**Treatment**	**Round 1**	**Round 2**	**Round 3**	**Round 1**	**Round 2**	**Round 3**
Green Ash	-	26.3 ± 2.0 a	9.0 ± 2.2 a	13.8 ± 1.0 a	0.21 ± 0.02 b	0.04 ± 0.18 b	0.25 ± 0.01 b
White Ash	-	24.1 ± 1.6 a	9.0 ± 0.7 a	13.5 ± 1.1 a	0.47 ± 0.12 a	0.42 ± 0.05 a	0.37 ± 0.03 a

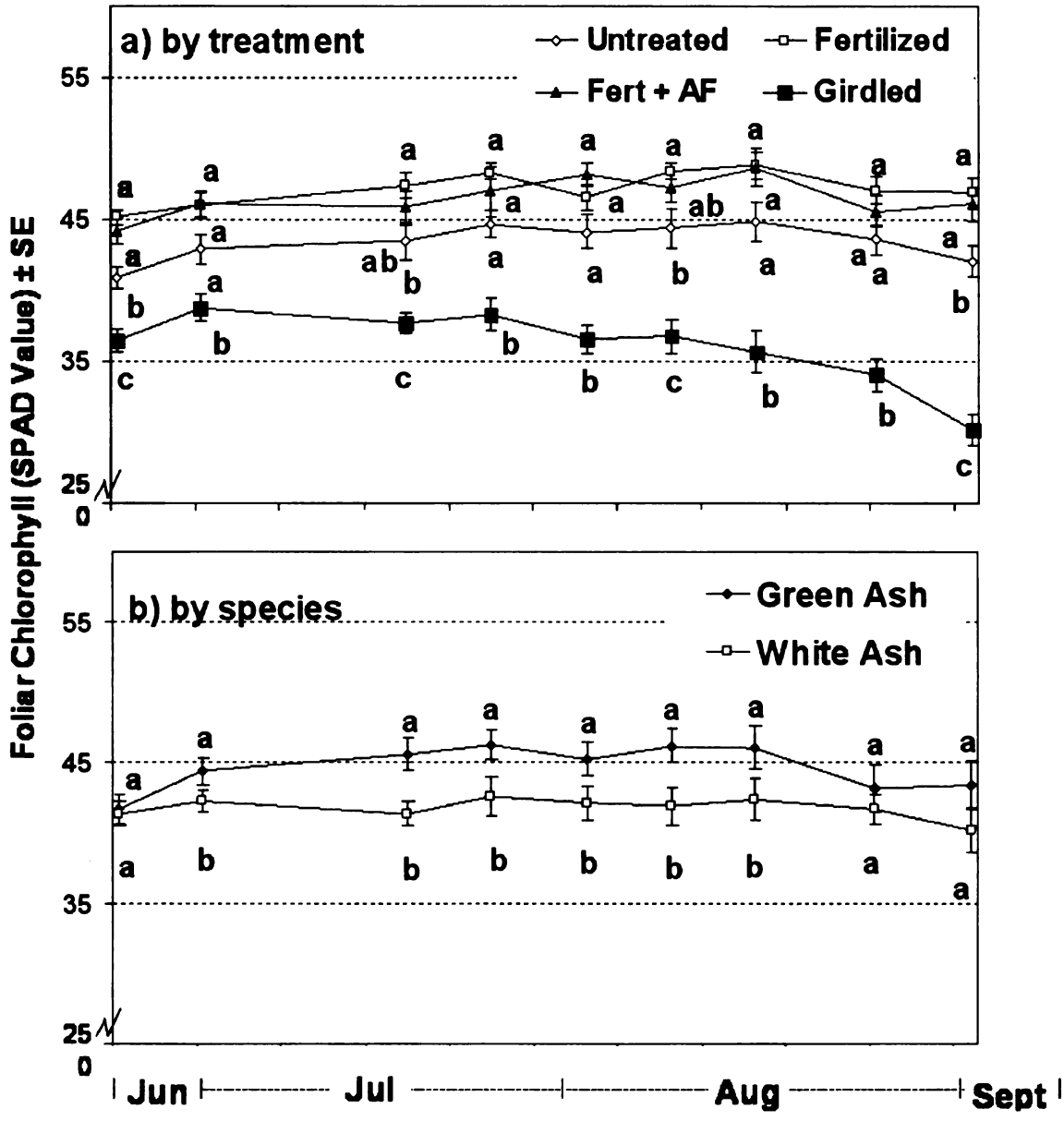

Figure 1.1: Mean (± SE) chlorophyll index in green and white ash foliage by (a) treatment and (b) species in 2008. Significance letters are based on log (x+1) transformed values. Points with different letters are significantly different from each other within each date (2-way ANOVA, $p<0.05$). Species p-values by date: 23 Jun $p=0.71$, 30 Jun $p=0.030$, 17 Jul $p<0.001$, 24 Jul $p=0.003$, 1 Aug $p=0.014$, 8 Aug $p<0.001$, 15 Aug $p=0.004$, 25 Aug $p=0.13$, 2 Sept $p=0.11$. (N=38 for Jun, Jul, Aug dates; N=36 for 2 Sept).

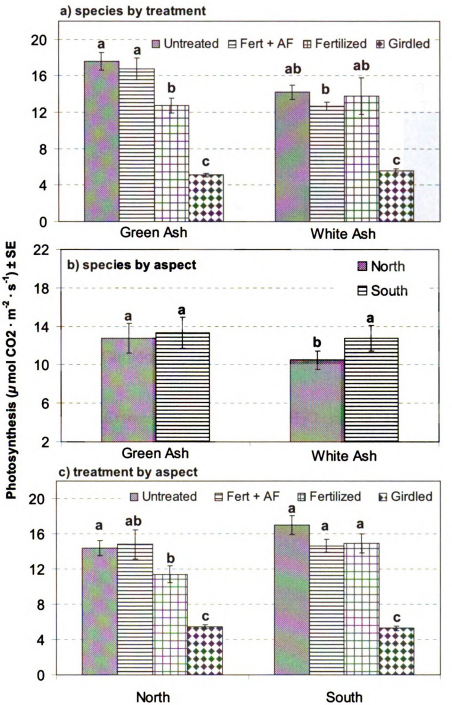

Figure 1.2: Mean (± SE) photosynthesis rates (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) for green and white ash trees by (a) species · treatment, (b) species · aspect, and (c) treatment · aspect in 2008. Significance letters are based on log (x) transformed values. Bars with different letters are significantly different from each other (3-way ANOVA, $p<0.05$). (N=48).

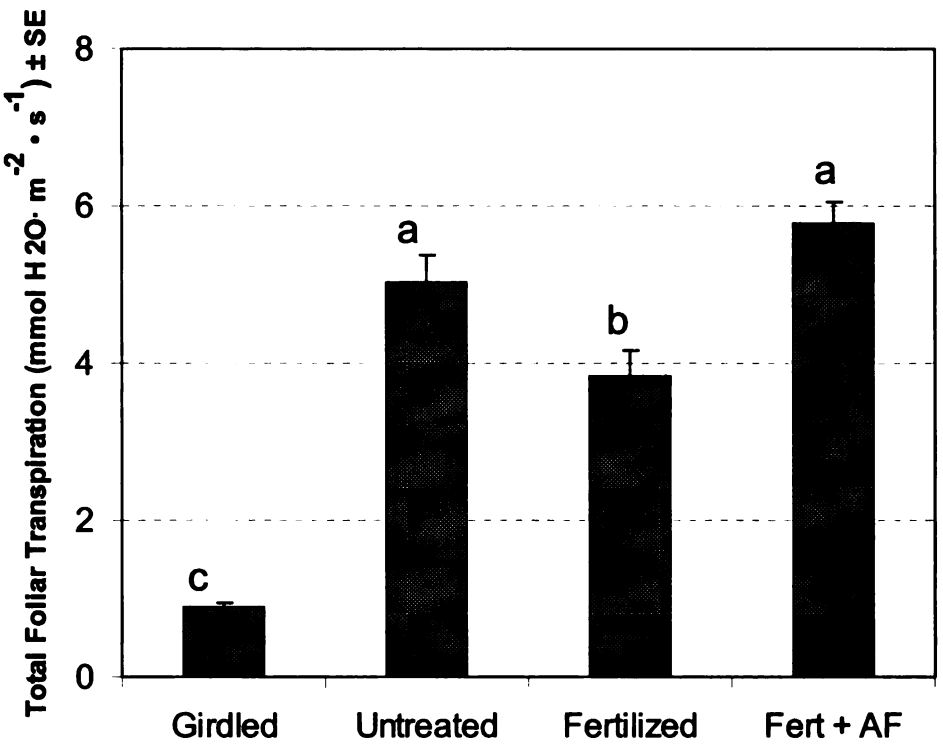

Figure 1.3: Mean (± SE) transpiration rates (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) by treatment in 2008. Bars with different letters are significantly different from each other (3-way ANOVA, $p<0.05$). (N=48).

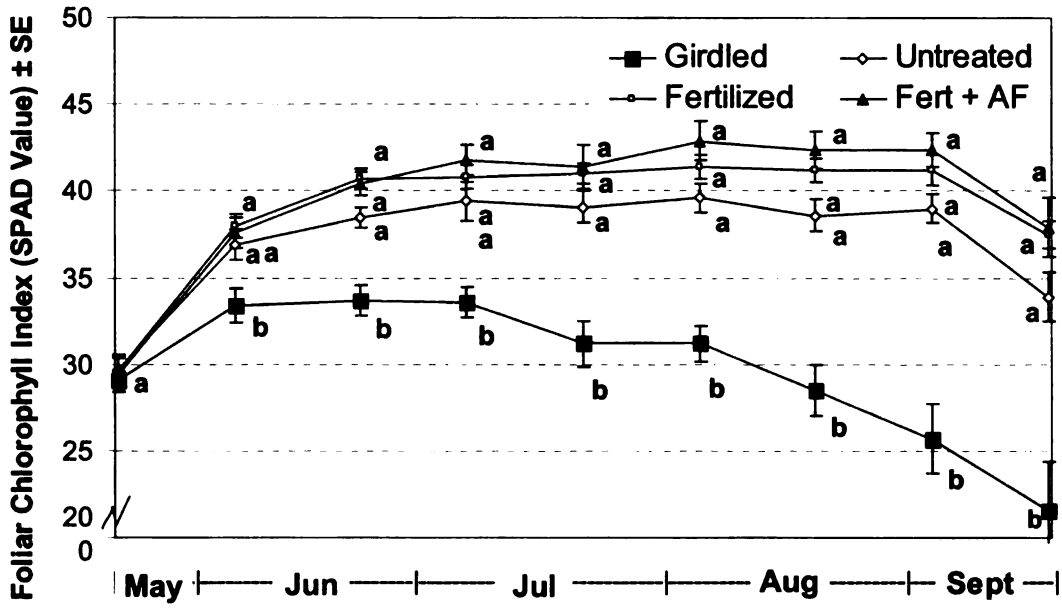

Figure 1.4: Mean (± SE) chlorophyll levels in green and white ash foliage by treatment in 2009. Significance letters are based on log (x+1) transformed values. Points with different letters are significantly different from each other within each date (2-way ANOVA, $p<0.05$). Species p-values by date: 26 May $p=0.031$, 9 Jun $p=0.82$, 24 Jun $p=0.004$, 7 Jul $p=0.97$, 21 Jul $p=0.31$, 4 Aug $p=0.76$, 18 Aug $p=0.78$, 1 Sept $p=0.97$, 15 Sept $p=0.15$. Treatment p-values by date: 26 May $p=0.93$, 9 Jun $p=0.004$, 24 Jun – 15 Sept $p<0.001$. (N=38, 40, 39, 40, 39, 39, 39, 39, 37 by date).

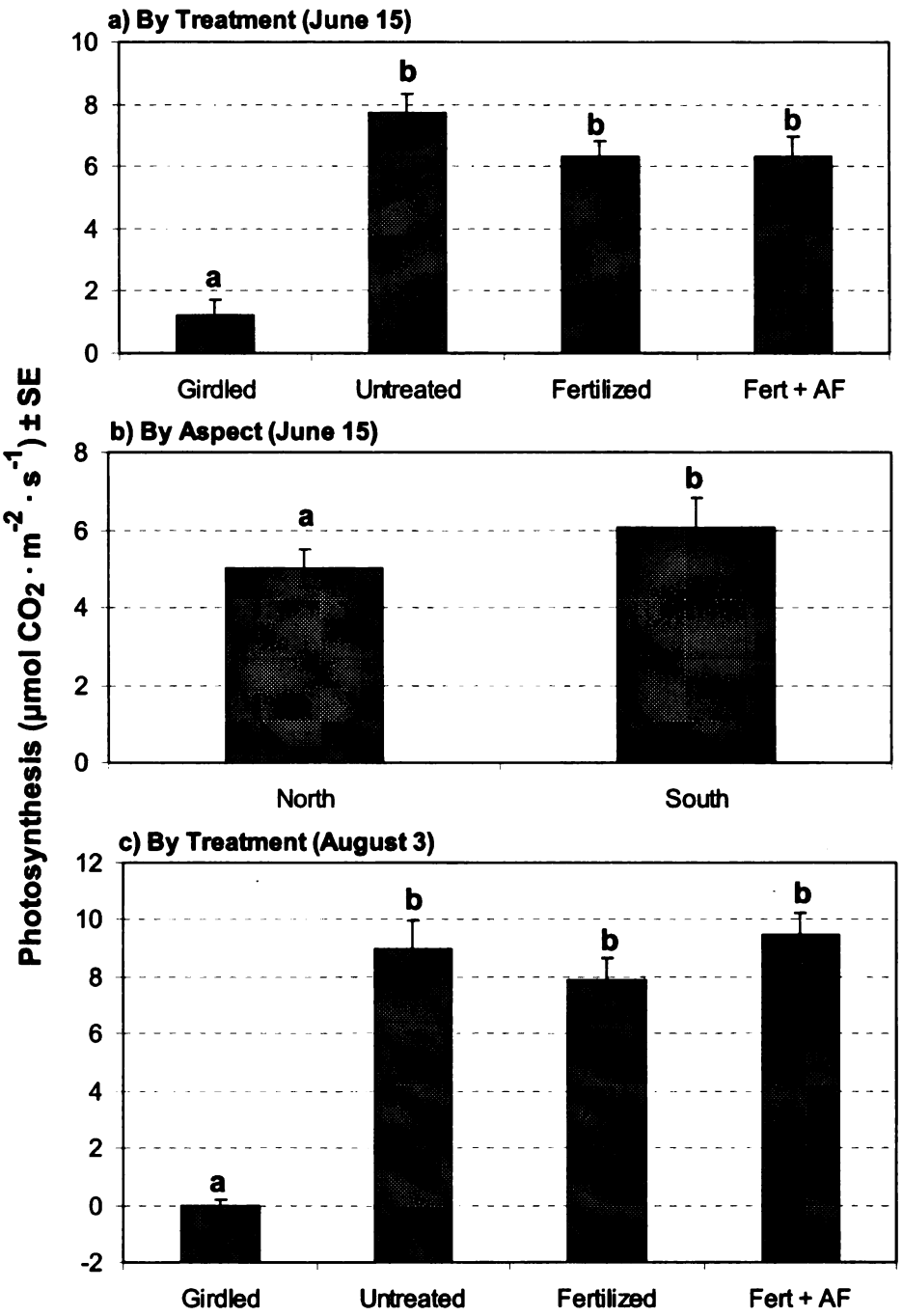

Figure 1.5: Mean (± SE) photosynthesis rates (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) for green and white ash trees by (a) treatment on June 15, (b) aspect on June 15, and (c) treatment on August 3 in 2009. Bars with different letters are significantly different from each other (3-way ANOVA, $p<0.05$). (N=47, 46 by date).

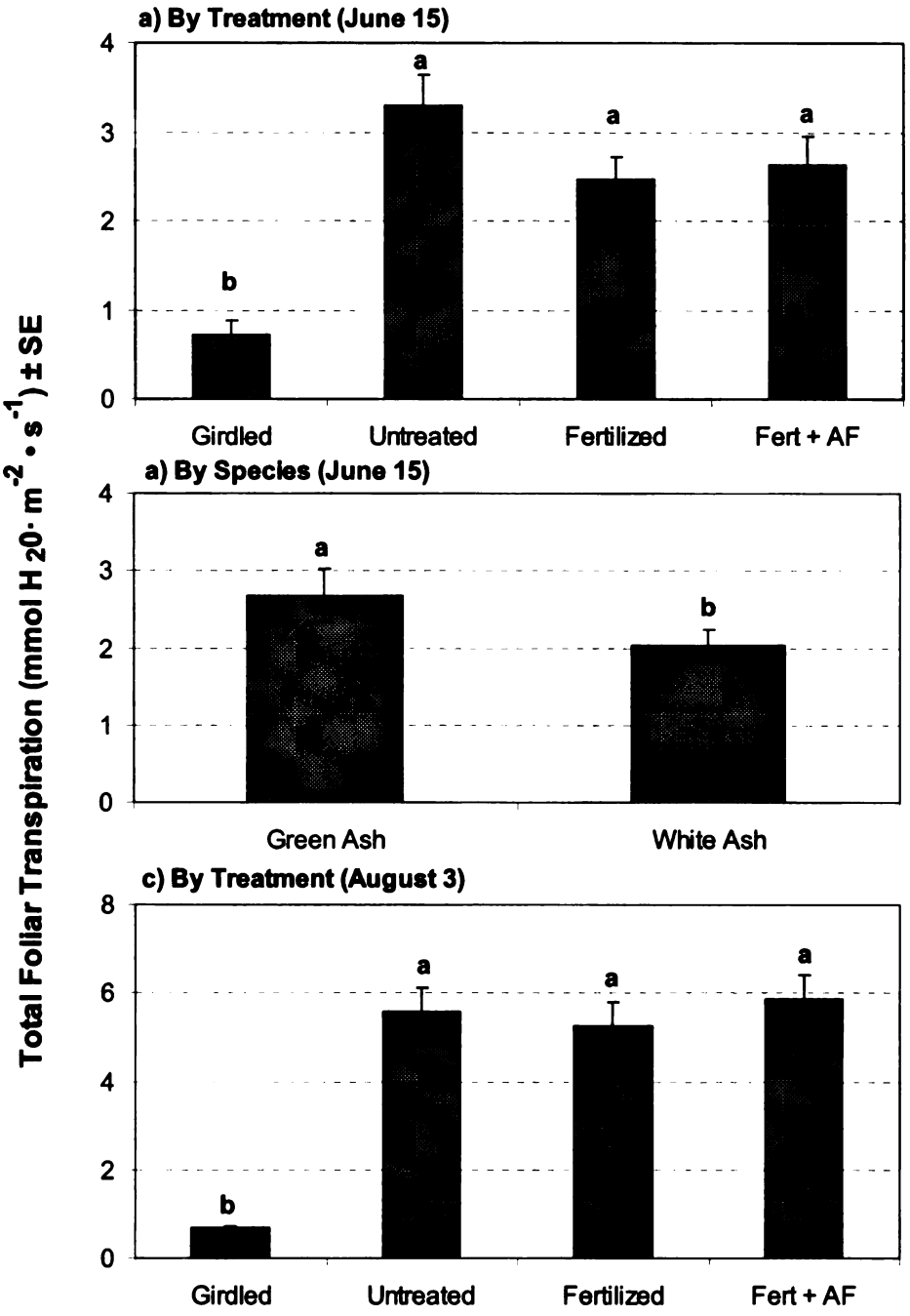

Figure 1.6: Mean (± SE) transpiration rates (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) for green and white ash trees by (a) treatment on June 15, (b) species on June 15, and (c) treatment on August 3 in 2009. Bars with different letters are significantly different from each other (3-way ANOVA, $p<0.05$). (N=47, 46 by date).

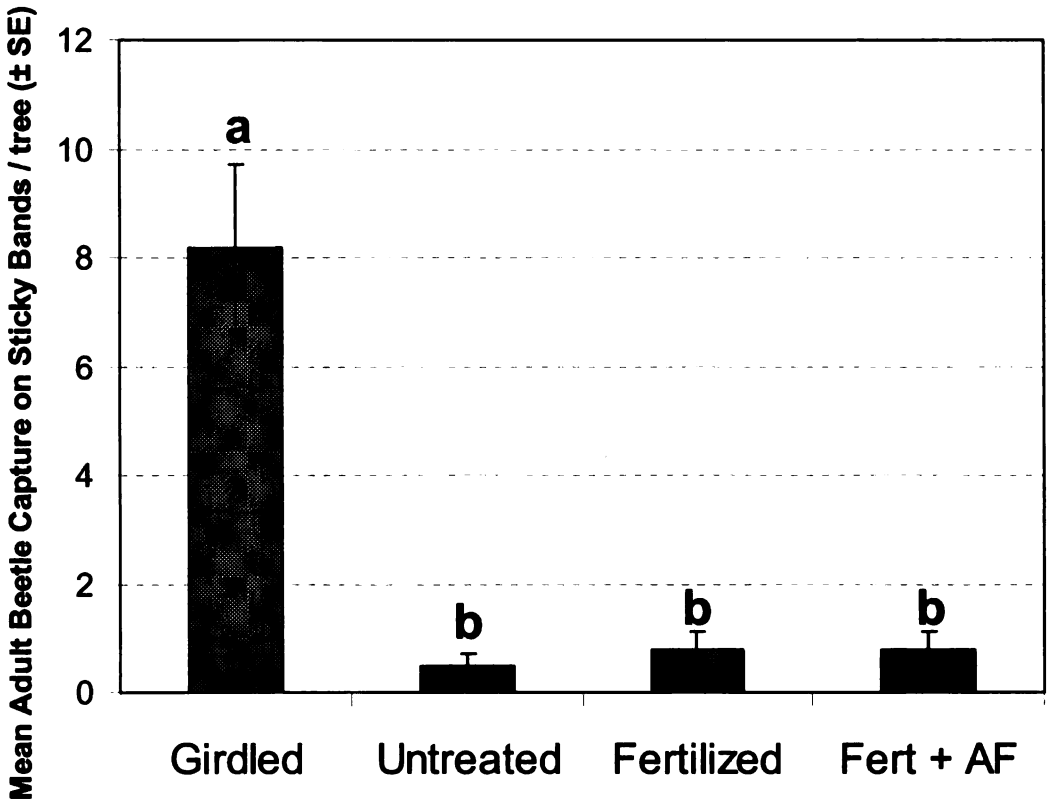

Figure 1.7: Mean (± SE) number of adult *A. planipennis* captured on sticky bands by treatment in 2009. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other (Kruskal-Wallis test, $p<0.05$). (N=40).

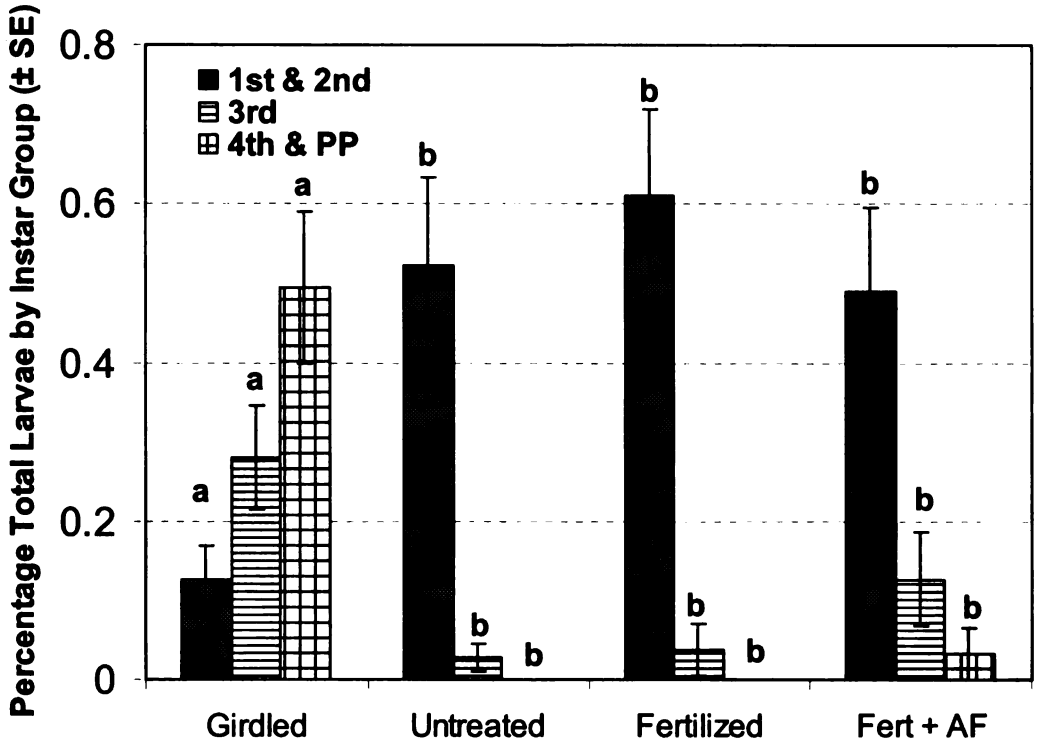

Figure 1.8: Mean (± SE) percentage total larvae grouped by instar (1st and 2nd instars, 3rd instars, 4th instars and prepupal larvae) which hatched from eggs laid by caged adults in 2009. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other within instar group (Kruskal-Wallis test, $p<0.05$). (N=80).

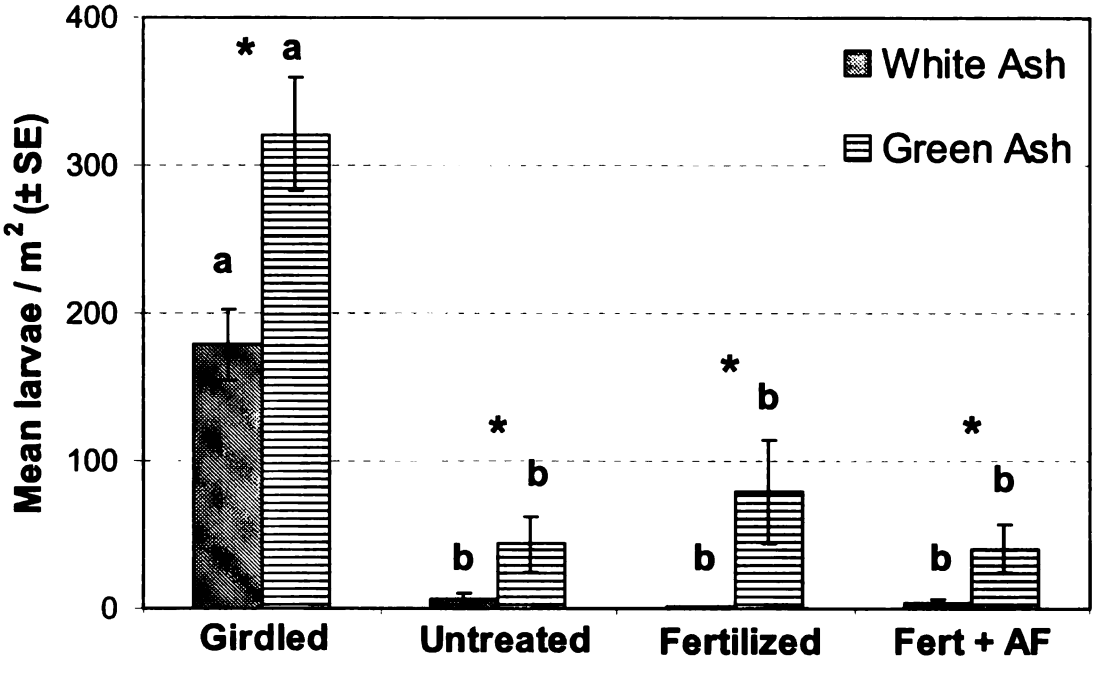

Figure 1.9: Mean (± SE) larval density (m^2) for species and treatment in 2008. Bars with different letters are significantly different from each other within treatment. Asterisks indicate a significant difference between species (2-way ANOVA, $p<0.05$) (N=38).

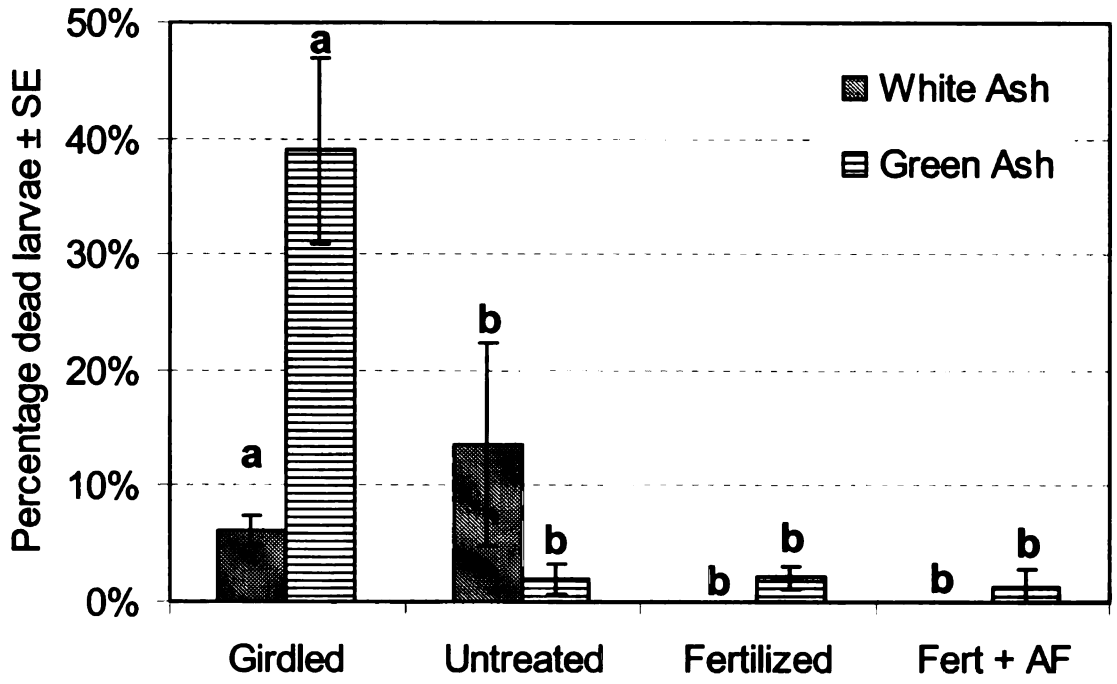

Figure 1.10: Mean (± SE) percentage of larvae that died for species and treatment in 2008. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other by treatment (Kruskal-Wallis test, $p<0.05$). (N=38).

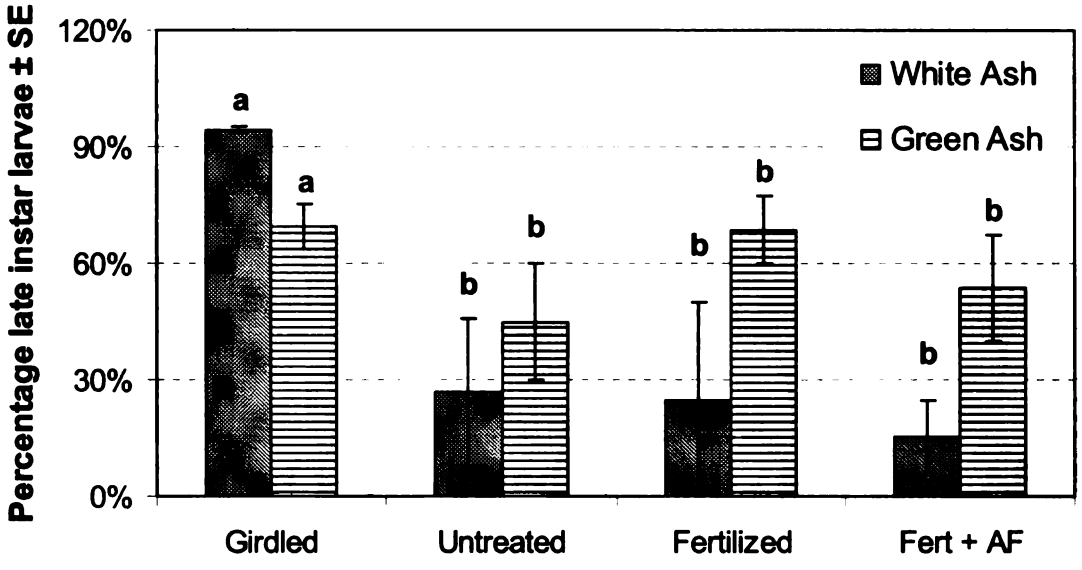

Figure 1.11: Mean (± SE) percentage late instar larvae (fourth instars – prepupal larvae) for species and treatment in 2008. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other by treatment (Kruskal-Wallis test, $p<0.05$). (N=38.)

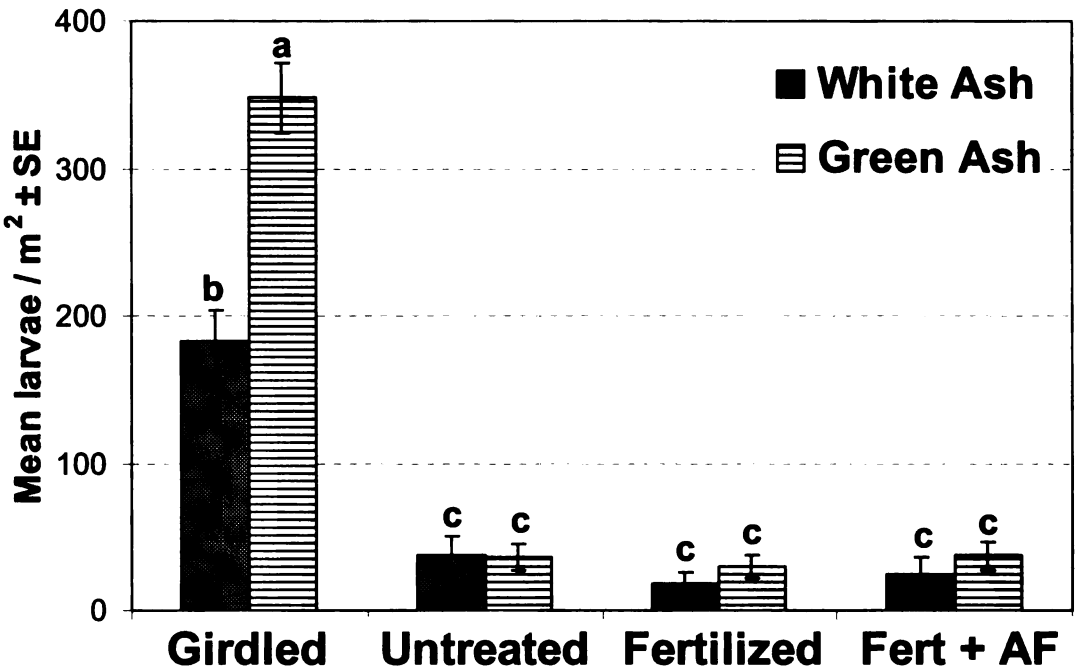

Figure 1.12: Mean (± SE) larval density (m^2) for species by treatment in 2009.

Significance letters are based on log (x+1) transformed values. Bars with different letters are significantly different from each other (2-way ANOVA, $p<0.05$). (N=40).

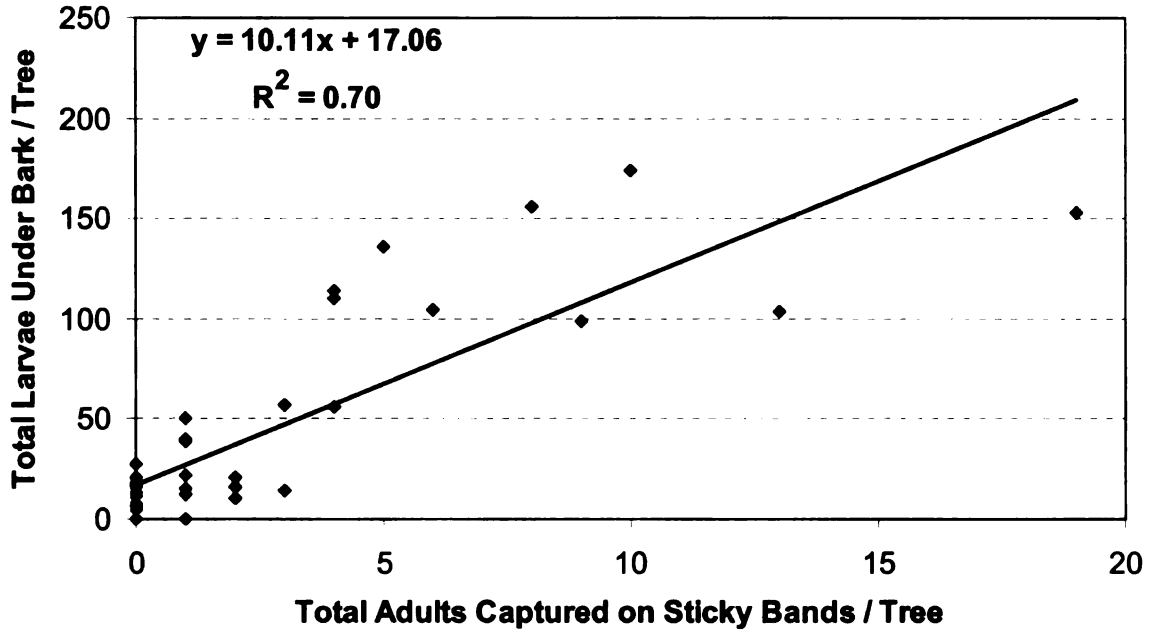

Figure 1.13: Regression of total wild larvae found per tree on total adults captured on sticky bands per tree in 2009 (Regression analysis, $p<0.05$). (N=40).

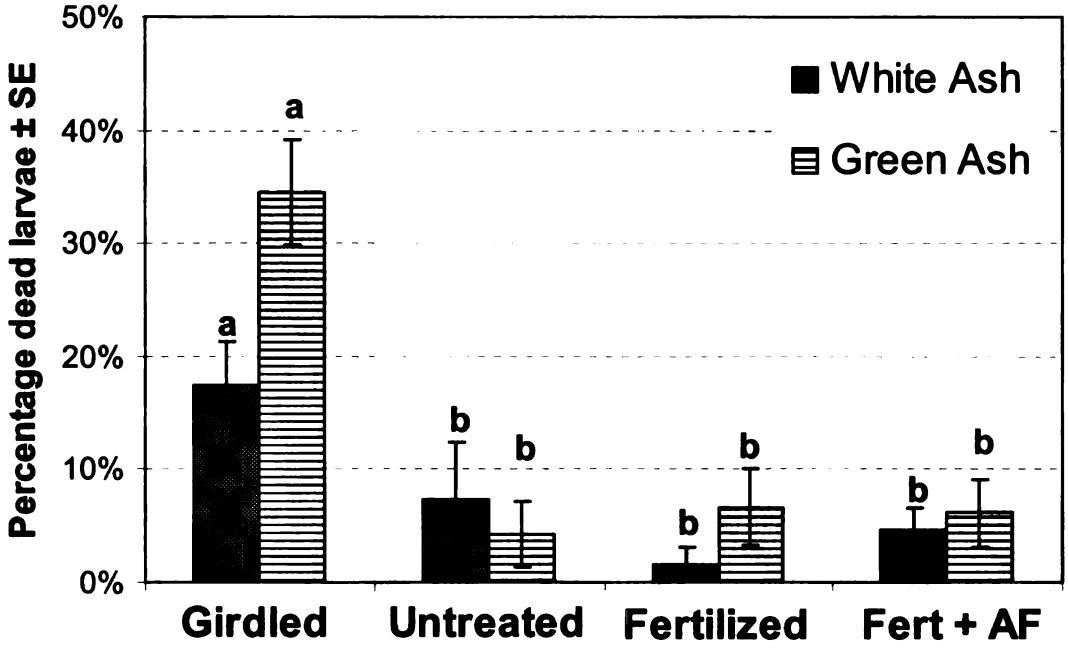

Figure 1.14: Mean (± SE) percentage of larvae that died for species and treatment in 2009. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other by treatment (Kruskal-Wallis test, $p<0.05$). (N=40).

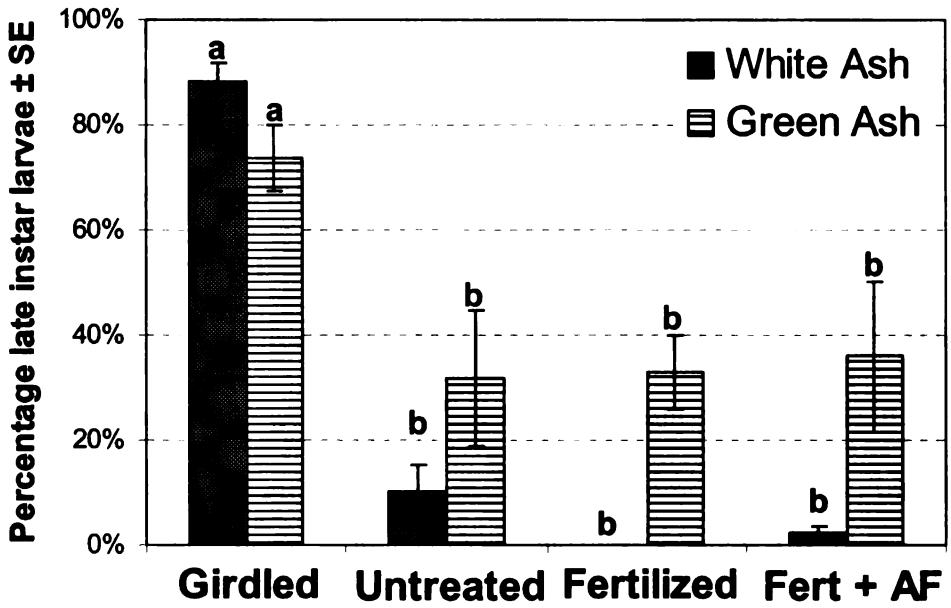

Figure 1.15: Mean (± SE) percentage late instar larvae (fourth instars – prepupal larvae) for species by treatment in 2009. Bars with different letters are significantly different from each other (2-way ANOVA, $p<0.05$). (N=40).

CHAPTER 2

Feeding Efficiency and Host Preference of Emerald Ash Borer (*Agrilus planipennis* Fairmaire) Adults on Stressed and Vigorous Green Ash Seedlings.

Introduction

The emerald ash borer (*Agrilus planipennis* Fairmaire), a devastating invasive pest of ash (*Fraxinus* spp.) trees in the United States and Canada, was first discovered in Michigan and Windsor, Ontario, Canada in 2002. It has since been found in 14 other states and Quebec (Poland and McCullough 2006, www.emeraldashborer.info 2010). More than 40 million ash *(Fraxinus* spp.) trees in Michigan have been killed to date (www.emeraldashborer.info 2010) and, if not controlled, the ash resource in the northeastern US may be largely eliminated.

Tree injury to *Fraxinus* spp. is caused by *A. planipennis* larvae feeding in the cambial region, creating galleries in the phloem and scoring the outer sapwood, which disrupts nutrient flow and water conduction, respectively (Cappaert et al. 2005b). Adult beetle emergence begins in late spring and continues through much of early summer. Adult female *A. planipennis* require 5-7 d of feeding before mating, and 5-7 d more before beginning oviposition (Bauer et al. 2004, Lyons et al. 2004). Beetles will continue to feed and oviposit during the remainder of their 3-6 wk lifespan (Bauer et al. 2004, Cappaert et al. 2005b). *A. planipennis* adults feed on ash leaves, but cause no significant defoliation (Cappaert et al. 2005b).

Like other *Agrilus* spp., *A. planipennis* prefer to oviposit on stressed trees (McCullough et al. 2009a). Native *Agrilus* spp. including the bronze birch borer, *Agrilus anxius* Gory, and the two-lined chestnut borer, *Agrilus bilineatus* (Weber), are secondary

pests that feed on stressed and dying trees (Anderson 1944, Haack and Benjamin 1982, Dunn et al. 1986). Likewise, *A. planipennis* is a secondary pest throughout its native range, presumably because it shares an evolutionary history with its host trees, which have greater defenses against it than do North American ash species (Yu 1992; Akiyama and Ohmomo 2000; Gould et al. 2005; Herms et al. 2005; Schaefer 2005; Williams et al. 2005, 2006, Eyles et al. 2007). Although healthy North American ash trees can succumb to high densities of *A. planipennis*, adult beetles display a stronger attraction to trees stressed by girdling, and this attraction has even affected dispersal habits as beetles are more likely to fly to areas occupied by girdled trees (McCullough et al. 2009a, Mercader et al. 2009, Siegert et al. 2010). In previous studies, girdled trees had higher larval densities than healthy trees or trees stressed by herbicide, wounding, or exposure to the stress elicitor methyl jasmonate (McCullough et al. 2009a, 2009b; Tluczek 2009).

Although adult foliage feeding causes little damage to the trees, examining these feeding habits could provide insight to the basic biology of *A. planipennis* and the relationship between host selection for feeding and oviposition. Historically, *Fraxinus* spp. has been free from major damage caused by defoliating pests (Solomon et al. 1993). In fact, one of the most devastating invasive generalist defoliators in the northeastern USA, the gypsy moth (*Lymantria dispar* L.), does not use ash as a host tree likely due to chemical deterrents in the foliage (Markovic et al. 1997). Little research to date has focused on adult *A. planipennis* host selection for foliage feeding. Adult beetles may be more likely to oviposit on trees where they choose to feed. One study suggested that beetles preferentially fed on clipped green, white, and black ash leaves over blue, European (*Fraxinus excelsior* L.), and Manchurian (*Fraxinus mandshurica* Rupr.) ash

leaves, possibly due to differences in volatiles produced by these species (Pureswaran and Poland 2009a). However, volatile production may differ between clipped and intact ash leaves. A similar study reported that lower feeding on Manchurian ash may reflect higher nutritional quality or stronger defenses in foliage from those trees. In comparison, greater feeding on green ash foliage may be compensatory (Pureswaran and Poland 2009b). Other phytophagous insects benefit from feeding on plants with high levels of nutrients, particularly nitrogen, amino acids, and protein: carbohydrate ratios (Mattson 1980, Doi et al. 1981, Kytö 1996, Fisher et al. 2001, Bi et al. 2003, Chen et al. 2009, Chen and Poland 2009). High chlorophyll content or photosynthesis rates are functions of high nitrogen levels, and may also contribute to increased foliar nutrition and better beetle success on these trees. Leaves with lower nutritional quality may require more compensatory feeding by adults, while leaves with higher nutrition (e.g. foliage from fertilized trees) may be consumed in smaller quantities (Mattson 1980, Scriber and Slansky 1981, Chen et al. 2009, Chen and Poland 2009, Pureswaran and Poland 2009b).

Other studies suggest that phytophagous insects may feed more on fertilized plants due to a preference for hosts with more nutrients (Kytö 1996, Glynn et al. 2003). The Growth/Differentiation Balance Hypothesis suggests that under certain conditions, allocation of nutrients to foliage for growth in fertilized trees may reduce the energy available for use as secondary defense metabolites. This would cause foliage with more nutrients to be less resistant to insect feeding damage (Loomis 1932, Lorio 1986, Herms and Mattson 1992). However, this hypothesis has had mixed support in studies (Kytö 1996, Glynn et al. 2003). Similarly, feeding on leaves with lower nutrition may prove less

efficient and result in increased frass production from undigested leaf material (Mattson 1980, Scriber and Slansky 1981, Chen and Poland 2009, Pureswaran and Poland 2009b).

Chen and Poland (2009) compared foliar nutrients on green ash seedlings. Variables considered included age of leaves, leaves grown in sun vs. shade, and girdled vs. ungirdled seedlings. The study revealed an increase in non-structural carbohydrates in girdled seedlings but a decrease in protein:carbohydrate ratios. This is consistent with the observation that girdling causes an accumulation of carbohydrates above the girdle while the trunk below the girdle receives none (Noel 1970, Roper and Williams 1989, Li et al. 2003, Mostafa and Saleh 2006, Chen and Poland 2009). Results of Chen and Poland's (2009) study suggest that younger leaves may contain more nitrogen and other nutrients than older leaves, as these nutrients are necessary for growth and expansion (Mattson 1980, Harper 1989, Chen et al. 2009, Chen and Poland 2009). Chen and Poland (2009) did not report that any of the factors they studied had an effect on beetle survival, and they did not test for differences in amount of leaf material consumed.

I examined the effects of girdling and fertilization on green ash seedlings and on *A. planipennis* foliage feeding behavior on these seedlings. I hypothesized that (1) *A. planipennis* would be attracted to girdled seedlings and would spend more time feeding on foliage from these seedlings when given a choice; (2) girdled seedlings would have lower chlorophyll, photosynthesis, and nutrition than untreated seedlings; (3) fertilized seedlings would have higher foliar chlorophyll, photosynthesis, and nutrition than untreated seedlings; and (4) *A. planipennis* would require extra feeding on girdled foliage to compensate for its lower nutritional value. Study objectives to test these hypotheses were to (1) assess effects of girdling and fertilization on the foliar chlorophyll,

photosynthesis rates, and nutrient concentration of leaves of green ash seedlings; and (2) evaluate adult *A. planipennis* feeding behavior on the green ash seedlings in choice and no-choice bioassays.

Materials and Methods

Seedling Establishment

Green ash seedlings (45-61 cm) were acquired on 30 January 2009 from Lawyer Nursery in Plains, MT. Seedlings were potted in 1-gallon containers with Fafard Heavyweight Mix #52, consisting of processed pine bark, peat moss, vermiculite, and perlite (Conrad Fafard, Inc; Agawam, MA, USA). The mix contained a water-soluble nutrient starter charge which was leached out by the time of treatment applications, as verified by lower readings on an electric conductivity meter in comparison to newly fertilized seedlings. Seedlings were stored in a polyhouse at Michigan State University's Tree Research Center in East Lansing, MI. Conditions in the polyhouse were approximately 15.5-21°C and 50-70% RH. Seedlings were watered twice weekly and grown for eight weeks before treatments began. Seedlings were randomly assigned to two groups comprised of 48 and 36 seedlings each. The 48 seedlings in Group 1 were used for no-choice bioassays and were maintained in the polyhouse throughout the study. The 36 seedlings in Group 2 were used for the choice assay and were moved on 15 May 2009 to an outdoor plot. These seedlings were planted pot in pot and provided with drip irrigation.

Treatment Applications

Group 1 seedlings were randomly assigned to one of three treatments: girdling, fertilization, and untreated control (16 seedlings per treatment). Seedlings assigned to the fertilization treatment were fertilized weekly beginning on 31 March 2009 via a liquid feed with 200 ppm nitrogen, 60 ppm phosphorous, 150 ppm potassium, and pH 6.5 (Peters 20-10-20 Peat Lite Special, Scotts, Marysville, OH, USA). On girdled seedlings, a pocket knife was used to remove a 5 cm length of outer bark and phloem on the main stem below branches on 31 March 2009. Untreated seedlings were irrigated throughout the study, but received no nutrient supplementation.

Group 2 seedlings were randomly assigned to one of three treatments: girdling, fertilization, and untreated control (12 seedlings per treatment). Seedlings assigned to the fertilization treatment were fertilized weekly beginning on 28 April 2009 via the same liquid feed applied to Group 1 seedlings. This liquid fertilizer was no longer used after seedlings were moved outdoors. A one-time granular application of Harrell's Pro-Blend with Micronutrients custom-mixed 19-4-8 controlled-release fertilizer (Harrell's, Inc., Sylacauga, AL, USA) was applied on 22 May 2009 to the pot around the base of each seedling at a rate of 5.6 g N per seedling (approximately 599 kg per ha). Seedlings were girdled on 5 May 2009 as described above. Untreated seedlings were irrigated throughout the study, but received no nutrient supplementation.

Foliar Nutrients and Photosynthesis

Chlorophyll content of Group 1 seedling foliage was analyzed weekly from 7 April to 12 May 2009 using the Minolta SPAD 502 Chlorophyll Meter (Spectrum Technologies, Inc., Plainifield, IL, USA). Four readings were taken per seedling; two from leaves on opposite sides of the lower portion of the seedling and two from leaves on opposite sides of the upper portion of the seedlings. These readings were averaged to obtain a mean value for each seedling. The Li-Cor LI-6400 (Li-Cor Biosciences, Lincoln, NE, USA) portable photosynthesis system was used to measure photosynthesis and transpiration rates on an upper and lower leaf from ten randomly selected Group 1 seedlings per treatment on 9, 16, and 30 April 2009. Photosynthesis rates were measured via foliage gas exchange rates (A_{max}) (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). Transpiration rates were measured as mmoles $H_2O \cdot m^{-2} \cdot s^{-1}$. Seedlings were analyzed with the Li-Cor in the polyhouse in mid-afternoon in sunny conditions. Quantum flux was set at 1500 μmols/m^2/s for use as a light source and machine temperature was set at that of the average daily temperature in °C. Flow was set to 500 μms and the mixer set at 400 μms CO_2R.

One leaf from each of ten randomly selected Group 1 seedlings per treatment was removed on 1 May 2009, flash frozen in liquid nitrogen, and stored at -20 °C until processing. If leaves were so small that one leaf would not provide enough leaf tissue for processing, two opposite leaves of the same age were selected. Leaf tissue was finely ground in liquid nitrogen and approximately 50 mg was extracted for analysis. Protein (mg/g fresh weight) was determined via Bradford protein assay and amino acid

concentration (μmol/g fresh weight) was determined colorimetrically via cadmium-ninhydrin procedure (Doi et al. 1981, Fisher et al. 2001, Bi et al. 2003, Chen et al. 2009). Total non-structural carbohydrates (mg/g fresh weight), calculated as the sum of glucose and starch, were determined using the glucose (HK) assay kit (Sigma-Aldrich, St. Louis, MO, USA) and methods from Jones (1979). Starch was estimated as glucose equivalents (Marquis et al. 1997, Chen et al. 2009).

On 19 May 2009, an upper and lower leaf on each of six, randomly selected no-choice seedlings per treatment were removed for total nitrogen determination. If leaves were small, two opposite leaves of the same age were selected to obtain ≥1 g leaf material after oven drying. Leaves were oven dried at 65.5°C for 72 hr in a model 30 GC lab oven (Quincy Lab, Inc., Chicago, IL, USA). Samples were sent for total nitrogen analysis via micro-Kjeldahl digestion procedure at Michigan State University's Soil and Plant Nutrient Laboratory.

Foliar chlorophyll content of Group 2 seedlings was analyzed weekly from 28 April to 28 July 2009 using the Minolta SPAD 502 Chlorophyll Meter (Spectrum Technologies, Inc., Plainifield, IL, USA) and methods described above. The Li-Cor LI-6400 (Li-Cor Biosciences, Lincoln, NE, USA) portable photosynthesis system was used to measure photosynthesis and transporation rates on an upper and lower leaf of ten and five randomly selected Group 2 seedlings per treatment on 21 May 2009 and 16 June 2009 respectively. Number of seedlings was reduced on the second date due to many seedlings having lost their leaves by that time. Photosynthesis rates were measured via foliage gas exchange rates (A_{max}) (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) with a fluorescent leaf chamber. Transpiration rates were measured as mmoles $H_2O \cdot m^{-2} \cdot s^{-1}$. Group 2 seedlings were

analyzed with the Li-Cor outdoors in mid-afternoon in sunny conditions via the same methods as Group 1 seedlings.

No-Choice Bioassays

I conducted four no-choice bioassays with adult *A. planipennis* beetles on Group 1 seedling foliage. Bioassays began on 12 April, 20 April, 4 May, and 7 May 2009. Beetles were reared from logs which were harvested in fall 2008 from naturally infested trees near Lansing, MI and maintained in cold storage at 3.9°C. After removing logs from cold storage, they were placed in 76.2 cm long, 15.2-30.5 cm diam cardboard tubes (Saginaw Tube Co., Saginaw, MI, USA) in a rearing room maintained at 26.7° C. Adult beetle emergence from logs followed in approximately 21 days. Emerging adults were collected daily and immediately transferred to bioassay material. Four bioassays were conducted to evaluate possible changes as the seedlings aged and continued to be affected by the fertilization and girdling treatments. Two leaves opposite each other on the same whorl of each seedling were collected and scanned in a flatbed scanner to determine leaf area using WinFOLIA software (Regent Instruments, Inc.; Quebec, Qc, Canada). After scanning, the petiole of each leaf was cut on a slant to provide a fresh surface area for water uptake. Leaves were inserted into water-filled microcentrifuge tubes to maintain moisture and placed individually in 150 mm diam Petri dishes. Two newly-emerged male *A. planipennis* were placed in a dish with one of the leaves from each seedling, and two newly-emerged female beetles were placed in a separate dish with the second leaf. Beetles were allowed to feed for three days. Petri dishes were checked daily for beetle mortality. After feeding, leaves were re-scanned and total leaf area consumed was

determined by comparing original leaf area to the remaining area. Leaf area consumed was divided by total "beetle days" (sum of the number of days, to 0.5 d, that each beetle survived) to obtain a value for total leaf area consumed per beetle per day. Frass was collected from each dish and weighed to the nearest mg to estimate feeding efficiency on the leaves.

Choice Assay

A choice assay was conducted using the 36 outdoor seedlings from 4 June through 13 August 2009. Seedlings used in the choice assay were exposed to feeding by the wild *A. planipennis* population at the Tree Research Center. Each week, total leaves were counted per seedling. Any live adult beetles were noted and, whenever possible, sexed. Feeding on each leaf of a given seedling was visually examined and recorded on a 1 (very little feeding) to 5 (extensive feeding) scale. Ratings for each leaf were summed to obtain a feeding estimate per seedling for each date. These values were compared by treatment. The cumulative number of leaves assigned to each feeding rank was recorded weekly. At the end of the study period, the proportion of total leaves per seedling assigned to each feeding rank was recorded and the means were compared by treatment across all dates.

Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots (PROC GLIMMIX, SAS Institute 2001). All parametric statistics were run using SAS 9.1 software (PROC GLIMMIX, SAS Institute 2001). One-

way or two-way analysis of variance (ANOVA) were applied to data that followed a normal distribution (SAS Institute 2001). If ANOVA results were significant ($p<0.05$), Tukey's honestly significant difference (HSD) multiple comparison test was used to assess differences among treatments (Tukey 1953, SAS Institute 2001).

Percentage foliar nitrogen was tested using two-way ANOVA to assess effects of tree treatment and leaf location. Remaining foliar nutrient concentrations, chlorophyll content in both seedling groups, and mean feeding ranks in Group 2 seedlings were tested using one-way ANOVA to assess the effects of seedling treatment. Photosynthesis rates in both groups and transpiration rates in Group 1 and in Group 2 on 21 May were tested using two-way ANOVA to assess effects of seedling treatment and leaf location. Total frass consumed per beetle per day was tested using two-way ANOVA to assess effects of tree treatment and beetle sex.

Protein concentration and Group 2 seedling photosynthesis rates on 16 June were normalized by log (x+1) transformation. Protein: carbohydrate ratios, Group 1 seedling photosynthesis rates on 9 and 16 April, and mean feeding ranks in Group 2 seedlings on 25 June, 2 July, 9 July, and 16 July were normalized by log (x) transformation. Group 1 seedling photosynthesis rates on 30 April and mean frass produced per beetle per day in no-choice bioassays were normalized by square root (x) transformation.

If transformations did not normalize variables, nonparametric tests were used. Friedman's two-way nonparametric ANOVA (SAS Institute 2001) was used to test non-normal data involving potential interactions. When significant, nonparametric multiple comparison tests were applied following methods from Conover (1971) and Zar (1984). If the interaction term in Friedman's test was $p\geq 0.50$ or no potential interactions existed,

Wilcoxon Rank Sum tests (Ott and Longnecker 2001) and Kruskal-Wallis tests (Kruskal and Wallis 1952) were used. When significant, multiple comparison tests were applied following the same methods (Conover 1971, Zar 1984). Friedman's two-way nonparametric ANOVA was used to test Group 2 seedling transpiration rates on 16 June and mean leaf area consumed per beetle per day in no-choice bioassays. Kruskal-Wallis tests were used to analyze percentage leaves consumed at each feeding rank on Group 2 seedlings. All analyses were conducted at the $p<0.05$ level of significance.

Results

Foliar Nutrients and Photosynthesis – Group 1

Total percentage foliar nitrogen was higher in fertilized seedlings than in untreated seedlings and over two times higher than in girdled seedlings (F=29.70; df = 2, 28; $p<0.001$) (Table 2.1). No significant differences in nitrogen between upper and lower leaves were observed (p=0.82). Protein concentration of foliage from fertilized seedlings was approximately half that of girdled or untreated seedlings (F=5.27; df=2, 27; p=0.012) (Table 2.1). Total amino acid concentration was nearly twice as high in foliage from fertilized seedlings when compared to foliage from untreated seedlings (F=3.77, df=2, 27, p=0.036) (Table 2.1). Total foliar non-structural carbohydrates were higher in foliage from girdled seedings than from fertilized seedlings (F=24.11, df=2, 27, $p<0.001$) (Table 2.1). There were no significant differences in protein:carbohydrate ratios among treatments (p=0.72) (Table 2.1).

Chlorophyll content of Group 1 seedlings fluctuated over the course of the study. Fertilized and untreated seedlings had consistently higher chlorophyll levels than girdled

seedlings by mid-May, approximately one month after treatments began (Fig. 2.1). By the end of May the average chlorophyll content of fertilized seedlings had peaked and the chlorophyll content of girdled seedlings was at its lowest (Fig. 2.1). Photosynthesis rates were consistently lower on girdled seedlings than on seedlings of other treatments, which were at least twice as photosynthetically active (9 April: F=64.00; df=2,47; p<0.001; 16 April: F=74.06; df=2,52; p<0.001; 30 April: F=73.79; df=2,51; p<0.001) (Fig 2.2a). On 16 April, photosynthesis rates on fertilized seedlings were also higher than those on untreated seedlings, but this was not the case on 9 or 30 April (Fig 2.2a). Lower, older leaves were more photosynthetically active than upper leaves on 9 April (F=4.07; df=1,47; p=0.049), 16 April (F=11.21; df=1,51; p=0.002), and 30 April (F=5.28; df=1,51; p=0.026) (Fig 2.2b). Transpiration rates on girdled seedlings were also at least half that of transpiration rates on other seedlings (9 April: F=8.97; df=2,45; p<0.001, 16 April: F=59.34; df=2,51; p<0.001, 30 April: F=95.16; df=2,51; p<0.001) (Fig. 2.3). There were no transpiration differences between upper and lower leaves on 9 April (p=0.47) or 16 April (p=0.06). On 30 April transpiration was slightly higher on lower leaves (3.30 ± 0.36 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) than on upper leaves (2.97 ± 0.28 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) ($F$=4.81; df=1,51; p=0.033).

Foliar Nutrients and Photosynthesis – Group 2

The chlorophyll content of Group 2 seedlings was affected by an interaction between date and treatment (F=6.91; df=14,271; p<0.001) (Fig. 2.4). Chlorophyll levels peaked in fertilized seedlings and were higher than in seedlings of other treatments by mid-May (Fig. 2.4). By mid-June, all of the girdled seedlings had lost their leaves and all

untreated seedlings lost their leaves by the end of July (Fig. 2.4). Photosynthesis was lower on girdled seedlings compared with seedlings of other treatments on 21 May (F=34.73; df=2,53; p<0.001) and 16 June (F=19.51; df=2,19; p<0.001) (Fig 2.5a). On 21 May, photosynthesis rates of fertilized seedlings were also higher than those on untreated seedlings. Differences were more difficult to detect on 16 June due to high variability (Fig 2.5a), and by this time average photosynthesis rates of all treatments had decreased by half due to seedling stress. As on no-choice seedlings, photosynthesis rates on seedlings in the choice assay were lower on upper leaves than on lower leaves on 21 May (F=4.26; df=1,53; p=0.044). This difference was not significant on 16 June (p=0.81) (Fig 2.5b). Transpiration rates on girdled trees were at least half that of rates on other trees on 21 May (F=32.11; df=2,51; p<0.001) but high variability obscured significance on 16 June (p=0.77) (Fig 2.6). Transpiration rates were higher on lower leaves (2.46 ± 0.30 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) than on upper leaves (1.70 ± 0.22 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) on 21 May, but high variability again obscured significance on 16 June (p=0.006).

No-choice bioassays

No significant differences in total leaf area consumed by *A. planipennis* were apparent in rounds 1 (p=0.14) and 4 (p=0.41). In rounds 2 and 3, beetles consumed more leaf material from girdled seedlings than from fertilized seedlings (Round 2 F=9.65; df=2,89; p<0.001; Round 3 F=8.79; df=2,83; p<0.001). Feeding was also higher on foliage from untreated seedlings than from fertilized seedlings in round 3 (Fig. 2.7). Frass production did not differ among treatments in rounds 1 (p=0.40) and 4 (p=0.27). In rounds 2 and 3, beetles produced over twice as much frass when feeding on girdled

seedlings than they did when feeding on fertilized seedlings (Round 2 F=23.27; df=2,90; p<0.001; Round 3 F=11.86; df=2,86, p<0.001). In round 2, beetles also produced more frass when feeding on untreated seedlings than they did when feeding on fertilized seedlings (Fig. 2.8). No differences between male and female beetles were ever observed for total leaf area consumption (Round 1 p=0.85; Round 2 p=0.88; Round 3 p=0.06; Round 4 p=0.42) or frass production (p=0.32; Round 2 p=0.71; Round 3 p=0.14, Round 4 p=0.37).

Choice assay

Girdled seedlings lost their leaves soon after pots were moved outdoors and were not included in the study. Beetles from wild *A. planipennis* populations in the area began feeding on leaves of the seedlings in mid-June. Examination of the seedlings revealed over twice as much feeding on fertilized seedlings than on untreated seedlings through most of the summer (2 July: F=6.23; df=2,24; p =0.007; 8 July: F=7.93; df=1,22; p=0.010; 16 July: F=6.45; df=1,20; p=0.020; 23 July: F=6.37; df=1,19; p=0.021; 30 July: F=10.37; df=1,17; p=0.005) (Fig. 2.9). Similarly, percentage of leaves fed at any rank was either not affected by species (Rank 1 χ^2=0.30, df=1,12, p=0.58, Rank 4 χ^2=1.49, df=1,12, p=0.22, Rank 5 χ^2=2.29, df=1,12, p=0.13) or was highest in fertilized seedlings (Rank 2 χ^2=5.42, df=1,12, p=0.02, Rank 3 χ^2=5.74, df=1,12, p=0.017). Most leaves had senesced by mid-August 2009. Fertilized seedlings maintained their leaves the longest (Fig. 2.10). Beetles were occasionally observed on seedlings but not often enough for statistical analysis, although beetles were usually found on fertilized seedlings. On 25

June three beetles were on fertilized seedlings and one beetle was flying in the vicinity of an untreated seedling; on 2 July two beetles were on fertilized seedlings; and on 16 July one beetle was on a fertilized seedling.

Discussion

In general, girdling caused nutrient stress while fertilization increased foliar nutrition. Girdling reduced the levels of nitrogen and chlorophyll as well as photosynthesis and transpiration rates of foliage. Girdling also increased foliar protein and carbohydrate levels, probably due to the inability of these nutrients to move down through the phloem to the roots (Noel 1970, Chen and Poland 2009). However, protein: carbohydrate ratios in girdled foliage were unaffected. Girdled seedling nutrition was consistent with observations made by Chen and Poland (2009) on girdled seedlings, which showed an increase in carbohydrate concentration of foliage but a decrease in protein: carbohydrate ratios. This protein: carbohydrate ratio seems to have an effect on the quality of nutrition beetles can obtain from leaves. No significant differences in protein: carbohydrate ratios were observed among treatments in this study. In contrast to girdling, fertilization increased foliar nitrogen and amino acid concentrations in comparison to foliage from girdled or untreated seedlings. It is unclear why protein levels were lowest in fertilized seedling foliage when amino acids were highest; perhaps there was a lower rate of conversion of amino acids to proteins in these seedlings, but no tests were run to confirm this.

Fertilization increased chlorophyll content in foliage of green ash seedlings in both the polyhouse and outdoors compared with girdled seedlings. Higher photosynthesis

rates on the lower leaves are probably due to greater leaf age. These results are consistent with observations made by Chen and Poland (2009) that older leaves had higher chlorophyll content, which should correlate with photosynthetic capabilities. Water loss via transpiration was also highest on girdled seedlings, suggesting seedlings were stressed and photosynthesis rates were low (Chaves et al. 2003). Overall, girdling appears to be an efficient stressor of green ash seedlings, while fertilization may serve to increase seedling vigor.

The higher consumption of leaf material on girdled seedlings by *A. planipennis* when compared with fertilized seedlings in two of four no-choice bioassays may reflect compensatory feeding to make up for lower levels of nitrogen or amino acids in foliage from girdled seedlings (Mattson 1980, Scriber and Slansky 1981). Compensatory feeding to make up for nutrition differences have also been observed in green ash in comparison to Manchurian ash (Pureswaran and Poland 2009b, Chen and Poland 2009).

Frass production was also highest when beetles fed on foliage from girdled seedlings. In general, relative frass amounts compared among treatments appeared to closely match the relationships observed in amount of leaf material fed. Lower feeding and frass production on fertilized seedlings may indicate beetles feeding on these seedlings better utilized water and nutrients. Sex of beetles had no discernible effect on adult feeding habits. The lack of significant differences in feeding or frass production in no-choice bioassay round 1 may be due to treatments not yet having affected seedling vigor at such an early date. Reasons for the lack of any significant differences in round 4 are unclear.

The amount of *A. planipennis* adult feeding damage was greater on fertilized seedlings over untreated seedlings in outdoor choice tests, indicating that adults preferred to feed on foliage with higher nutrient levels. Girdled seedlings did not tolerate exposure to outdoor conditions, probably due to their weakened state. The fact that fertilized seedlings maintained their leaves the longest is probably a result of their enhanced vigor. No-choice bioassays with Group 1 seedlings revealed that compensatory feeding is necessary on girdled foliage. If compensatory feeding makes up for the effects of lower foliar nutritional quality, this may explain Chen and Poland's (2009) observation that no significant differences exist in adult *A. planipennis* survival on foliage from girdled vs. ungirdled seedlings in the laboratory. In wild conditions, however, compensatory feeding may decrease survival by increasing the amount of time during which beetles must feed and therefore also be exposed to predation or severe weather. Preferential feeding on fertilized foliage is likely because feeding on these seedlings is more energy efficient. Optimal foraging theory states that animals will choose feeding habits which maximize energy obtained from food and reduces time spent obtaining that food (MacArthur and Pianka 1996). Therefore, feeding on girdled foliage which is both less energy efficient and increases time spent feeding should not be adaptive for these beetles. It would appear to be more beneficial in the wild to feed on healthy foliage.

Choice assay results appear to contradict the observation that adult beetles are most attracted to stressed or girdled trees for mating and oviposition (Cappaert et al. 2005b, McCullough et al. 2009a, 2009b; Tluczek 2009). It is worthwhile to note, however, that even the fertilized seedlings were stressed in these outdoor planting conditions when compared to their vigor in the greenhouse in Group 1. It is possible that

beetles exhibited no preference on which plants to land, but simply fed longer on fertilized seedlings after landing due to better food quality. However, this has not been tested for and beetles observed in the plantation were almost always on fertilized seedlings. It may be beneficial to repeat these studies on larger, more mature trees that are suitable for oviposition. Chapter 1 briefly touches on this issue, but more research is needed to compare adult *A. planipennis* foliage feeding choices with oviposition preferences. It is unclear what the association may be between adult host choices where both leaf feeding and oviposition are concerned. Understanding the relationship between host choice for oviposition and host choice for foliage feeding may shed light on *A. planipennis* adult behavior and host relations.

Table 2.1: Mean (±SE) nutrient concentration of foliage from green ash seedlings in 2009. Protein significance letters are based on log (x+1) transformed values. Protein: TNC ratio significance letters are based on log (x) transformed values. Values followed by different letters within columns are significantly different from each other (2-way ANOVA, $p<0.05$). (N=34, 30, 30, 30, 30 respectively by column). (TNC = Total non-structural carbohydrate).

Treatment	Total Nitrogen (ppm NH_4 N)	Protein (mg/g fresh weight ± SE)	Total Amino Acid (µmol/g fresh weight ± SE)	TNC (mg/g fresh weight ± SE)	Protein:TNC (ratio by weight ± SE)
Girdled	26.5 ± 1.8 b	17.3 ± 2.0 a	4.5 ± 0.9 ab	12.2 ± 1.5 a	1.7 ± 0.4 a
Untreated	34.7 ± 2.8 b	15.6 ± 1.6 a	3.5 ± 0.7 b	10.3 ± 1.0 ab	1.4 ± 0.2 a
Fertilized	59.7 ± 3.8 a	9.5 ± 1.2 b	6.6 ± 0.9 a	7.2 ± 0.6 b	1.7 ± 0.3 a

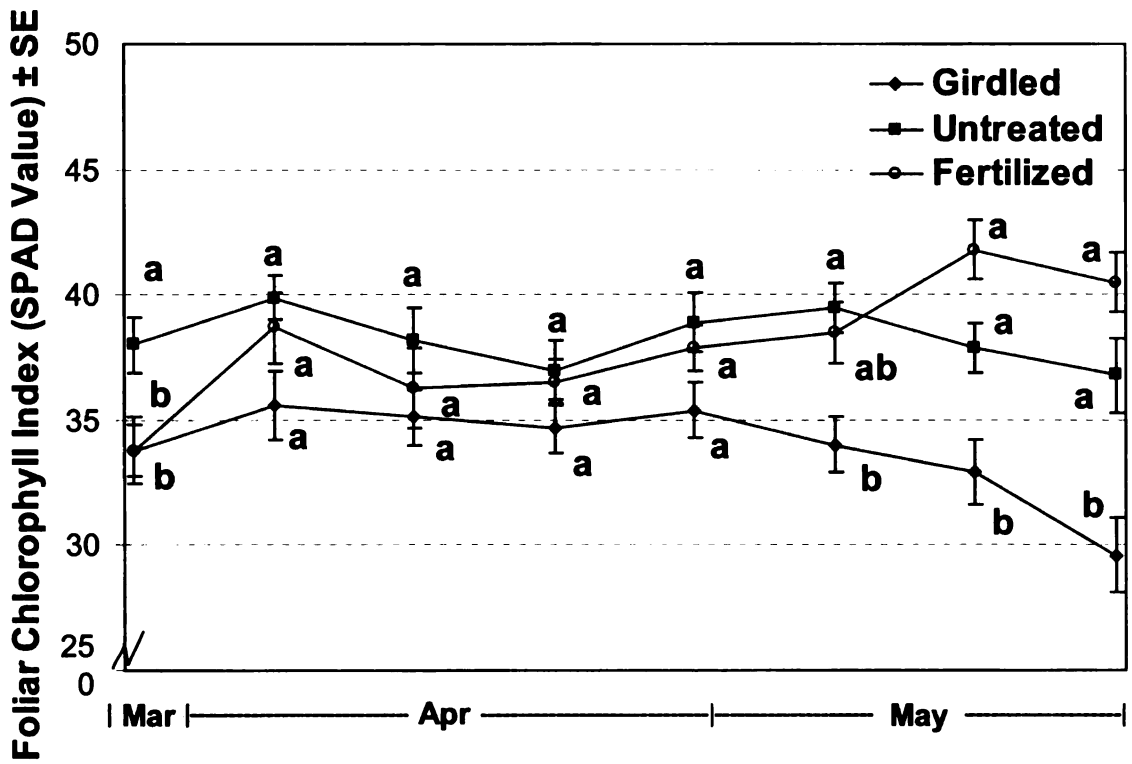

Figure 2.1: Mean (± SE) foliar chlorophyll index by treatment over time for Group 1 seedlings. Points with different letters are significantly different from each other within each date (ANOVA, $p<0.05$). Treatment p-values by date: 31 Mar $p=0.025$, 7 Apr $p=0.07$, 14 Apr $p=0.24$, 21 Apr $p=0.23$, 28 Apr $p=0.07$, 5 May $p=0.020$, 12 May $p<0.001$, 19 May $p<0.001$. (N=48 for 31 Mar-12 May dates, N=47 for 19 May).

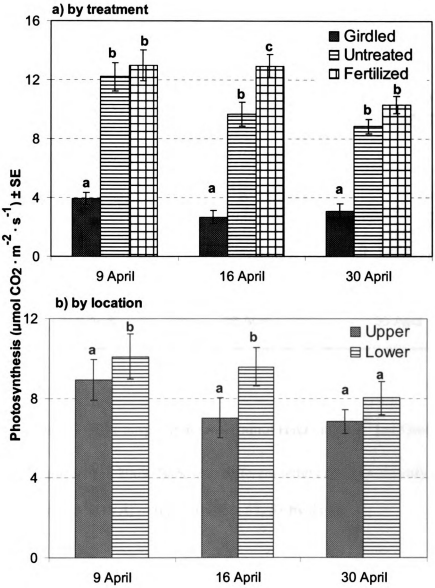

Figure 2.2: Mean (± SE) photosynthesis rates (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) of Group 1 green ash seedlings by treatment (a) and leaf location (b) in 2009. Significance letters for 9 and 16 April are based on log (x+1) transformation. Significance letters for 30 April are based on square root (x) transformed values. Bars with different letters are significantly different from each other (2-way ANOVA, $p<0.05$). (N=59, 57, 57 by date).

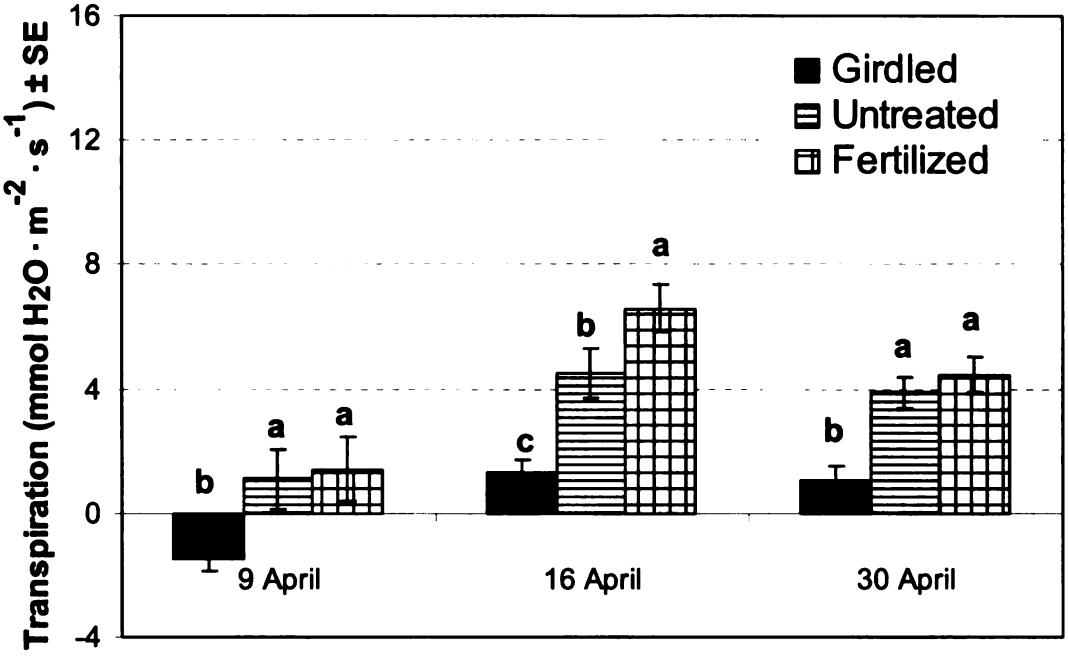

Figure 2.3: Mean (± SE) transpiration rates (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) of Group 1 green ash seedlings by treatment in 2009. Bars with different letters are significantly different from each other (2-way ANOVA, $p<0.05$). (N=59, 57, 57 by date).

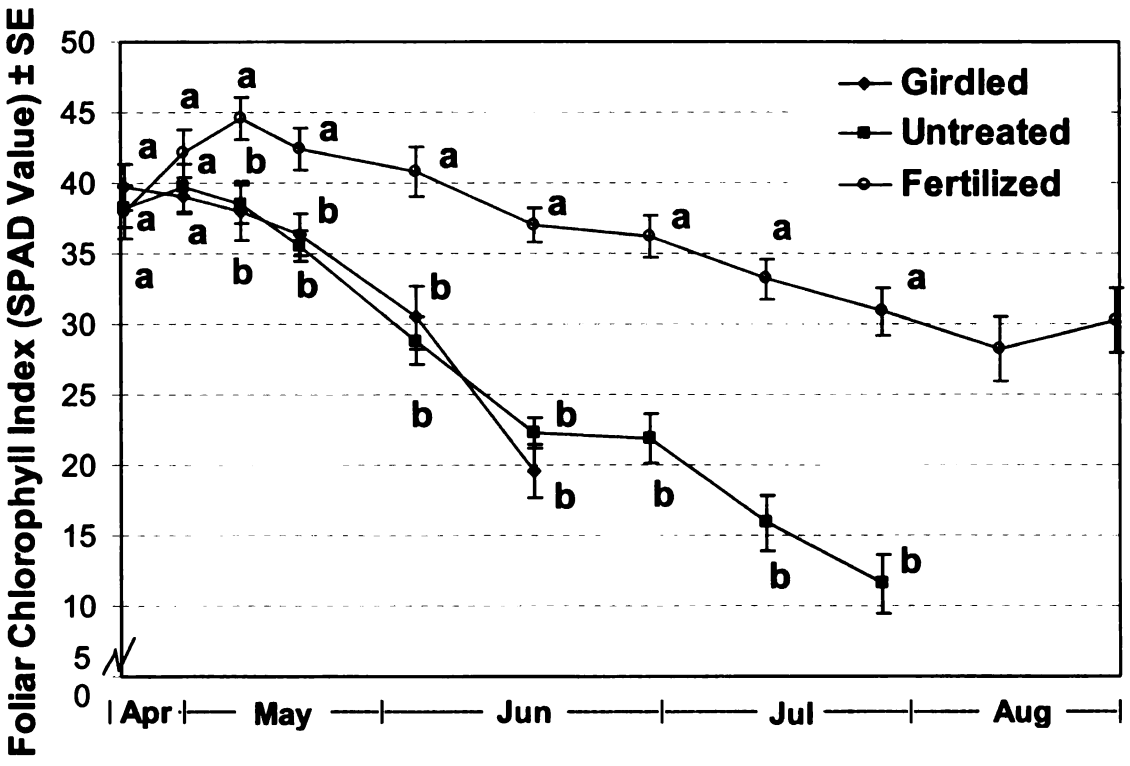

Figure 2.4: Mean (± SE) foliar chlorophyll index by treatment over time for Group 2 seedlings. Points with different letters are significantly different from each other within each date (ANOVA, $p<0.05$). Treatment p-values by date: 28 Apr $p=0.636$, 5 May $p=0.005$, 12 May $p=0.014$, 19 May $p=0.257$, 2 Jun $p<0.001$, 16 Jun $p<0.001$, 30 Jun $p<0.001$, 14 Jul $p<0.001$, 28 Jul $p<0.001$. (N=36 for 28 Apr-2 Jun, N=33 for 16 Jun, N=24 for 30 Jun, N=23 for 14 Jul, N=19 for 28 Jul).

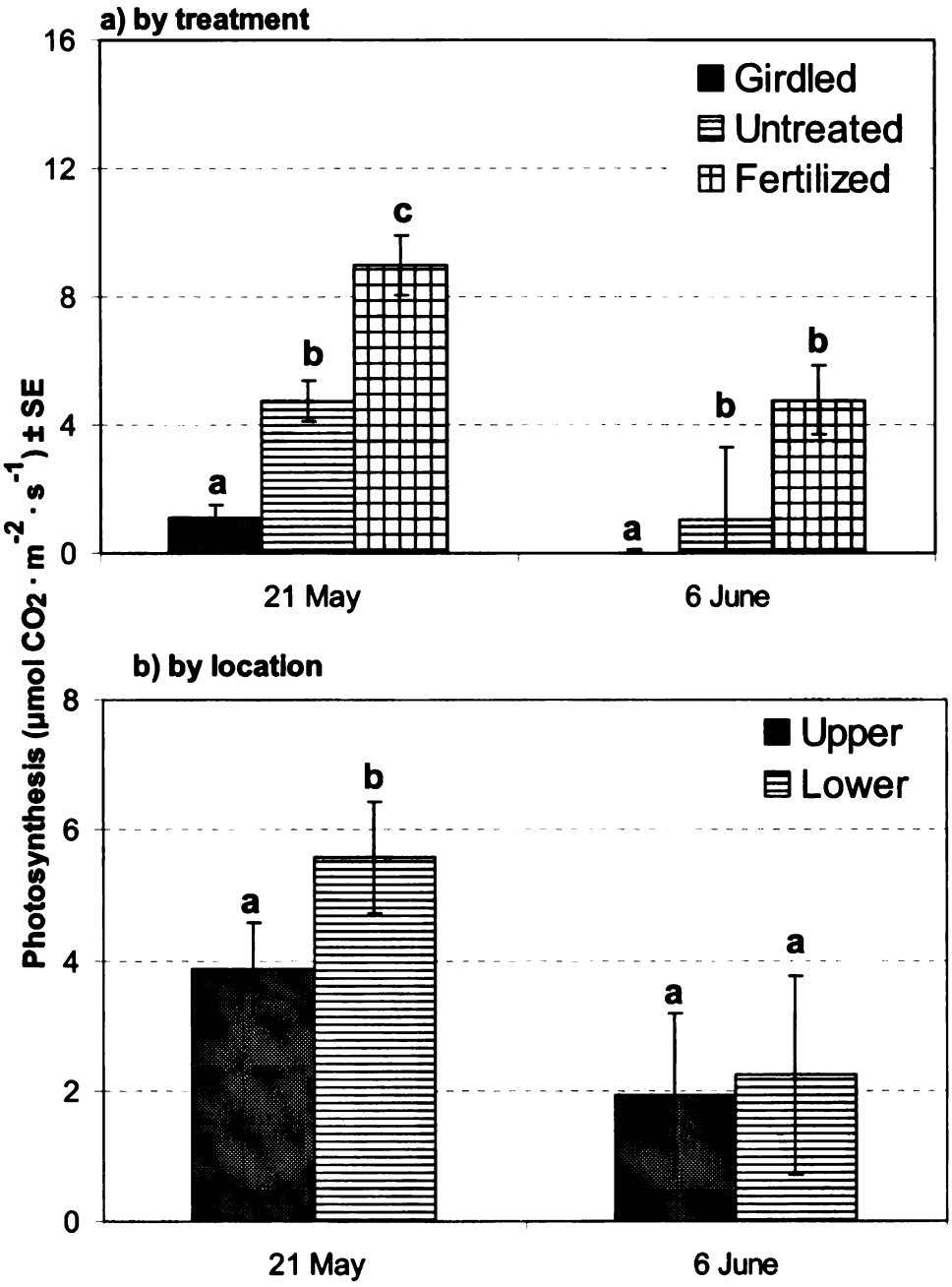

Figure 2.5: Mean (± SE) photosynthesis rates (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) of Group 2 green ash seedlings by (a) treatment and (b) and leaf location in 2009. Significance letters on 16 June are based on log(x+1) transformed values. Bars with different letters are significantly different from each other (2-way ANOVA, $p<0.05$). (N= 57, 23 by date).

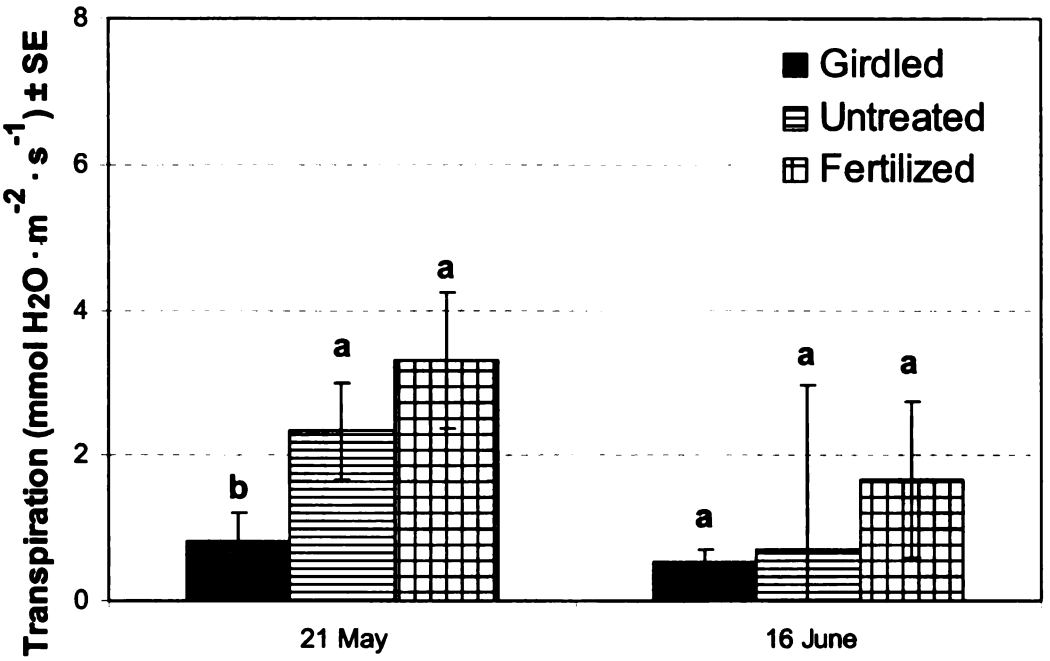

Figure 2.6: Mean (± SE) transpiration rates (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) of Group 2 green ash seedlings by treatment in 2009. Significance values on 16 June were determined nonparametrically. Bars with different letters are significantly different from each other (2-way ANOVA, Friedman's 2-way nonparametric ANOVA, $p<0.05$). (N= 57, 25 by date).

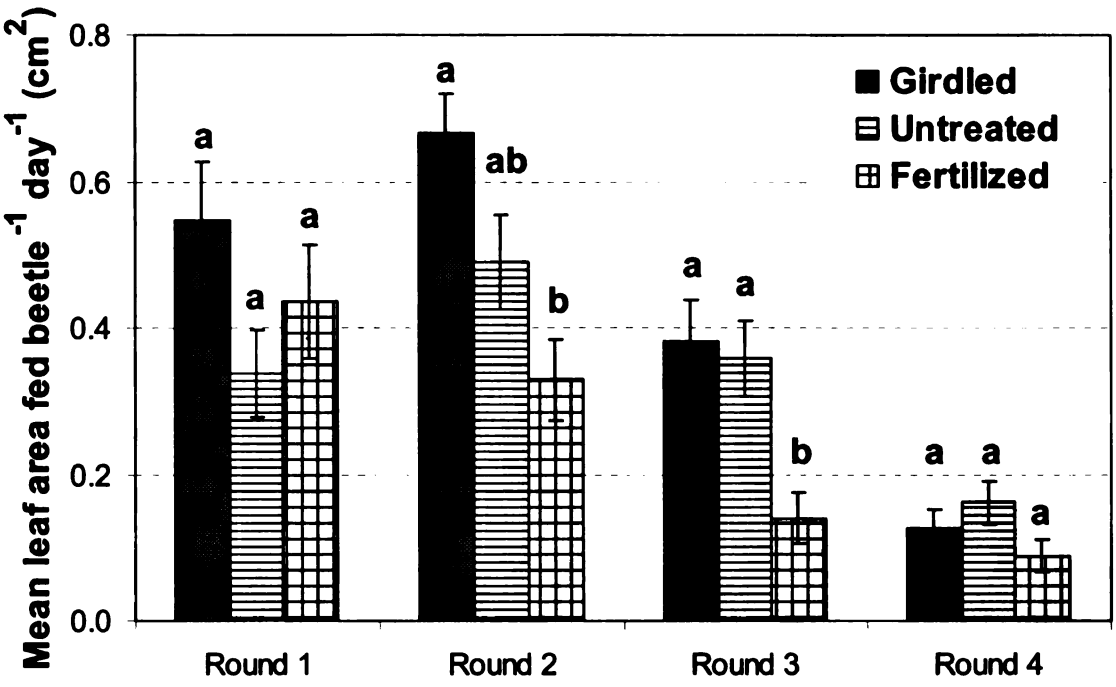

Figure 2.7: Mean (± SE) leaf area consumed (cm^2) per beetle per day in no-choice bioassays in 2009. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other (Friedman's 2-way nonparametric ANOVA, $p<0.05$). (N=84, 95, 89, 86 respectively by round).

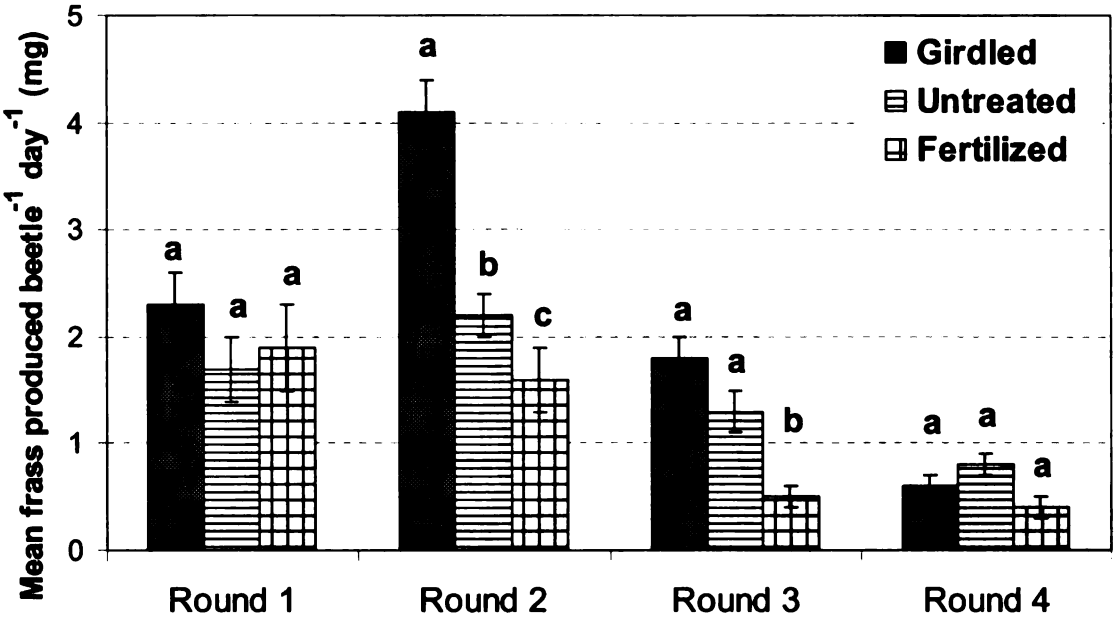

Figure 2.8: Mean (± SE) weight of frass produced (mg) per beetle per day in no-choice bioassays in 2009. Significance letters are based on square root (x) transformed values. Bars with different letters are significantly different from each other (2-way ANOVA $p<0.05$). (N=96, 96, 92, 88 respectively by round).

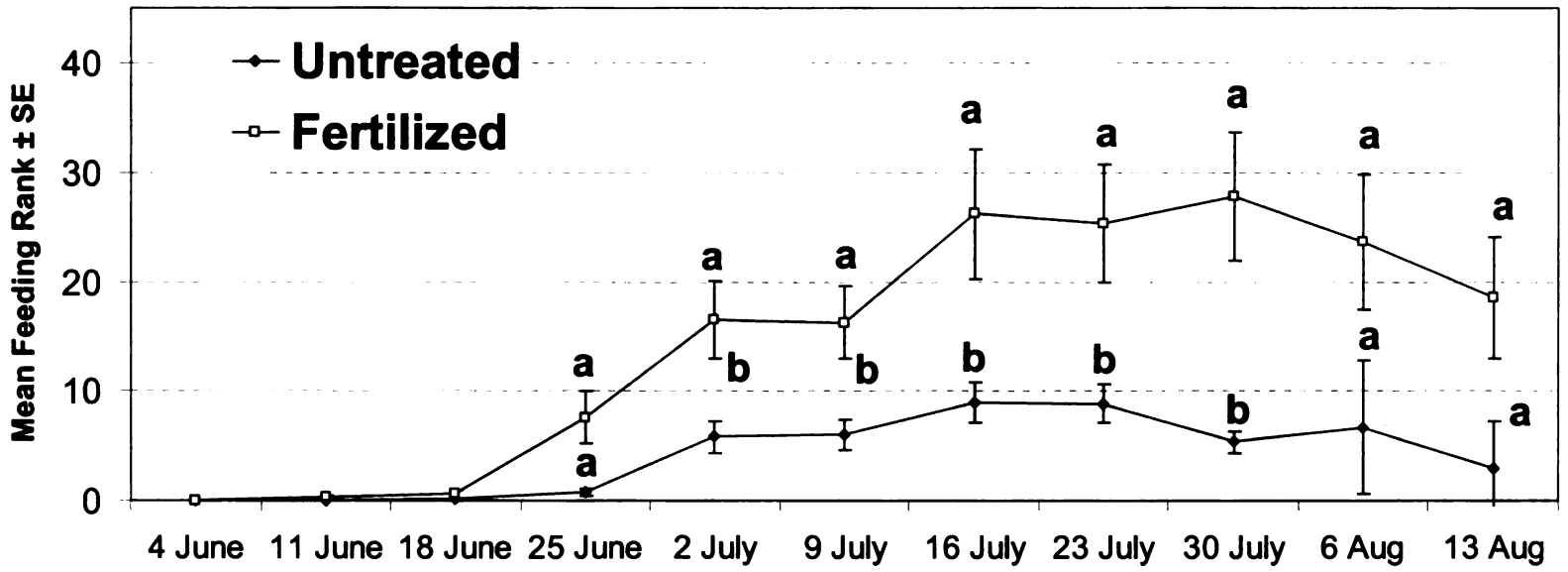

Figure 2.9: Mean (± SE) feeding rank by treatment on Group 2 seedlings. Significance letters on 25 June, 2 July, 19 July, and 16 July are based on log (x) transformed values. Points with different letters are significantly different from each other (ANOVA, $p<0.05$). (N=24, 24, 24, 24, 24, 24, 24, 22, 21, 19, 18, 13 by date).

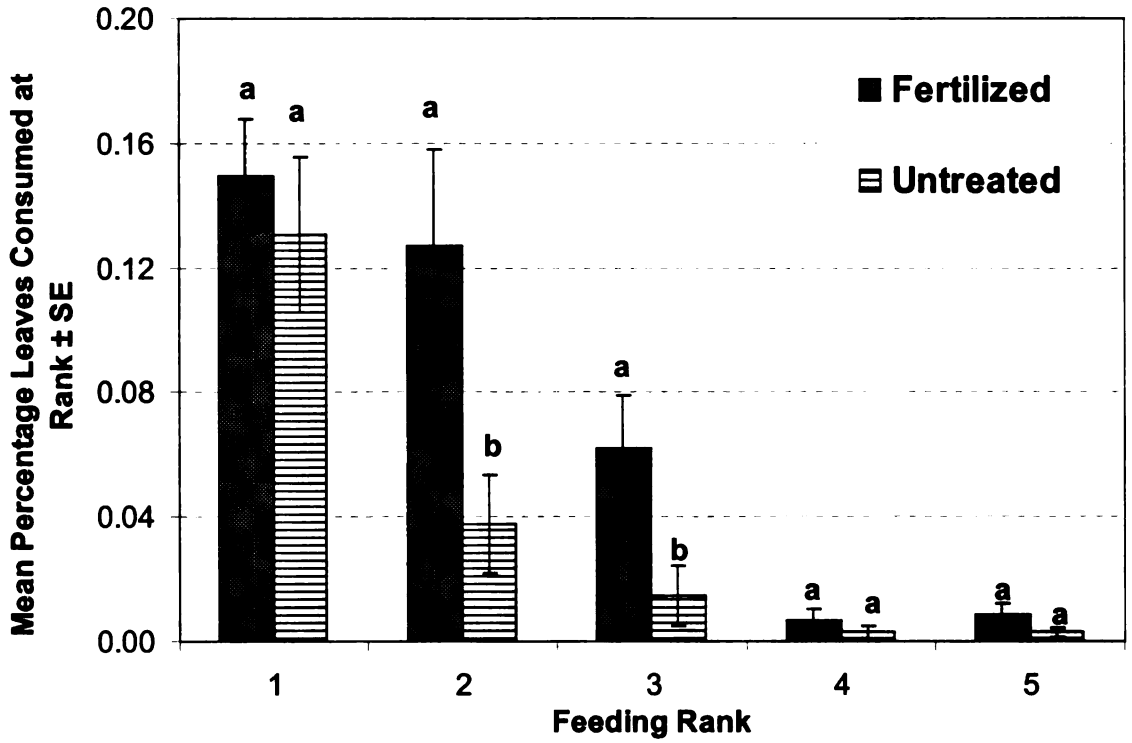

Figure 2.10: Mean (± SE) percentage leaves consumed at each rank level by treatment on Group 2 seedlings. Significance values were determined nonparametrically. Bars with different letters are significantly different from each other (Kruskal-Wallis test, $p<0.05$). (N=24).

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.: 2010-05

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

TREE VIGOR AND ITS RELATION TO EMERALD ASH BORER (*AGRILUS PLANIPENNIS* FAIRMAIRE) ADULT HOST PREFERENCE AND LARVAL DEVELOPMENT ON GREEN AND WHITE ASH TREES

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Chenin Kathleen Limback

Date 06 August 2010

*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: Shiawassee Co. Emerged from green ash logs 23-Jul-10 det. by: C.K. Limback MICHIGAN: Ingham Co. MSU Tree Research Center Corner of Hagadorn and Jolly Ex: Under bark of white ash (*Fraxinus americana* L.) (70% ETOH) 14-Feb-10 C.K. Limback, coll.		4			7	8		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Chenin Kathleen Limback

Date 06-Aug-2010

Voucher No. 2010-05
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 26 Aug 2010
Date

LITERATURE CITED

Akiyama, K. and S. Ohmomo. 2000. The Buprestid beetles of the world. Iconographic Series of Insects 4. Gekkan-Mushi Co., Ltd. 341 pp.

Anderson, R.F. 1944. The relation between host condition and attacks by the bronzed birch borer. Journal of Economic Entomology 37: 588-596.

Anonymous. 2009. Cooperative Emerald Ash Borer Project *http://www.emeraldashborer.info.* Accessed August 2010.

Anulewicz, A.C., D.G. McCullough, and D.L. Cappaert. 2007a. Emerald ash borer (*Agrilus planipennis*) density and canopy dieback in three North American ash species. Arboriculture and Urban Forestry 33 (5): 338-349.

Anulewicz, A.C., D.G. McCullough, T.M. Poland, and D.L. Cappaert. 2007b. Attraction of emerald ash borer to trap trees: Can MeJA or manuka oil compete with girdling? p. 83 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

Barter, G.W. 1957. Studies of the bronze birch borer, *Agrilus anxius* Gory, in New Brunswick. The Canadian Entomologist 89: 12-36.

Bauer, L.S., R.A. Haack, D.L. Miller, T.R. Petrice, and H. Liu. 2004. Emerald ash borer life cycle. p. 8 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

Bede, J.C., J.N. McNeil, and S.S. Tobe. 2007. The role of neuropeptides in caterpillar nutritional ecology. Peptides 28: 185-196.

Bernays, E.A. and R.L. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman and Hall, New York, NY.

Bi, J.L., N.C. Toscano, and M.A. Madore. 2003. Effect of urea fertilizer application on soluble protein and free amino acid contgent of cotton petioles in relation to silverleaf whitefly (*Memisia argentifolii*) populations. J. Chem. Ecol. 29: 747-761.

Cappaert, D., D. McCullough, and T. Poland. 2005a. Emerald ash borer life cycle: a reassessment. P. 19 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

Cappaert, D., D.G. McCullough, T.M. Poland, and N.W. Siegert. 2005b. Emerald ash borer in North America: A research and regulatory challenge. American Entomologist 51(3): 152-165.

Chabot, B.F. and D.J. Hicks. 1982. The ecology of leaf life spans. Ann. Rev. Eco. Syst. 13: 229-259.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant response to drought: from genes to the whole plant. Functional Plant Biology 30: 239-264.

Chapin, F. S. III, D.A. Johnson, and J.D. McKendrick. Seasonal movement of nutrients in plants of differing growth form in an Alaskan tundra ecosystem: implications for herbivory. J. Ecol 68: 189-209.

Chen, Y. and T.M. Poland. 2009. Interactive influence of leaf age, light intensity, and girdling on green ash foliar chemistry and emerald ash borer development. Journal of Chemical Ecology 35: 806-815.

Chen, Y. and T.M. Poland. 2010. Nutritional and defensive chemistry of three North American ash species: possible roles in host performance and preference by emerald ash borer adults. The Great Lakes Entomologist. (In press).

Chen, Y., X. Ni, and G.D. Buntin. 2009. Physiological, nutritional, and biochemical bases of corn resistance to foliage-feeding fall armyworm. Journal of Chemical Ecology 35: 297-306.

Conover, W.J. 1971. Practical nonparametric statistics. Texas Technical University, New York.

Crook, D.J., A. Khrimian, I. Fraser, J.A. Francese, T.M. Poland, and V.C. Mastro. 2008. Electrophysiological and behavioral responses of *Agrilus planipennis* (Coleoptera: Buprestidae) to host bark volatiles. Environ. Entomol. 37:356–365.

de Groot, P., G.G. Grant, T.M. Poland, R. Scharbach, L. Buchan, R.W. Nott, L. Macdonald, and D. Pitt. 2008. Electrophysiological response and attraction of emerald ash borer to green leaf volatiles (GLVs) emitted by Host Foliage. J. Chem. Ecol. 34: 1170-1179.

DePew, L.J. 1971. Effect of infurrow treatments of three systemic insecticides on grain sorghum emergence. J. Econ. Entomol. 64: 1321-1322.

DePew, L.J. and M.L. Hooker. Effect of insecticide placement at planting for control of greenbug (Homoptera, Aphididae) on grain-sorghum. J. Econ. Entomol. 80: 490-493.

Dicke, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. Bioch Syst & Ecol. 28: 601-617.

Doi, E., D. Shibata, and T. Matoba. 1981. Modified colorimetric ninhydrin methods for peptidase assay. Anal. Biochem. 118: 173-184.

Dunn, J.P., D.W. Kimmerer, G.L. Nordin. 1986. The role of host tree condition in attack of white oaks by the twolined chestnut borer, *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae). Oecologia 70: 596-600.

Eyles, A., W. Jones, K. Riedl, D. Cipolline, S. Schwartz, K. Chan, D.A. Herms, and P. Bonello. 2007. Comparative phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). J. Chem. Ecol. 33: 1430-1448.

Fisher, G.H., I. Arias, I. Quesada, S. D'aniello, F. Errico, M.M. Di Fiore, and A. D'aniello. 2001. A fast and sensitive method for measuring picomole levels of total free amino acids in very small amounts of biological tissues. Amino Acids 20: 163-173.

Foyer, C.H. 1987. The basis for source-sink interaction in leaves. Plant Physiology and Biochemistry 25: 649-657.

Garbelotto, M., D.J. Schmidt, and T.Y. Harnik. 2007. Phosphite injections and bark application of phosphite + Pentrabark™ control sudden oak death in coast live oak. Arboriculture and Urban Forestry 33 (5): 309-317.

Gould, J., J. Tanner, D. Winograd,and S. Lane. 2005. Initial studies on the laboratory rearing of emerald ash borer and foreign exploration for natural enemies. p. 73-74 in Emerald Ash Borer Research and Technology Development Meeting. Mastro, V., and R. Reardon (comps.).

Glynn, C., D.A. Herms, M. Agawa, R. Hansen, and W.J. Mattson. 2003. Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos 101: 385-397.

Guest, D.I. and B.R. Grant. 1991. The complex action of phosphonates as antifungal agents. Biological Reviews of the Cambridge Philosophical Society 66: 159-187.

Haack, R.A. and D.M. Benjamin. 1982. The biology and ecology of the twolined chestnut borer, *Agrilus bilineatus* (Coleoptera: Buprestidae), on oaks, *Quercus* spp., in Wisconsin. The Canadian Entomologist 114: 385-396.

Haack, R.A., E. Jendek, H. Liu, K.R. Marchant, T.R. Petrice, T.M. Poland, and H. Ye. 2002. The emerald ash borer: a new exotic pest in North America. Newsletter Mich. Entomol. Soc. 47 (3and4): 1-5.

Harper, J.L. 1989. The value of a leaf. Oecologia 80: 53-58.

Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67: 283-335.

Herms, D.A., E. Rebek, D. Smitley, P. Bonello, and D. Cipollini. 2005. Interspecific variation in ash resistance to emerald ash borer. P. 33 in Emerald Ash Borer Research and Technology Development Meeting. Mastro, V., and R. Reardon (comps.).

Iles, J.K. and A.M. Vold. 2003. Landscape tree cultivar preferences in Iowa, U.S. Journal of Arboriculture 29: 331-336.

Jendek, E. 1994. Studies in the East Palearctic species of the genus *Agrilus* Dahl, 1823 (Coleoptera: Buprestidae). Part 1. Entomol. Prob. 25: 9-25.

Jones, M.G.K. 1979. An enzymic microassay for starch. Plant, Cell and Environ. 2: 227-234.

Kenward, M.G. and J.H. Roger. 1997. Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. Biometrics 53: 983-997.

Kovas, K.F., R.G. Haight, D.G. McCullough, R.J. Mercader, N.W. Siegert, and A.M. Liebhold. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. Ecological Economics 69: 569-578.

Kruskal, W.H. and W.A. Wallis. 1952. Use of Ranks in One-Criterion Variance Analysis. Journal of the American Statistical Association 47: 583-621.

Kytö, M., P. Niemelä, and S. Larsson. 1996. Insects on trees: population and individual response to fertilization. Oikos 75: 148-159.

Lee, K.P., S.T. Behmer, S.J. Sempson, and D.A. Raubenheimer. 2002. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). J. Insect Physiol. 48: 655-665.

Li, C., D. Weiss, and E.E. Goldschmidt. 2003. Girdling affects carbohydrate-related gene expression in leaves, bark and roots of alternate-bearing citrus trees. Ann. Bot. 92: 137-143.

Liu, H., and L.S. Bauer. 2006. Susceptibility of *Agrilus planipennis* (Coleoptera: Buprestidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. J. Econ. Entomol. 99: 1096-1103.

Loomis, W.E. 1932. Growth-differentiation balance vs. carbohydrate-nitrogen ratio. Proc. Am. Soc. Hort. Sci. 29: 240-245.

Lorio Jr., P.L. 1986. Growth-differentiation balance: a basis for understanding southern pine beetle-tree interactions. For Ecol. Manage. 14: 259-273.

Lyons, D.B., G.C. Jones, and K. Wainio-Keizer. 2004. The biology and phenology of the emerald ash borer, *Agrilus planipennis*. p. 5 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

MacArthur, R.H. and E.R. Pianka. 1966. On Optimal Use of a Patchy Environment. The American Naturalist 100: 603-609.

Markovic, I., D.M. Norris, and E.V. Nordhiem. 1997. Gypsy moth (*Lymantria dispar*) larval development and survival to pupation on diet plus extractables from green ash foliage). Entomologia Experimentalis et Applicata 84: 247-254.

Marquis, R.J., E.A. Newell, and A.C. Villegas. 1997. Nonstructural carbohydrate accumulation and use in an understory rain-forest shrub and relevance for the impact of leaf herbivory. Funct. Ecol. 11: 636-643.

Mattson, W.J. Jr. 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11: 119-161.

McCullough, D.G., D.A. Cappaert, T.M. Poland, P. Lewis, and J. Molongoski. 2007. Evaluation of neo-nicotinoid insectidies applied as non-invasive trunk sprays. pp. 52-54 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

McCullough, D.G., D.L. Cappaert, T.M. Poland, A.C. Anulewicz, P. Lewis, and J. Molongoski. 2008. Evaluation of non-invasive trunk sprays and trunk-injected Emamectin Benzoate. pp. 40-42 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

McCullough, D.G. and N.W. Siegert. 2007. Estimating potential emerald ash borer (Coleoptera : Buprestidae) populations using ash inventory data. Journal of Economic Entomology 100: 1577-1586.

McCullough, D.G., T.M. Poland, and D. Cappaert. 2009a. Attraction of the emerald ash borer to ash trees stressed by girdling, herbicide treatment, or wounding. Can. J. For. Res. 39: 1331-1345.

McCullough, D.G., T.M. Poland, A.C. Anulewicz, and D. Cappaert. 2009b. Emerald ash borer (Coleoptera: Buprestidae) attraction to stressed or baited ash trees. Environmental Entomology 38: 1668-1679.

Mercader, R.J., N.W. Siegert, A.M. Liebhold, and D.G. McCullough. 2009. Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. Agricultural and Forest Entomology 11: 421-425.

Mostafa, E.A.M. and M.M.S. Saleh. 2006. Response of balady Mandarin trees to girdling and potassium sprays under sandy soil conditions. Res. J. Agric. Biol. Sci. 2: 137-141.

Noel, A.R.A. 1970. The girdled tree. Botanical Review 36: 162-195.

Ott, R.L., and M. Longnecker. 2001. An Introduction to Statistical Methods and Data Analysis. Duxbury Thomson Learning, Inc.

Poland, T.M. and D.G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104 (3): 118-124.

Pureswaran, D.S. and T.M. Poland. 2009a. Host selection and feeding preference of *Agrilus planipennis* (Coleoptera: Buprestidae) on ash (*Fraxinus* spp.). Environmental Entomology 38: 757-765.

Pureswaran, D.S. and T.M. Poland. 2009b. Effects of visual silhouette, leaf size and host species on feeding preference by adult emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). The Great Lakes Entomologist 42: 62-71.

Rebek, E.J., D.A. Herms, and D.R. Smitley. 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). Environ. Entom. 37: 242-246.

Rodriguez-Saona, C., T.M. Poland, J.R. Miller, L.L. Stelinski, G.G. Grant, P. de Groot, L. Buchan, and L. Macdonald. 2006. Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis*, to induced volatiles of Manchurian ash, *Fraxinus mandshurica*. Chemoecology 16: 75-86.

Roper, T.R. and L.E. Williams. 1989. Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. Plant Physiol. 89: 1136-1140.

SAS Institute. 2001. PROC user's manual, version 6th ed. SAS Institute, Cary, NC.

Schaefer, P.W. 2005. Foreign exploration for emerald ash borer and its natural enemies. P. 67-68 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

Scriber, J.M. and F. Slansky, Jr. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183-211.

Siegert, N.W., D.G. McCullough, D.W. Williams, I. Fraser, T.M. Poland, and S.J. Pierce. 2010. Dispersal of *Agrilus planipennis* (Coleoptera: Buprestidae) from discrete epicenters in two outlier sites. Environmental Entomology 39: 253-265.

Simpson, S.J. and D. Raubenheimer. 1993. The central role of the haemolymph in the regulation of nutrient intake in insects. Physiol. Entomol. 18: 395-403.

Shapiro, S.S. and M.B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591-611.

Solomon, J.D., T.D. Leininger, A.D. Wilson, R.L. Anderson, L.C. Thompson, and F.I. McCracken. 1993. Ash pests: a guide to major insects, diseases, air pollution injury and chemical injury. Gen. Tech. Rep. SO-96. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 45 p.

Tluczek, A.R. 2009. Influence of host vigor on larval distribution, development, and mortality of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Masters Thesis, Michigan State University. 132p.

Tukey, J.W. 1953. Multiple Comparisons. Journal of the American Statistical Association 48: 624–625.

Wilde, G., T. Mize, J. Stuart, J. Whitworth, and R. Kinsinger. 1984. Comparison of planting-time applications of granular or liquid insecticides and liquid fertilizer plus insecticide combinations for control of chinch bugs (Heteroptera: Lygaeidae) and greenbugs (Homoptera: Aphididae) on seedling sorghum. J. Econ. Entomol. 77: 706-708.

Williams, D., H.P. Lee, and Y.S. Jo. 2005. Explorations for natural enemies of emerald ash borer in South Korea during 2004. p. 66 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

Williams, D., H.P. Lee, and Y.S. Jo. 2006. Exploration for emerald ash borer and its natural enemies in South Korea during May-June 2005. p. 52 in Emerald Ash Borer Research and Technology Development Meeting, Mastro, V., and R. Reardon (comps.).

[USDA–FS FIA] USDA–Forest Service, North Central Research Station, Forest Inventory and Analysis. 2006. http://www.ncrs.fs.fed.us/4801/.

Yu, C. 1992. *Agrilus marcopoli* Obenberger pp. 400-401 in G. Xiao (ed.) Forest insects of China, 2nd ed. China Forestry Publishing, Beijing, China.

Zar, J.H. 1984. Biostatistical analysis. Prentice Hall, Englewood Cliffs, NJ.


MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 03063 6843