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Influence of host vigor on larval distribution, development, and mortality of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America

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INFLUENCE OF HOST VIGOR ON LARVAL DISTRIBUTION, DEVELOPMENT, AND MORTALITY OF *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE) IN NORTH AMERICA

By

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ABSTRACT

INFLUENCE OF HOST VIGOR ON LARVAL DISTRIBUTION, DEVELOPMENT, AND MORTALITY OF *AGRILUS PLANIPENNIS* FAIRMAIRE (COLEOPTERA: BUPRESTIDAE) IN NORTH AMERICA AS INFLUENCED BY HOST VIGOR

By

Andrew Roy Tluczek

Effects of host stress on the density, development, and mortality of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) larvae were evaluated in field and laboratory settings from 2006 to 2008. In field studies, *A. planipennis* larval density was consistently higher on girdled trees than non-girdled trees. *A. planipennis* larval development was determined by tree vigor, and not larval density.

Field data were used to construct a life-table for *A. planipennis* larvae for 2006 and 2007. Woodpecker predation was the largest source of *A. planipennis* mortality. Woodpeckers fed on larvae most commonly near the tree top and most predation occurred after the month of November. Mortality from unknown causes, such as desiccation, pathogen, or infection, and from cannibalism was rare. A higher percentage of larvae died from unknown causes and cannibalism below the girdle compared with above the girdle on girdled trees.

Laboratory studies of *A. planipennis* larvae showed that cannibalism was rare, even at high densities. Individual larvae each consumed about 10 cm^2 of phloem before reaching the prepupal stage.

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PREFACE

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a phloem-feeding buprestid native to Asia, was first discovered in Southeastern Michigan and Essex County, Ontario in June 2002. Initial observations in Michigan indicated that *A. planipennis* larvae had a univoltine life cycle; eggs laid in June and July, larvae fed in the summer, overwintered as prepupae, and then emerged as adults the following spring. However, subsequent studies have found that at least some *A. planipennis* larvae take two years to develop. There is limited information available on the factors that influence *A. planipennis* mortality and no life-tables have been developed. Understanding factors that affect larval development and influence mortality would be helpful for modeling *A. planipennis* spread and population dynamics, and for improving survey activities of program managers.

This thesis focused on evaluating effects of host vigor on the density, development, and mortality of larval *A. planipennis* in Michigan. In Chapter One, field studies were conducted to evaluate the effects of host stress on density and development of larvae on green ash, *Fraxinus pennsylvanica* Marshall (Oleaceae), trees. In Chapter Two, I assessed the factors that affect *A. planipennis* larval mortality in the same field site and constructed life-tables in 2006 and 2007. Chapter Three addressed the mechanisms that may affect larval development using larvae produced by adult beetles caged on trees. The goal was to determine whether larval density or host stress determined the rate of

larval development. I also calculated the amount of phloem required for *A. planipennis* larvae to complete development.

The goal of Chapter 4 was to determine if larval cannibalism occurred, whether cannibalism was related to population density, and whether it increased rate of development of the surviving larvae. For this laboratory study, white ash, *Fraxinus americana* Linnaeus (Oleacea), phloem arenas sandwiched between two pieces of Plexiglas were used to observe *A. planipennis* larvae. In a second study, larval feeding and mortality on small sections of white ash stems were observed. Results were used to calculate the amount of phloem necessary for *A. planipennis* larvae to complete development.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Density, Development, and Distribution of *Agrilus planipennis* Adults and Larvae as Influenced by Host Vigor

INTRODUCTION

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a phloem-feeding beetle native to Asia that was first discovered in southeastern Michigan, USA, and Essex County, Ontario, Canada in June 2002. To date, the host range of *A. planipennis* in North America is known to include *Fraxinus americana* Linnaeus (white ash), *F. pennsylvanica* Marshall (green ash), *F. nigra* Marshall (black ash), *F. quadrangulata* Michaux (blue ash) and *F. profunda* (Bush) (pumpkin ash). *A. planipennis* is thought to be able to colonize all ash species native to North America (Anulewicz et al. 2006a, 2008a).

Initial studies in southeast Michigan indicated that the life cycle of *A. planipennis* was univoltine (Bauer et al. 2004). Adults oviposited during the summer, larvae fed from late summer to fall, then overwintered as pre-pupal larvae. Pupation occurred in spring and adults emerged in summer (Cappaert et al. 2005).

Recent observations, however, have indicated that at least some larvae feed for two summers. For example, in newly established infestations with low densities of *A. planipennis*, only 18% of 282 larvae had developed in one year (Cappaert et al. 2005). Additionally, data from a detection tree site indicated that

larvae in vigorous trees developed more slowly than larvae in girdled trees (Siegert and McCullough, unpublished data). Such prolonged development, if common, would strongly influence *A. planipennis* rate of spread, population dynamics, survey and control activities of program managers. More information is necessary to determine what effects host vigor and larval density have on larval development.

This study had three objectives including (1) to determine the role of host vigor on *A. planipennis* larval development; (2) to determine if density affects the rate of development of *A. planipennis* larvae and (3) to assess the distribution of captured adults and larvae among trees experiencing different levels of stress.

MATERIALS AND METHODS

Study Site

This study was conducted using green ash (*Fraxinus pennsylvanica*) trees in a well-stocked plantation established in 1980 and located in the Rose Lake State Wildlife Research area in Clinton County, Michigan. The plantation was organized into 54 rows of 10 trees each, spaced at 1.5 meters. Occasional trees were missing, but 363 trees with an average DBH of 10.8 ± 0.20 cm were available for this study. Original planting records were lost, but the plantation was presumably established as a replicated provenance test (J. Durling, Rose Lake Plant Material Center, pers. comm.). Initial observations in fall 2005 and spring 2006 indicated that emerald ash borer was not yet established in this plantation. In May 2006, upon closer inspection, however, 11 trees infested with a very low-density population of emerald ash borers (mean larval density 12.8 ± 7.2 larvae per m^2, range 1 to 197 larvae per tree) were identified, felled and removed.

2006 Study

In May 2006, ten blocks of nine trees each were established for a total of 90 trees. Since all trees were in similar growing conditions and were of similar size, blocks were assigned based on the proximity of trees to each other. Trees were chosen in a way to ensure that their canopies did not contact or overlap

with each other. Diameter at breast height (DBH) was measured in May of 2006 (Table 1.1).

Three trees within each block were randomly assigned to one of three stress treatments. Trees were either girdled (30 trees), exposed to the stress-elicitor methyl jasmonate (30 trees), or left as untreated controls (30 trees). Girdled trees had a 20 cm band of outer bark and phloem removed between 0.85 m and 1.0 m on 10 May. Methyl jasmonate a volatile derivative of jasmonic acid, can elicit stress responses from plants (Gols et al. 2003, Rodriguez-Saona and Thaler 2005), including ash seedlings (Poland 2005, Rogriguez-Saona et al. 2006). To expose trees to methyl jasmonate, a line with 20 bubble caps spaced 20 cm apart was suspended in the canopy. Each bubble cap had 150 µl of methyl jasmonate with a release rate (per bubblecap) of 0.38 mg per day determined in the laboratory at 20 °C (Con Tech Inc., Delta, BC). Bubble caps were suspended through the canopy on 17 May, using a Big Shot Line Launcher™ (WesSpur Tree Equipment, Bellingham WA). DBH and tree height did not vary significantly among treatments and so were not included in further analyses.

During the summer of 2006, experimental trees were qualitatively ranked from 1 to 5 based on the amount of canopy exposure. A score of 1 indicated an open grown tree, 2 indicated that the tree was super-dominate and most of the leaves extended above the rest of the canopy, 3 indicated that the tree was bordered by one or two trees, 4 indicated that the tree was bordered by at least

three trees, and 5 indicated that the tree was suppressed, and little or no foliage extended into the canopy.

I captured adult *A. planipennis* by wrapping a 20 cm band of plastic wrap between 1.3 m and 1.5 m above the ground on each tree. Tanglefoot™ (The Tanglefoot Company, Grand Rapids, MI) was applied to the band. Over the summer, the bands become less sticky, so Tanglefoot™ was reapplied in July. We collected adult *A. planipennis* from the sticky bands once a week from 21 June until 16 August. Adults were cleaned with 70% ethanol and sexed under a stereoscope. The first and second abdominal sections of the male are narrower than those of the female. A male also has a visible layer of setae on the ventral side of the thorax which females lack (Rodriguez-Saona et al. 2007).

Trees were felled and debarked from December 2006 through March 2007 to quantify larval density and development. Trees were felled in blocks so that trees in each block were felled within a few days of each other. After trees were felled, tree height was measured, then each tree was bucked into one m sections, up to at least 8 m aboveground. Bark area was estimated by measuring the diameter of each section at the midpoint then calculating the surface area. The one m logs were returned to the lab and debarked with chisels and drawknives.

The numbers and life stage of *A. planipennis* larvae were recorded and density was standardized per m^2 of exposed surface area for each tree. Instars were identified by head capsule width (Cappaert et al. 2005b). It was assumed that larvae found as 1^{st}, 2^{nd}, or 3^{rd} instar would probably not reach the prepupal

stage by spring, and would require another summer of feeding resulting in a two year life cycle. Observations by Cappaert et al. (2005a) supported this; 2nd and 3rd instars present in April did not complete development before the end of the summer. Larvae that are 4th instars or prepupae in late fall or winter are expected to emerge in the spring as adults, resulting in a one year life cycle (Cappaert et al. 2005a).

Up to three galleries of prepupal larvae were randomly selected on each log by choosing a prepupa that was closest to the top, the bottom and the middle of the log. The maximum width and length of each gallery was measured.

2007 Study

The study was replicated in 2007 with a few modifications. Ten blocks of nine trees were selected using the same parameters as in 2006 for a total of 90 trees. Trees were girdled on 10 May. A line with ten methyl jasmonate bubble caps spaced 20 cm apart was suspended through the canopies on 25 May, and a second line with 10 methyl jasmonate bubble caps was suspended on 25 June. While this did not change the total amount of methyl jasmonate released, it was expected that this would create a more sustained level over the summer. Sticky bands were checked for *A. planipennis* adults once a week from 29 May until 29 August. Trees were felled and debarked from October 2007 through February 2008. Phloem thickness was measured on the cut ends of each log in 2007 using a caliper. Two measurements were taken on each end and the mean of

the four measurements was used for analysis. Bark thickness did not vary among treatments and so was not used in further analyses (Table 1.1).

Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Four response variables were evaluated including number of captured adults, larval density, the proportion of larvae that developed in one year, and the horizontal gallery dimensions. Number of captured adults, larval density, and horizontal gallery dimensions were $\log_{10}(x+1)$ transformed to normalize data. Proportion of larvae that developed in one year required no transformation.

All response variables were analyzed using the GLIMMIX procedure for mixed models in SAS statistical software (PROC GLIMMIX; SAS Institute 2003). Effects of three factors: tree vigor with three levels (control, methyl jasmonate, or girdled), canopy exposure with five levels (1, 2, 3, 4, 5), and height in tree (in one m increments) were analyzed. Block and block crossed with treatment were used as random factors. Differences among treatment means were tested as unplanned comparisons and multiple comparison tests were applied only when overall analysis of variance (ANOVA) was significant ($P<0.05$). When significant differences among levels occurred, the Tukey protected least significant difference (LSD) test was used to evaluate those differences (Ott and Longnecker 2001). The $\log_{10}(x+1)$ relationship between larval density and adult catch was analyzed using a general linear model (PROC GLM, SAS institute

2003). The linear relationship between larval density and the proportion of one year larvae on each of the three vigor treatments was analyzed separately using a general linear model with transformed data (PROC GLM, SAS institute 2003).

RESULTS

2006 Study

Overall, a total of 81 adults were captured on sticky bands in 2006. At least one *A. planipennis* adult was captured on 5, 9, and 24 of the control, methyl jasmonate, and girdled trees, respectively. Most adult beetles were captured in July; 16, 64, and 4 adults were captured in June, July, and August, respectively. Peak captures occurred during the week preceding 7 July, with 27.4% of all beetles captured, and during the week preceding 19 July when 26.2% of all beetles were captured. Girdled trees captured significantly more adults than trees in other treatments, with an average of 0.2 ± 0.1, 0.4 ± 0.1, and 2.1 ± 0.5 adults on control, methyl jasmonate, and girdled trees, respectively ($F_{2,18}$=23.70, P<0.001). Adult capture did not vary among canopy exposure classes ($F_{3,57}$=2.69, P=0.055). Of the total beetles captured, 79.1% were female.

A total of 4,621 *A. planipennis* larvae were recorded when the 90 trees were debarked; counts ranged from 0 to 514 larvae per tree. Overall, the mean density of *A. planipennis* larvae was 34.5 ± 5.8 larvae per m^2. At least one larva was found on 24, 21, and 30 of the control, methyl jasmonate, and girdled trees, respectively. Larval density was significantly higher on girdled trees than on trees treated with methyl jasmonate or untreated control trees ($F_{2,18}$=85.02, P<0.001) (Fig. 1.1). Larval density averaged 3.7 ± 0.9, 6.7 ± 4.4, and 71.1 ± 10.9 larvae per m^2 on control, methyl jasmonate, and girdled trees respectively. Average larval density was not significantly lower below the girdle than above it on girdled trees (T_{27}=-2.32, P=0.118) (Fig. 1.1). Larval density did not vary

among canopy exposure ranks ($F_{3,54}$=2.55, p=0.066). A $\log_{10}$ relationship existed between larval density and adults captured was significant ($F_{1,87}$=59.64, P<0.001) but relatively weak (Fig 1.2A).

Height significantly affected larval density on girdled trees only ($F_{7,644}$=10.46, P<0.001). Significantly lower densities of *A. planipennis* larvae were found between 2-4 m above ground compared with densities found at other heights (Fig 1.3A). The interaction between height and treatment was not significant with respect to larval density ($F_{14, 647}$=1.48, P=0.115). The percentage of total larvae in the tree also varied by height ($F_{7,474}$=11.53, P<0.001) for all treatments. The highest proportions of larvae were found between two to four meters above ground (Fig. 1.3B). The interaction between height and treatment significantly affected the percent of total larvae ($F_{14, 424}$=1.83, P=0.032). Larvae were more uniformly distributed with height on girdled compared to control and methyl jasmonate trees, which had a stronger peak between three and four m above ground (Fig. 1.3B). The proportion of logs with at least one larva did not vary significantly by height among vigor treatments ($F_{14,859}$=2.67; P<0.001) (Fig. 1.4A). After larval mortality was taken into account, the mean density of *A. planipennis* larvae that survived until the trees were debarked averaged 2.9 ± 0.7, 3.6 ± 1.4, and 47.9 ± 6.8 larvae per m^2 on control, methyl jasmonate, girdled trees, respectively.

Overall, an average of 28.1% ± 3.2% larvae per tree had reached the 4^{th} instar or were prepupal larvae when trees were debarked in fall or winter.

Densities of larvae were higher on girdled trees, and had a larger proportion of larvae reached the 4th instar or prepupal stage on these trees than on control and methyl jasmonate trees ($F_{2,18}$=19.95, P<0.001). On average, 16.0% ± 3.8%, 12.1% ± 4.2%, and 49.0% ± 4.3% of larvae were 4th instars or prepupae on control, methyl jasmonate, and girdled trees, respectively (Fig. 1.5). Within girdled trees, larvae below the girdle developed at more than two times the rate of larvae feeding above the girdle ($F_{3,27}$=40.16, P<0.001) (Fig. 1.5). Interestingly, larvae above the girdle did not develop significantly faster than larvae on control and methyl jasmonate trees (Fig. 1.5). Larval development did not vary among canopy exposure levels ($F_{3,42}$=1.80, P=0.162). When all trees were analyzed, larval development was not significantly related to with larval density ($F_{2,34}$=2.35, P=0.103). However, a significant relationship did occur between larval density and development when only girdled trees were analyzed ($F_{1,29}$=13.03, P=0.001) (Fig 1.6C). There was no significant relationship between larval density and development on control ($F_{1,18}$=1.22, P<0.285) (Fig 1.6A) or methyl jasmonate trees ($F_{1,19}$=0.00, P<0.949) (Fig. 1.6B).

Dimensions of galleries made by prepupal larvae averaged 3.8 ± 0.2 cm wide and 7.8 ± 0.4 cm long. Gallery width was correlated with gallery length ($F_{1,46}$=31.98, P<0.001 r^2=0.42). Gallery width did not vary by treatment ($F_{2,11}$=1.96, P=0.187) or canopy exposure ($F_{3,21}$=1.04, P=0.395). However, galleries were significantly wider when larvae fed below the girdle than above the girdle ($F_{3,57}$=52.74, P=0.001) (Fig. 1.7). Similarly, average length of galleries

was greater below the girdle than above, with mean lengths of 13.7 ± 1.1 cm and 6.9 ± 0.2 cm, respectively.

2007 Study

The number of *A. planipennis* adults captured on sticky bands increased substantially in 2007. A total of 426 adults were captured and at least one *A. planipennis* adult was captured on 20, 21, and 30 control, methyl jasmonate, and girdled trees, respectively. The largest number of beetles were captured in June, with 0, 269, 145, and 12 captured in May, June, July, and August, respectively. Peak capture occurred during the week preceding 21 June when 261 adults, or 35.7% of all beetles were captured. Girdled trees captured significantly more adults than control and methyl jasmonate trees. On average, 1.3 ± 0.2, 2.0 ± 0.4, and 10.9 ± 2.0 adults were captured on control, methyl jasmonate, and girdled trees, respectively ($F_{2,18}$=52.63, P<0.001). The number of adults captured did not vary by canopy exposure ($F_{4,56}$=1.48, P=0.222). Overall, 55.8% of adult beetles captured were female.

A total of 18,891 larvae were recorded on the 90 trees that were debarked, ranging from 0 to 1,862 larvae per tree. Overall, the mean density of *A. planipennis* larvae was 79.6 ± 9.8 larvae per m^2, a two-fold increase over 2006. At least one larva was found on 29, 29, and 30 of the control, methyl jasmonate, and girdled trees, respectively. A significantly higher density of larvae occurred on girdled trees than on control or trees treated with methyl jasmonate ($F_{2,18}$=30.87; P<0.001) (Fig. 1.1). Larval density averaged 38.4 ±

10.4, 25.8 ± 9.7, and 174.5 ± 14.3 larvae per m^2 on control, methyl jasmonate, and girdled trees, respectively (Fig. 1.1). The average larval density was not significantly lower below than above the girdle on girdled trees (T_{27}=-0.64, P=0.920) (Fig 1.1). Larval density did not vary by canopy exposure ($F_{6,50}$=1.08; P=0.386). A significant relationship existed between larval density and adults captured ($F_{1,89}$=66.40; P<0.001) (Fig 1.2B).

Significantly lower densities of *A. planipennis* larvae were feeding near the top of the tree than in other sections ($F_{7,859}$=26.09, P<0.001) (Fig 1.8A). With respect to density of *A. planipennis* larvae, there was no interaction between treatment and tree height ($F_{14,\ 859}$=1.34, P=0.178). The percentage of total larvae in the tree varied by height ($F_{7,583}$=26.53, P<0.001) for all treatments; the highest proportion of larvae was consistently found 2-4 m above ground (Fig. 1.8B). The interaction between height and treatment significantly affected the distribution of larvae by height ($F_{14,\ 583}$=2.67, P=0.001). As in 2006, larvae on girdled trees were more evenly distributed along the trunk and leader than on control and methyl jasmonate trees (Fig 1.8B). The proportion of logs that had at least one larva varied on the control and methyl jasmonate trees with height ($F_{14,859}$=2.67; P<0.001), but not on girdled trees (Fig 1.4B). The highest proportion of infested logs was from trunk sections 3-4 m above ground and the lowest proportion of infested logs were from the basal trunk section, within 1 m above ground (Fig. 1.4B). After larval mortality was accounted for, the density of

A. planipennis larvae averaged 25.1 ± 4.6, 19.0 ± 7.3, and 111.2 ± 9.8 larvae per m^2 on control, methyl jasmonate, and girdled trees, respectively.

Overall, 88.7% of larvae that began feeding reached the 4^{th} instar or were prepupal larvae when trees were felled. Larvae developed significantly faster on girdled trees than on control and methyl jasmonate trees ($F_{2,18}$=18.07, P<0.001). On average 69.0% ± 4.2%, 64.1% ± 4.8%, and 81.3% ± 3.4% of larvae were 4^{th} instars or prepupae on control, methyl jasmonate and girdled trees, respectively. Unlike in 2006, larvae below the girdle did not develop significantly faster than larvae above the girdle on girdled trees (T-value$_{27}$=1.92, P=0.244) (Fig 1.5). Larval development did not vary among canopy exposure classes ($F_{4,54}$=2.24, P=0.077) and development rate was not significantly related to larval density ($F_{2,86}$=0.39, P=0.676) (Fig. 1.9). One data point appeared to be an outlier and was removed from the analysis, but this did not alter the result ($F_{2,86}$=1.05, P=0.356).

Galleries formed by larvae that had reached the prepupal stage averaged 3.5 ± 0.1 cm wide and 8.6 ± 0.3 cm long. Similar to 2006, there was a significant correlation between gallery width and length, but this relationship only accounted for a small amount of the variation ($F_{1,83}$=7.03; P=0.009; r^2=0.08). Gallery width did not vary by treatment ($F_{2,11}$=1.96; P=0.187). As in 2006, larvae that developed below the girdle on girdled trees had galleries that were significantly wider than larvae above the girdle ($F_{3,27}$=78.92; P<0.001) (Fig. 1.7). Similarly,

mean gallery length was longer below the girdle than above, with a length of 15.5 ± 1.5 cm and 7.3 ± 0.1 cm, respectively.

DISCUSSION

Adult and larval distribution

A. planipennis adults, including ovipositing females, were more attracted to girdled trees than to control or methyl jasmonate trees. These findings are similar to those from other field studies of *A. planipennis* and other buprestids. *Agrilus bilineatus* (Weber) (two-lined chestnut borer) (Haack and Benjamin 1982, Dunn et al. 1986a, 1986b), *Agrilus anxius* (bronze birch borer) (Barter 1957), and *Agrilus burkei* (flatheaded borer) (Svihra and Koehler 1993), are attracted to declining oaks (*Quercus sp.*), birch trees (*Betula*), and alders (*Alnus*), respectively. A meta-analysis found that wood boring insects were attracted to trees stressed by drought at higher rates than vigorous trees (Koricheva and Larsson 1998). *A. planipennis* follows this pattern in its native range China, attacking primarily stressed trees (Akiyama and Ohmomo 2000, Schaefer 2005, Williams et al. 2005, 2006). Field studies in North America using girdled trees to attract *A. planipennis* adults have produced similar results; girdled trees attracted significantly more adults than non-girdled trees (Poland et al., 2004, 2005, McCullough et al. 2006, Fraser and Mastro 2007, Anulewicz et al. 2007, Anulewicz et al. 2008b). However, these differences are obscured in sites with high densities of *A. planipennis* (McCullough et al. 2009 submitted). In this study, the relationship between the number of adults captured and the density of larvae was weak and highly variable.

Adult capture rates did not vary between control and methyl jasmonate trees. An earlier trap tree study also found that girdled trees captured significantly more larvae than either control or methyl jasmonate trees, which did not differ from each other (McCullough et al. 2006). While methyl jasmonate had no affect on *A. planipennis* distribution or development in this study, it can induce stress responses in other plants. Methyl jasmonate induced volatile emissions in cotton plants (Rodriguez-Saona et al. 2001) and ash seedlings in a laboratory setting (Poland et al. 2006). Subsequent tests showed *A. planipennis* adults were attracted to volatiles produced by ash seedlings treated with methyl jasmonate (Poland et al. 2006). Methyl jasmonate generates stress responses in conifer saplings by activating the jasmonic pathway (Hudgins and Franceschi 2004). It is possible that the effect of methyl jasmonate is minimal on large trees, or the dose used was too low to elicite a stress response. Many Previous studies using methyl jasmonate generally involved treating seedlings or small, herbaceous plants while the trees in this study were about 10 m tall. Aerts et al. (1994) found that methyl jasmonate vapor increased the synthesis of protective alkaloids in the herbaceous plant *Catharanthus roseus* L. G. Don, but only while the plant is a seedling. Henery et al. (2007) found that methyl jasmonate did not increase the production of defensive chemicals in 15-month-old *Eucalyptus grandis* Hill ex Maiden trees. Further studies will be needed to determine why methyl jasmonate had no effect.

Overall, adult and larval density increased by a factor of five from 2006 to 2007. This increase has been observed at other sites where adult *A. planipennis*

captures increase between three- to ten-fold between 2006 and 2007 (Anulewicz et al. 2008). There was a drop in the proportion of captured adults that were female from about 75% in 2006 to about 55% in 2007. Other trapping studies have not found similar divergences in sex ratios (McCullough et al. 2009). The sex ratio in 2006 may have been due to random chance, or more females were captured on the sticky bands because they were ovipositing.

McCullough and Siegert (2007) reported that the average density of *A. planipennis* in killed trees varied between 105 adults per m^2 on trees with a DBH of $\geq$13 cm and 69 adults per m^2 on trees with a DBH of <13 cm. Similar results were found in 2007 on girdled trees after taking mortality into account. Larval densities on non-girdled trees were lower than the adult yield reported by McCullough and Siegert (2007). The control and methyl jasmonate trees did not appear to be declining when they were felled, and would certainly have produced additional *A. planipennis* adults in later years. Larval densities were also lower on girdled trees in 2006 than the adult yield reported by McCullough and Siegert (2007). While the girdled trees in 2006 would not likely survive for a second year to be infested again because of the artificial wounding, the phloem was probably under-utilized by *A. planipennis* larvae due to the low density that existed that year.

While adult catches and larval density did not differ due to canopy exposure in this study, previous studies have shown that buprestids are attracted to trees exposed to sun or in open areas (Anulewicz et al. 2007, Wermelinger et al. 2007). Anulewicz et al. (2007) found about 90% of all captured *A. planipennis*

adults were on trees fully exposed to the sun or exposed on three of four sides. Wermelinger et al. (2007) found that buprestid species such as *Anthaxia nitidula* (Linné), *Agrilus olivicolor* Kiesenwetter, *Agrilus viridus* (Linnaeus), *Agrilus convexicollis* Redtenbacher, and *Agrilus laticollis* Kiesenwetter prefered edge habitats to the interior of the forest. The plantation in my study was small and intermixed with canopy gaps. Sunlight was able to penetrate most of the stand and likely obscured effects from canopy exposure rank that were found to be significant in other studies.

Larval densities were significantly lower near the top of the tree than in lower portions of the tree. Previous studies found no differences in buprestid adult capture by height (Wermelinger et al. 2007). McCullough et al. (2006) placed sticky bands at the height of 1.5 m, and at 3-5 m above ground in trap trees, as well as sticky panel traps in the canopies. They found no differences in adult capture of *A. planipennis* adults at different heights on trap trees. The proportion of logs infested varied by height for control and methyl jasmonate treated trees; significantly higher proportion of logs from the trunk between 2-5 m were infested compared with logs from other heights within trees. Differences in larval densities by height were not as pronounced on girdled trees. This may be because the high population density of *A. planipennis* larvae utilized more of the phloem on girdled trees. Additionally, more larvae were found on the lowest one-meter log from girdled trees than on one-meter logs from control or methyl jasmonate treated trees. This may have been more attractive to *A. planipennis* adults, therefore leading to higher oviposition. Larval survival may also be higher

on below the girdle. Haack and Benjamin (1982) found that *A. bilineatus* adults were captured at seven times the rate on girdled oaks compared with controls and that *A. bilineatus* larva were also found at higher densities on oak trees that had lower reserves of stored starch. Starch reserves in oak trees have been found to be lower after defoliation events, drought, and on trees infested with high levels of *A. bilineatus* (Haack and Benjamin 1982). Haack and Benjamin (1982) suggested that lower starch levels either led to higher levels of infestation, were the result of larval girdling, or a mix of the two. *A. planipennis* found below the girdle might have developed faster because the girdle cut off the flow of nutrients from leaves down the tree, creating a lower level of starch.

The trees used in this experiment were similar in size to trees used as detection trees in surveys for *A. planipennis* adults in Michigan and other states (Flint 2005, Storer et al. 2005, Harrison 2005). While trees used in this study were in a plantation, and detection trees are commonly located along roads, patterns of larval distribution are probably similar.

Larval Development

Overall, treatment was the primary factor affecting the larval development of *A. planipennis* in both 2006 and 2007, with larvae developing faster on girdled trees than on control and methyl jasmonate trees. Additionally, in 2006, larvae below the girdle developed significantly faster than larvae found above the girdle. Larvae found above the girdle in 2006 did not develop at significantly different rates than larvae on control and methyl jasmonate trees. Furthermore, larval

development rates do not appear to be heavily influenced by larval density. Even though a higher proportion of larvae reached the fourth instar or prepupae below the girdle than above the girdle, the larval density was lower below the girdle than above the girdle.

While *A. planipennis* larval density did not significantly affect larval development at the scale of the individual tree, there may be a relationship between larval density and development at a larger scale. The number of adults and larvae in the entire plantation increased by a factor of five from 2006 to 2007 which coincided with a five-fold increase in the proportion of larvae that reached 4^{th} instar or the prepupal stage. This pattern has been found in other field studies as well. Low density sites had large numbers of overwintering early instar, while larvae only occasionally overwintered as early instars in moderately to heavily infested sites (Cappaert et al. 2005b).

The paradox of different rates of larval development at the scale of the individual tree and the entire site might be explained by variables associated with the hosts or by changes in adult *A. planipennis* behavior at different population densities. Individual trees might vary genetically or inhabit a different microclimate, which may cause some trees to be under more stress, and therefore more susceptible to attack. *A. planipennis* adults might also behave differently in high density populations compared with low density populations. For example, Wallin and Raffa (2004) found that host preferences of adult spruce beetles, *Dendroctonus rufipennis* (Kirby), attacking *Picea glauca* (Moench) and *Picea engelmannii* (Parry and Engelm), differed between endemic and epidemic

populations. *D. rufipennis* in low population densities were found to only attack felled trees and *D. rufipennis* in high population densities attacked vigorous hosts as well (Wallin and Raffa 2004). *A. planipennis* might exhibit a similar adult density-dependent behavior, with larvae produced by adults in high adult density populations developing faster than larvae found in low adult density populations. Further studies will be needed to determine why larval development appears to be affected by larval density at the scale of the plantation but not at the scale of the individual tree.

Average gallery width was dramatically greater below the girdle, matching the faster larval development rate. Galleries found above the girdle and on non-girdled trees formed the standard serpentine pattern, while galleries found below the girdle did not follow this pattern. Larvae below the girdle usually fed in random directions. This pattern was also found by Barter (1957) in *A. anxius* larvae on birch trees. Barter (1957) speculated that larvae form serpentine galleries to help slow cambial activity, which may interfere with larval development. By blocking the downward flow of nutrients or defensive compounds, the gallery functions like a small girdle. It would be interesting to determine if *A. planipennis* larvae feed downwards more commonly than they feed upwards, and if feeding direction is affected by girdling. Future studies are needed to elucidate the effects that damage from artificial wounding and from *A. planipennis* feeding has on the nutritional and chemical content of phloem.

In North America, native buprestids are known to attack and kill stressed and wounded trees at far higher rates than vigorous trees. Barter (1957) found

that *A. anxius* had survival rates of only 7-25% on healthy birch trees and that all surviving larvae took at least two years to develop. Barter (1957) also found that as more larvae attacked a tree, with densities of 5 and 12 larvae per m^2, the mortality decreased and about 1-5% of larvae developed in one year.

Drought and water stress may have played a role in our study. Precipitation patterns varied during the first four to six weeks of larval feeding between 2006 and 2007. Larvae typically begin to feed in mid to late July (Cappaert 2005b). First instars were found in Jackson County on 17 July in 2006 (Chapter 4). Weather records for nearby Haslett, Michigan (www.wunderground.com) indicated a much drier July in 2007 compared with 2006, with 13.6 cm, and 4.1 cm of rain falling in all of July and the first two weeks of August in 2006 and 2007, respectively. Precipitation in the last two weeks of August was 5.4 cm and 13.9 cm for 2006 and 2007, respectively. Therefore, total rainfall was greater in 2007 compared with 2006, but it occurred later in the season. For comparison, the 30-yr precipitation average for the months of July and August are 8.5 ± 0.7 cm and 8.7 ± 0.9 cm, respectively. Further studies are needed to clarify whether short-term drought can affect the development of *A. planipennis*. Studies of other buprestids have found that water stress plays an important role in larval development of buprestids. Trees that are stressed by drought had higher densities of buprestid larvae (Koricheva and Larsson 1998). Mattson and Haack (1987) suggested that hosts may become more suitable during droughts because plant nutrients become either more concentrated or better balanced. Trees under drought stress have been known to form localized

zones of cambial and phloem tissue degeneration (Santamour 1990). Water and carbon manipulations using irrigation and girdling affect stem diameter growth in *Juglans nigra* (black walnut) trees (Daudet et al. 2005). Tree diameter was shown to grow at lower rates below the girdle and to slow dramatically during a drought. Stem diameter growth was also shown to recover quickly after a drought.

While no studies have been performed directly linking tree traits such as bark moisture or callus growth to *A. planipennis* development, several studies have been performed on other wood boring insects. Hanks et al. (1999) found that moisture content of bark affects feeding behavior of *Phoracantha semipunctata* (Fabricius) (Cerambycidae), with fewer larvae reaching the cambium on logs with moist bark compared with logs that had dry bark in a laboratory setting. Additionally, Hanks et al. (1999) found that *P. semipunctata* larvae feeding on an artificial substrate survived at higher levels in dry compared with wet conditions. Fierke and Stephen (2008) found that the number of attacks by *Enaphalodes rufulus* (red oak borer) larvae in the field were not affected by the level of bark moisture, but were affected by the level of callus overgrowth on wounds. This was also found in *A. bilineatus* on oaks, with adults attacking trees at higher rates that had lower levels of callus overgrowth (Dunn 1990). It is possible that *A. planipennis* development is responding in a similar fashion to tree stress.

Climate was found to be the strongest determining factor of whether *A. planipennis* larvae in China would develop in one or two years. Specifically,

larvae required at least 150 frost-free days in order to develop (Wie et al. 2007). Therefore, larvae took two years to develop in the northern province of Heilongjiang 45.45 °N (Yu 1992), one year to develop in the more southerly Liaoning Province 41.8 °N (Zhao et al. 2005), and either one or two years to develop in the transitional area of Jilin Province (Wie et al. 2007). My site in Clinton County is at 42.7 °N, corresponding to the transitional area in China where larvae take 1-2 years to develop.

Similarly, development of *A. anxius* has been found to vary by latitude. *A. anxius* larvae reportedly take at least two years to develop in New Brunswick and Quebec (Barter 1957) but one year to develop in Connecticut and New York. The development of *A. anxius* varies in Minnesota and Maine, taking one or two years to develop depending on host vigor (Anderson 1944, Nash et al. 1951). Barter (1957) found that even in northern locations, larvae could develop in one year on plant tissue that was fairly moribund, including on girdled trees that had been attacked repeatedly. To date, development of *A. planipennis* has not been shown to vary with climate or latitude in North America. Both one and two-year development has been found in sites in Michigan. It may be possible that as *A. planipennis* spreads further north and south, climate may have a larger impact on its larval development rate.

Table 1. Mean (± SE) tree DBH, height, surface area sampled per tree, phloem thickness, and the percent of larvae developing in one year for three treatments of plantation trees in 2006 and 2007

	Control	Methyl jasmonate	Girdled
2006 trees			
No. trees	30	30	30
Mean DBH (cm)	12.2 ± 0.6	12.4 ± 0.4	12.3 ± 0.5
Mean tree height (m)	11.1 ± 0.3	10.8 ± 0.3	10.6 ± 0.3
Mean surface area sampled (m^2)	2.4 ± 0.1	2.4 ± 0.2	2.3 ± 0.2
Mean % of 1 year larvae	16.0 ± 3.8%	12.1 ± 4.2%	49.0 ± 4.3%
2007 trees			
No. trees	30	30	30
Mean DBH (cm)	12.1 ± 0.5	12.7 ± 0.7	13.0 ± 0.5
Mean tree height (m)	10.7 ± 0.3	10.5 ± 0.4	10.9 ± 0.3
Mean phloem thickness (mm)	2.4 ± 0.6	2.5 ± 0.7	2.5 ± 0.5
Mean surface area sampled (m^2)	2.6 ± 0.2	3.2 ± 0.4	3.1 ± 0.3
Mean % of 1 year larvae	69.0 ± 4.2%	64.1 ± 4.8%	91.9 ± 1.8%

Was not significant for any variable in either 2006 or 2007 ($P>0.05$)

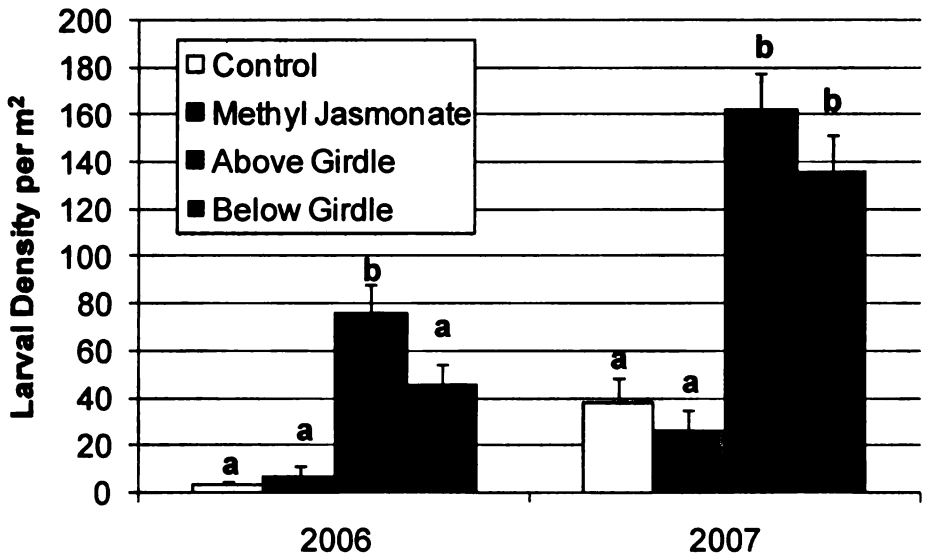


Fig. 1.1 Mean (± SE) density of *A. planipennis* larvae per m^2 on control and methyl jasmonate trees, and above and below the girdle on girdled trees in 2006 and 2007. Within year means with the same letter are not significantly different (Tukey protected LSD test; $P<0.05$).

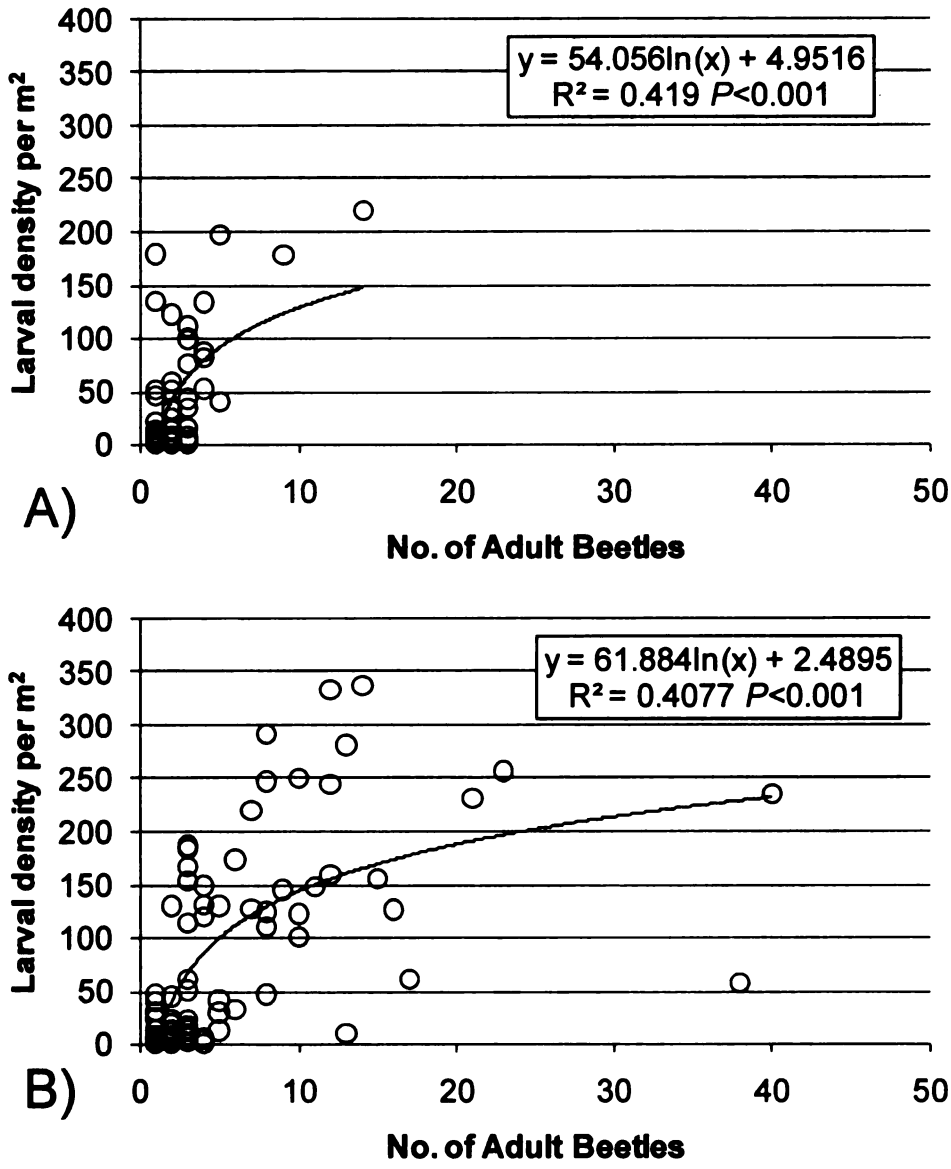
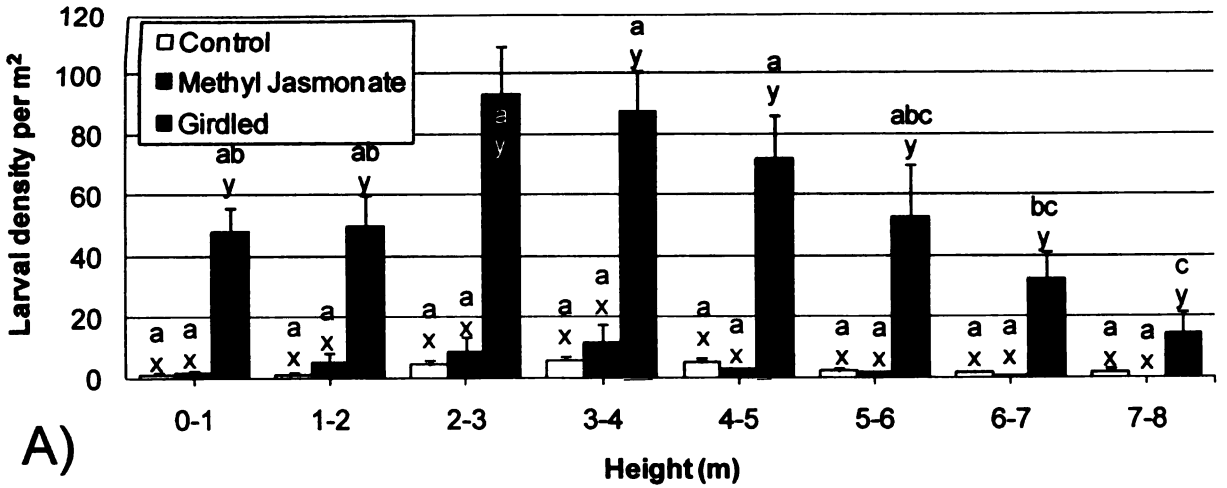
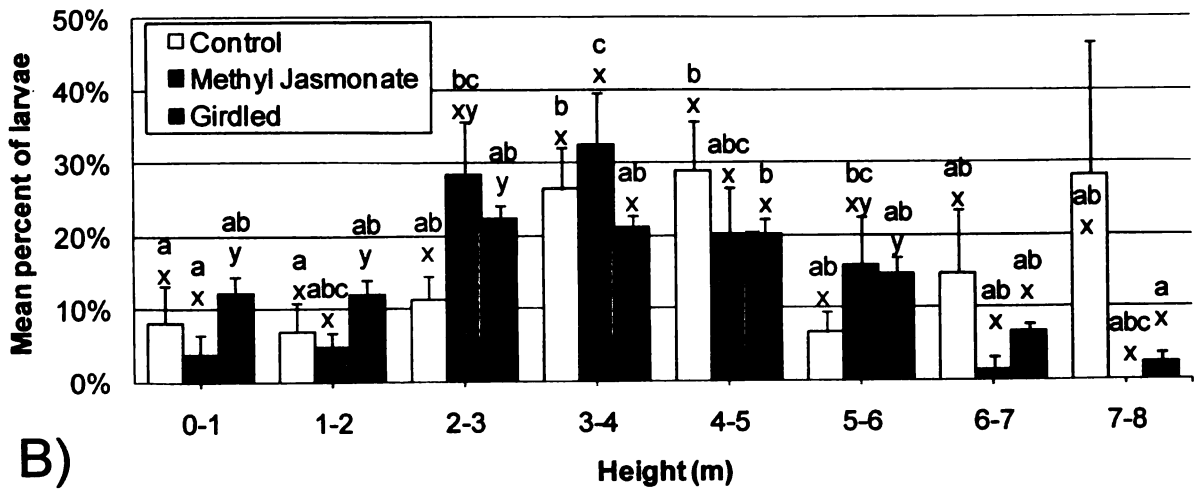


Fig. 1.2 $Log_{10}(x+1)$ relationship between *A. planipennis* larvae per m^2 and total adult capture on sticky bands on trees in 2006 (A) and 2007 (B).

Fig. 1.3 **A** Mean (± SE) larval density by height in trees in 2006. **B** Mean (± SE) percent of larvae by height in trees in 2006. Means for different heights within of the same treatment with the same letter (a, b, and c) are not significantly different. Means within the same height of different treatments with the same letter(x and y) are not significantly different (Tukey protected LSD test; $P < 0.05$).

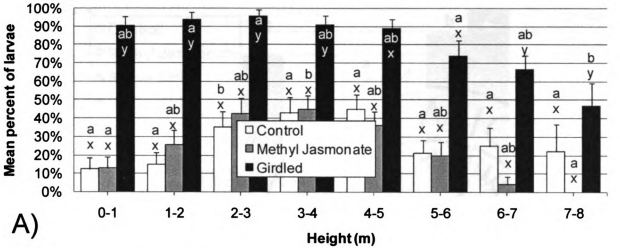

Control
Methyl Jasmonate
Girdled
Larval density per m²
120
100
80
60
40
20
0
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
Height (m)

A)

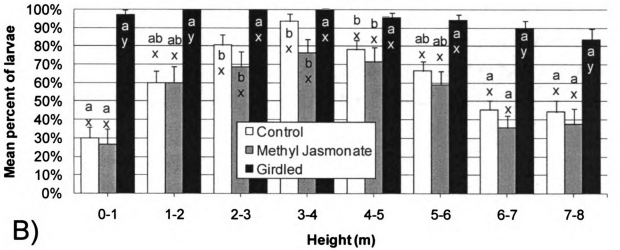

Control
Methyl Jasmonate
Girdled
Mean percent of larvae
50%
40%
30%
20%
10%
0%
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
Height (m)

B)

Fig. 1.4 Mean (± SE) percent of sections by height that were infested with *A. planipennis* larvae for 2006 (**A**) and 2007 (**B**). Means for different heights within the same treatment with the same letter (a, b, and c) are not significantly different. Means within the same height of different treatments with the same letter(x and y) are not significantly different (Tukey protected LSD test; $P < 0.05$).


Mean percent of larvae
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Control
Methyl Jasmonate
Girdled
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
Height (m)

A)


Mean percent of larvae
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Control
Methyl Jasmonate
Girdled
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
Height (m)

B)

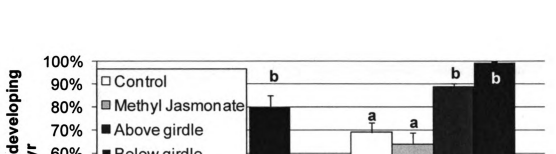
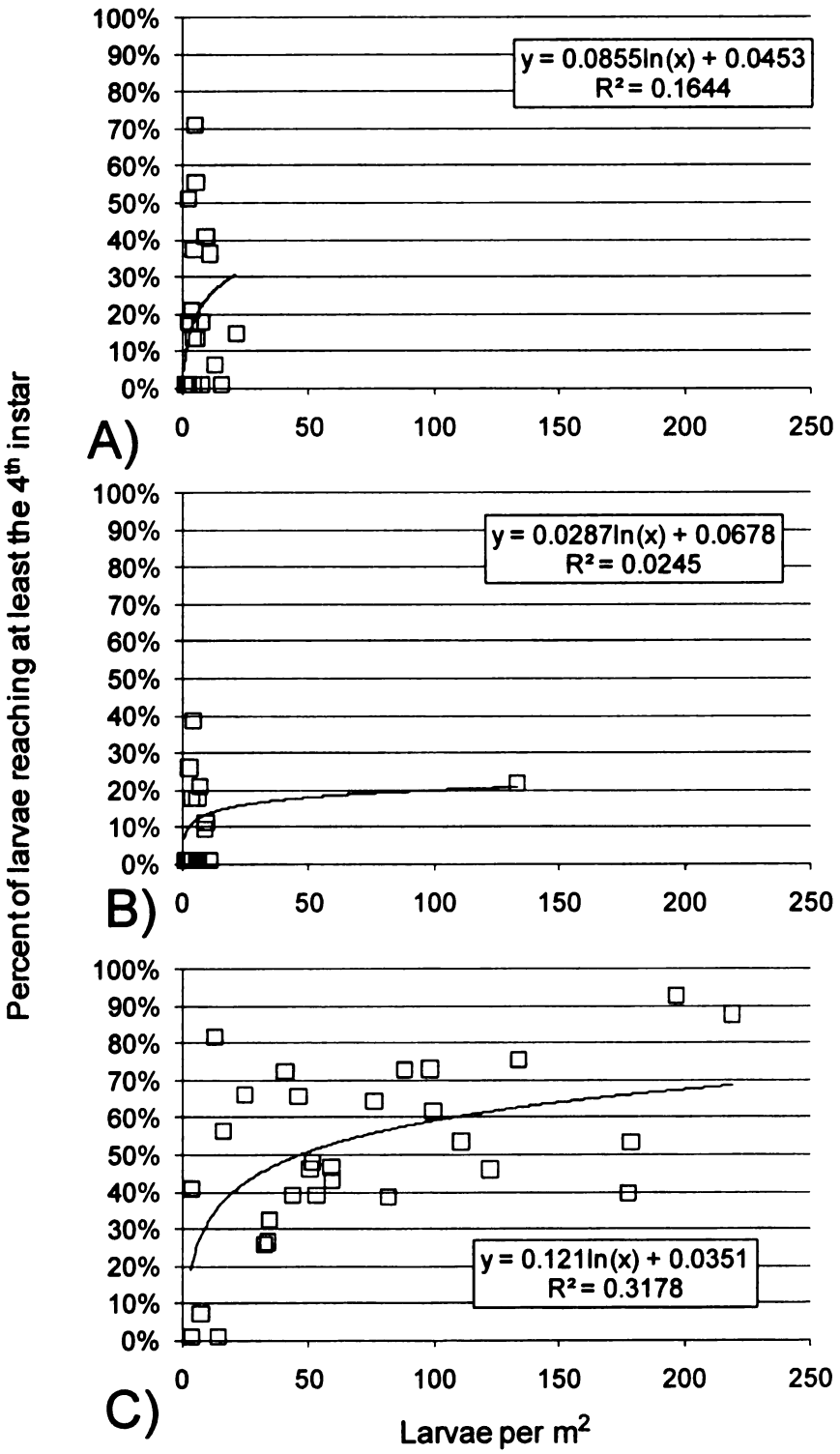


Fig. 1.5 Mean (± SE) percentage of *A. planipennis* larvae that had reached at least 4th instar on control and methyl jasmonate trees, and above and below the girdle on girdled trees in 2006 and 2007. Within year, means with the same letter are not significantly different (Tukey protected LSD test; $P < 0.05$).

Fig. 1.6 The $\log_{10}+1$ relationship between the percent of 1-yr larvae and larvae per m square on control (A), methyl jasmonate (B) and girdled (C) trees in 2006 (GLM; Only girdled trees were significant $P < 0.05$).


Percent of larvae reaching at least the 4th instar
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
y = 0.0855ln(x) + 0.0453
R² = 0.1644
A)
0
50
100
150
200
250
y = 0.0287ln(x) + 0.0678
R² = 0.0245
B)
y = 0.121ln(x) + 0.0351
R² = 0.3178
C)
Larvae per m2

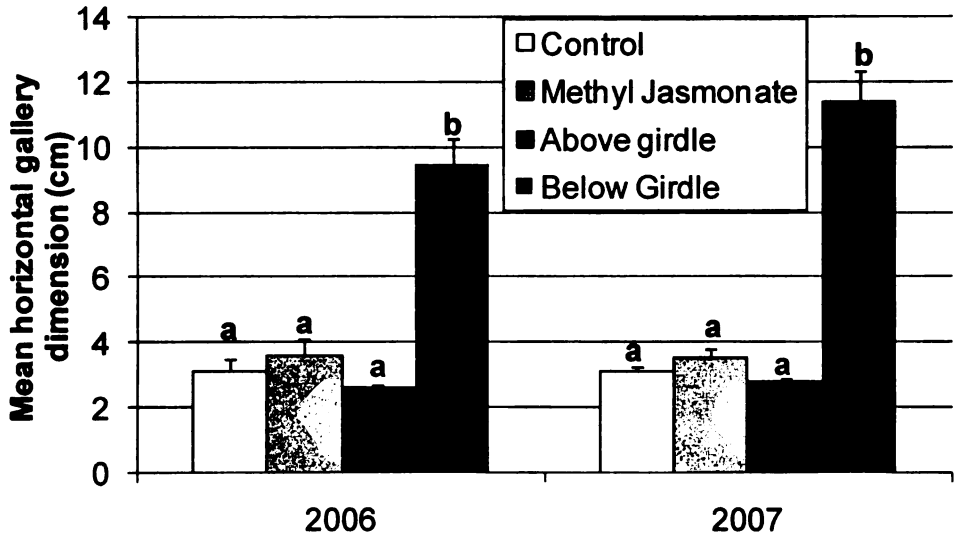


Fig. 1.7 Mean (± SE) width of *A. planipennis* prepupal galleries on control and methyl jasmonate trees, and above and below the girdle on girdled trees in 2006 and 2007. Within year, means with the same letter are not significantly different (Tukey protected LSD test; $P<0.05$).

Fig. 1.8 **A** Mean (± SE) larvae density m^2 by height in tree in 2007. **B** Mean (± SE) percent of total larvae by height in tree in 2007. Means for different heights within the same treatment with the same letter (a, b, c, and d) are not significantly different. Means within the same height of different treatments with the same letter(x and y) are not significantly different (Tukey protected LSD test; $P < 0.05$).

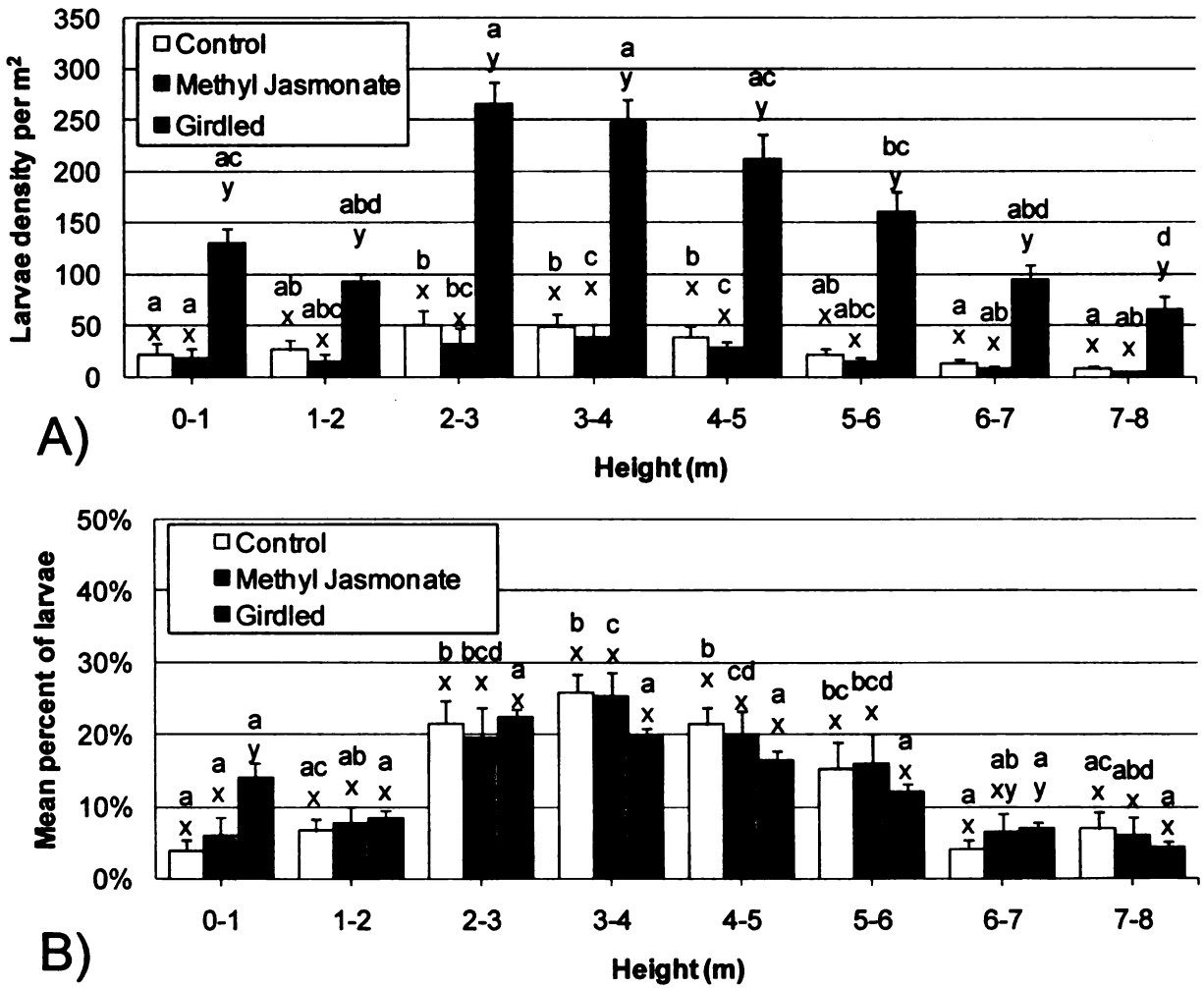
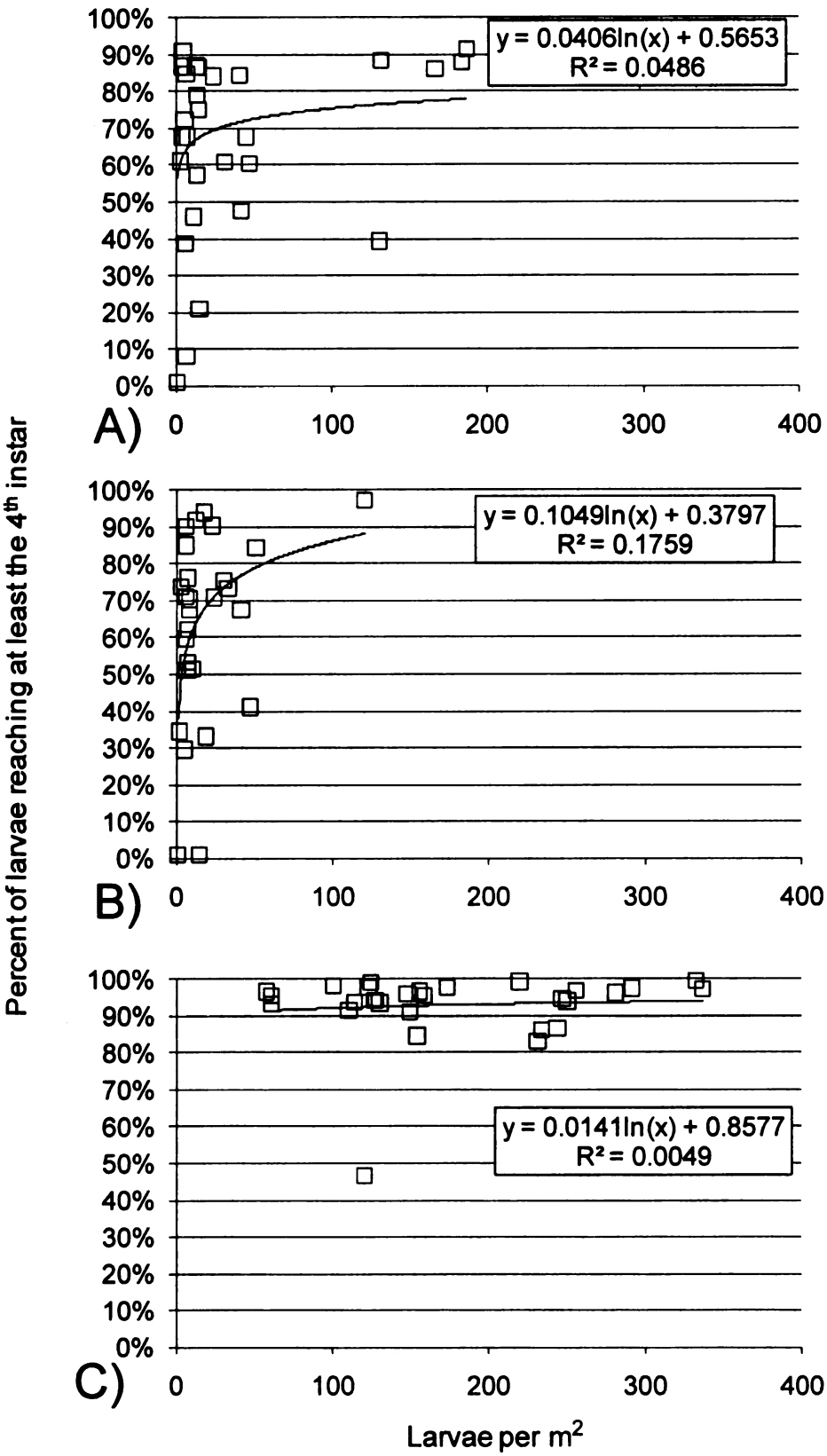

Control
Methyl Jasmonate
Girdled
Larvae density per m²
350
300
250
200
150
100
50
0
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
Height (m)
A)
Mean percent of larvae
50%
40%
30%
20%
10%
0%
Height (m)
B)

Fig. 1.9 The $\log_{10}+1$ relationship between the percent of 1-yr larvae and larvae per m square on control (A), methyl jasmonate (B) and girdled (C) trees in 2007 (GLM; No relationships were significant, $P < 0.05$).


Percent of larvae reaching at least the 4th instar
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
y = 0.0406ln(x) + 0.5653
R² = 0.0486
A)
0
100
200
300
400
y = 0.1049ln(x) + 0.3797
R² = 0.1759
B)
y = 0.0141ln(x) + 0.8577
R² = 0.0049
C)
Larvae per m2

CHAPTER 2

Mortality of *Agrilus planipennis* larvae in green ash trees

INTRODUCTION

Like other insects, Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), larvae can die from a variety of causes including woodpecker predation, pathogens, cannibalism, and parasitism. After the discovery of *A. planipennis* in southeastern Michigan in June 2002, about ten years after its introduction from northwest China, studies were conducted to determine the types and rate of mortality. Bauer et al. (2004) found that less than 2% of larvae were infected with entomopathogenic fungi. Cappaert et al. (2005b) and Lindell et al. (2008) reported that woodpeckers consume highly variable proportions of larvae; e.g., ranging from 0-95% of larvae (Cappaert et al. 2005b). Lindell et al. (2008) found that native woodpeckers have responded to new infestations of *A. planipennis* by spending more time foraging on ash trees than they did before the trees became infested.

Parasitoids that attack *A. planipennis* larvae and eggs have been identified in China and in North America (Yang et al. 2005, 2006, Bauer et al. 2005, Bauer and Liu 2006, Liu and Bauer 2007a, Gould et al. 2007, Liu et al. 2008, Cappaert and McCullough 2008). The parasitoids *Oobius agrili* Zhang and Huang, and *Tetrastichus planipennisi* Yang were estimated to reduce the total population of *A. planipennis* by an estimated 73.6% in four sites near Changchun

city, in the Jilin Province of China (Liu et al. 2007b). Trial releases of these parasitoids have now occurred in Michigan. Parasitoids native to North America infest less than 1%, of larvae and eggs (Bauer et al. 2004).

A comprehensive survey of *A. planipennis* larval mortality in a population in North America has not yet been performed. Data on the factors and rates of larval mortality would be useful for researchers developing models of *A. planipennis* rate of spread and population dynamics and for control or management efforts.

This study had four objectives including (1) identify the factors causing *A. planipennis* larvae to die; (2) determine the mortality rates for each factor; (3) determine whether host vigor affects mortality factors or rates; and (4) determine whether mortality factors and rates vary with *A. planipennis* larval density.

MATERIALS AND METHODS

For this study, I used the green ash, *Fraxinus pennsylvanica* Marshall (Oleaceae), trees at the Rose Lake plantation described in Chapter 1. As explained in Chapter 1, ten blocks of 9 trees each (total of 90 trees) were selected and treated in spring 2006, then felled and debarked the following winter. In 2007, an additional ten blocks of 9 trees each were similarly selected, treated, then felled and debarked the following winter. Trees within each block were randomly assigned to one of three stress treatments each year. Trees were either girdled (30 trees), exposed to the stress-elicitor methyl jasmonate (30 trees), or left as untreated controls (30 trees) as described in Chapter 1.

The number and instar of each *A. planipennis* larvae on each log were recorded. Larval density was standardized per m^2 of exposed surface area per tree as in Chapter 1. Developmental instar was determined by head capsule width (Cappaert et al. 2005b). When a larva was missing from its gallery, instar was estimated based on the gallery size compared to galleries constructed from larvae of known instar. The month in which blocks of trees were felled was recorded.

Larval mortality caused by woodpecker predation, unknown causes, and cannibalism was recorded for each log. *A. planipennis* larvae preyed on by woodpeckers were identified by matching holes made by woodpeckers with galleries of removed larvae. Mortality from unknown causes was recorded when the larva apparently died from disease or desiccation but was still present in the

gallery. Cannibalism was previously defined by Elgar and Crespi (1992) as the killing and consumption of intraspecific individuals. A larvae was considered to have been cannibalized when either; a remnant of a larva was discovered with a gallery from another larva intersecting its body or when a gallery abruptly ended at another gallery and the associated larva was absent.

I assumed that larvae overwintering as 1st, 2nd or 3rd instars would probably not reach the prepupal stage by the spring, and would require another summer of feeding, resulting in a two year life cycle (Cappaert et al. 2005a). Larvae overwintering as 4th instars or as prepupae would likely emerge in the spring as an adult, resulting in a one year life cycle (Cappaert et al. 2005a). Larvae hatching in 2006 but requiring two years to develop would not contribute to the adult population in 2007. Therefore, larvae with delayed development would not reproduce in the subsequent year and were effectively "lost" from the current years' cohort. A few larvae were found in 2006 that had hatched and begun developing during the summer of 2005. Larvae that required two years to develop were identified by gallery appearance and callus tissue that had begun to grow over the older section of the gallery (Cappaert et al. 2005b, Siegert et al 2007). These larvae were recorded separately from larvae that hatched and began developing in 2006.

The 2006 study was replicated with trees felled and debarked trees from October 2007 through February 2008.

Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Three response variables were evaluated including the proportion of larvae fed upon by woodpeckers, the proportion of larvae that died due to unknown causes, and the proportion of larvae that died due to cannibalism. Larval density was used as a covariate and required a $\log_{10}(x+1)$ transformation to achieve normality.

All response variables were analyzed using the GLIMMIX procedure for mixed models in SAS statistical software (PROC GLIMMIX; SAS Institute 2003). Effects of three factors: tree vigor with three levels (control, methyl jasmonate, or girdled), canopy exposure rank with five levels (1, 2, 3, 4, 5), and height in tree with eight levels (one m increments above ground) were analyzed. Block and block crossed with treatment were used as random factors. Differences among treatment means were tested as unplanned comparisons and multiple comparison tests were applied only when overall analysis of variance (ANOVA) was significant ($P<0.05$). When significant differences occurred among levels, the Tukey protected least significant difference (LSD) test was used to evaluate those differences (Ott and Longnecker 2001). The $\log_{10}$ relationship between larval density and the three classifications of mortality was analyzed using a general linear model with a $\log_{10}(x+1)$ transformation (PROC GLM, SAS institute 2003).

Life-tables

Life-tables were constructed using *A. planipennis* larvae recorded when trees were debarked each year. The total number of larvae found was used as the initial cohort for the population of *A. planipennis* for that year. My study focused on *A. planipennis* larvae and did not attempt to quantify eggs. *A. planipennis* adults oviposit in bark crevices or under bark flaps, making detection difficult on trees with flaky periderm (Anulewicz et al. 2008a). The apparent loss, or age-specific mortality, was a measure of the mortality within a life stage. Apparent loss was calculated by dividing the total number of dead individuals for each stage by the total number of larvae in that life stage. The real mortality, or real loss, measured the total contribution of mortality at a life stage to the total population. Real mortality was calculated by dividing the total number of dead individuals for each stage by the total number of larvae found. Live larvae overwintering as 1^{st}, 2^{nd}, or 3^{rd} instars were counted as a loss for the adult population of the following year but were added to the potential adult population for the following year.

RESULTS

2006 Study

Mean density of *A. planipennis* larvae was 34.5 ± 5.8 larvae per m^2 per tree and at least one larva was found on 24, 21, and 30 of the control, methyl jasmonate, and girdled trees, respectively. Larval density averaged 3.7 ± 0.9, 6.7 ± 4.4, and 71.1 ± 10.9 larvae per m^2 on control, methyl jasmonate, and girdled trees respectively. Larval density was significantly higher on girdled trees than control and methyl jasmonate trees ($F_{2,18}$=85.02, P<0.001).

Out of 4,621 larvae recorded in 2006, 1438, or 30.1%, of all larvae died. On average, 17.1% ± 2.1% of the larvae per tree died (Table 2.1). Mortality varied by vigor treatment, with a slightly higher proportion of larvae dying on girdled trees than control and methyl jasmonate trees ($F_{2,18}$=5.62, P=0.013). Average larval mortality was 10.3% ± 2.9%, 15.0% ± 3.9% and 24.0% ± 3.0% dying on control, methyl jasmonate, and girdled trees, respectively. The proportion of apparent mortality, or the total loss within an instar, was highest 4^{th} instars; overall, 9.7%, 36.9%, 71.6%, and 30.4% of 1^{st} and 2^{nd}, 3^{rd}, 4^{th} instars, and prepupae died, respectively (Table 2.1). The real loss, or the total loss in the population, by instar was highest at the 4^{th} instar and the prepupae stage; 3.0%, 6.5%, 10.3%, and 11.4 of 1^{st} and 2^{nd}, 3^{rd}, 4^{th} instars, and prepupae died, respectively (Table 2.1).

Woodpecker predation caused the highest larval mortality, accounting for 9.9% ± 2.0% of all larvae. A significantly higher proportion of woodpecker predation occurred on girdled trees than on control or methyl jasmonate trees

($F_{2,18}$=16.30, P<0.001). On average, woodpeckers killed 4.8% ± 2.2%, 2.6% ± 1.3%, and 19.1% ± 2.7% of larvae on control, methyl jasmonate, and girdled trees, respectively. A significant $\log_{10}(x+1)$ relationship existed between larval density and woodpecker predation ($F_{1,40}$=21.82, P<0.001) (Fig. 2.1). Woodpeckers preyed on 4th instars at significantly higher rates than prepupae, 3rd instars, and 1st and 2nd instars ($F_{3,175}$=18.80, P<0.001) (Table 2.1). Height in tree significantly affected woodpecker predation rates ($F_{7,280}$=7.42, P<0.001). More predation occurred near the top of the tree than near the bottom of the tree (Fig. 2.2). The proportion of larvae killed by woodpeckers was not affected by month ($F_{3,42}$=0.82, P=0.492) (Fig. 2.3). Canopy exposure rank also did not significantly affect woodpecker predation ($F_{3,42}$=2.92, P=0.045).

Larval mortality from unknown causes accounted for 6.9% ± 1.5% of all larvae and did not vary by vigor treatment ($F_{2,18}$=2.62, P=0.100). The proportion of mortality from unknown causes averaged 5.5% ± 1.9%, 9.2% ± 2.6%, and 4.3% ± 0.9% of larvae on control, methyl jasmonate, and girdled trees, respectively. On girdled trees, however, larvae died at significantly higher rates from unknown causes below the girdle than above the girdle ($F_{3,27}$=3.21, P=0.003). The proportion of mortality from unknown causes averaged 3.9% ± 1.2% above the girdle and 12.3% ± 4.3% below the girdle. Larval density and mortality from unknown causes were not significantly related ($F_{1,42}$=2.84, P=0.100). Larvae in the prepupal stage died due to unknown causes at significantly lower rates compared to 4th, 3rd, and 1st and 2nd instars ($F_{3,171}$=5.18,

P=0.002) (Table 2.1). Height in tree ($F_{7,280}$=1.27, P=0.267), month of felling ($F_{3,42}$=1.04, P=0.383) and canopy exposure rank ($F_{3,42}$=1.92, P=0.141) did not significantly affect mortality from unknown causes.

Cannibalism killed only 0.3% ± 0.1% of all larvae and did not significantly vary among vigor treatments ($F_{2,18}$=1.28; P=0.302). The proportion of cannibalized larvae averaged 0.0%, 0.2% ± 0.1%, and 0.6% ± 0.2% on control, methyl jasmonate, and girdled trees, respectively. Larval density and cannibalism had a weak but significant relationship ($F_{1,40}$=12.59, P=0.001) (Fig. 2.4A). Rates of cannibalism did not vary significantly by instar ($F_{2,114}$=1.69, P=0.190), height in tree ($F_{6,274}$=1.96, P=0.072), month of tree felling ($F_{3,40}$=1.65, P=0.192) or canopy exposure rank ($F_{3,40}$=1.76, P=0.169).

Mortality from parasitism or pathogens was not observed. While mortality from unknown causes could have been caused in part by pathogens, external symptoms were not apparent. We did not confirm the presence of pathogens in the larvae.

A total of 1791, or 38.8%, of live larvae from the 2006 cohort had not reached the 4th instar or the prepupal stage by winter and were expected to require an additional year to develop (Table 2.1). We also recorded 81 larvae that hatched and began feeding in 2005. These individuals would contribute to the adult population in 2006 and were included in the life table (Table 2.1). Woodpeckers predated 67.9% of the 81 larvae from 2005 larvae in 2006 (Table 2.1).

2007 Study

Mean density of *A. planipennis* larvae was 79.6 ± 9.8 larvae per m^2 per tree and at least one larva was found on 29, 29, and 30 of the control, methyl jasmonate, and girdled trees, respectively. Larval density averaged 38.4 ± 10.4, 25.8 ± 9.7, and 174.5 ± 14.3 larvae per m^2 on control, methyl jasmonate, and girdled trees respectively. Larval density was significantly higher on girdled trees than control and methyl jasmonate trees ($F_{2,18}$=30.87, P<0.001).

Out of 18,891 larvae recorded, 6738, or 35.7%, of all larvae died; average mortality was 34.2% ± 2.6% per tree (Table 2.2). Mortality did not vary significantly by vigor treatment ($F_{2,18}$=0.30, P=0.745). Larval mortality averaged 33.3% ± 4.3%, 31.5% ± 4.9%, and 34.0% ± 3.5% on control, methyl jasmonate, and girdled trees, respectively. The proportion of apparent mortality, or the total loss by instar, was highest for 4th instars; 44.8%, 69.7%, 88.0%, and 21.1% of 1st and 2nd, 3rd, 4th instars, and prepupae died, respectively (Table 2.2). The real loss, or the total loss in the population, was highest 4th instars and prepupae. Overall, 1.1%, 5.8%, 13.3%, and 15.7% of 1st and 2nd, 3rd, 4th instars, and prepupae died, respectively (Table 2.2). This level of stage-specific mortality was similar to that observed in 2006.

Woodpecker predation accounted for most of the larval mortality. Overall, 30.1% ± 2.5% of all larvae were preyed upon by woodpeckers. Unlike 2006, woodpecker predation did not vary by vigor treatment ($F_{2,18}$=0.17; P=0.843). Woodpecker predation averaged 29.9% ± 4.2%, 29.5% ± 5.1% and 28.2% ± 3.2% of larvae on control, methyl jasmonate, and girdled trees, respectively.

Larval density and the rate of woodpecker predation were not significantly related ($F_{1,51}$=0.57, P=0.454). Woodpeckers killed 4th instars at higher rates than prepupae and 3rd instars, which were predated at higher rates than 1st and 2nd instars ($F_{3,219}$=70.18; P<0.001) (Table 2.2). As in 2006, a significantly higher percentage of larvae were consumed by woodpeckers near the top of the tree compared to larvae near the bottom of the tree ($F_{7,594}$=28.04, P<0.001) (Fig. 2.2). Woodpecker predation rates were significantly higher on trees felled in late winter, compared with trees felled in fall ($F_{4,51}$=9.83, P<0.001) (Fig 2.3). Canopy exposure rank ($F_{4,51}$=0.43, P=0.789) did not significantly affect woodpecker predation.

Mortality from unknown causes accounted for 3.3% ± 0.4% of all larvae and did not vary by vigor treatment ($F_{2,18}$=0.45, P=0.643). As in 2006, mortality from parasitism or pathogens was not observed. On average, of mortality from unknown causes killed 3.3% ± 0.7%, 2.0% ± 0.6%, and 3.6% ± 0.7% of all larvae on control, methyl jasmonate, and girdled trees, respectively. However, as in 2006, larvae died at significantly higher rates from unknown causes below the girdle than above the girdle on girdled trees ($F_{3,27}$=7.34, P=0.001). Larval mortality above the girdle averaged 2.6% ± 0.4% and 11.1% ± 2.3% below the girdle. Larval density and mortality from unknown causes were not significantly related ($F_{1,51}$=1.74, P=0.193). Prepupal larvae died from unknown causes at significantly lower rates compared to 4th and 3rd instars, which died at significantly lower rates than 1st and 2nd instars ($F_{3,229}$=17.34, P<0.002) (Table

2.2). Height in tree ($F_{7,562}$=1.97, P=0.057), month of tree felling ($F_{3,51}$=0.86, P=0.496), and canopy exposure rank ($F_{3,51}$=0.07, P=0.990) did not significantly affect mortality from unknown causes.

As in 2006, mortality from cannibalism was rare, accounting for 0.8% ± 0.3% of all larvae. Cannibalism did not significantly vary with vigor treatment ($F_{2,18}$=1.50; P=0.249), averaging 0.1% ± 0.0%, 0.0% ± 0.0%, and 2.1% ± 0.7% of all larvae on control, methyl jasmonate, and girdled trees, respectively. Cannibalism did occurr at significantly higher rates below than above the girdle on girdled trees, with 9.2% ± 2.6% and 0.2% ± 0.1% of larvae cannibalized below and above girdle, respectively ($F_{3,27}$=7.44, P=0.001). Larval density and cannibalism had a weak, but significant relationship ($F_{1,53}$=10.32, P=0.002) (Fig. 2.4A). Cannibalism occurred at significantly higher rates during 1st and 2nd instars than during 3rd and 4th instars ($F_{2,177}$=8.35, P<0.001) (Table 2.3). Rates of cannibalism did not significantly vary by height in tree ($F_{7,567}$=0.74, P=0.639), month of tree felling ($F_{3,53}$=0.45; P=0.771) or canopy exposure rank ($F_{4,53}$=1.17; P=0.149).

A total of 697, or 3.7% of the total 2007 cohort, overwintered as 1st, 2nd, or 3rd instars (Table 2.2). We also recorded 710 larvae that began feeding in 2006 and would have contributed to the adult population in 2007 (Table 2.2). Woodpeckers predated 43.4% of the 710 larvae that had begun feeding in 2006 (Table 2.1).

DISCUSSION

Overall, 30.1% of the total larval cohort died in 2006 and 35.7% of the total larval cohort died in 2007. Mortality at the prepupal stage accounted for most the total mortality, followed by fourth instars and then third instars. Woodpeckers accounted for most of the mortality, followed by unknown causes and cannibalism.

Woodpecker predation was the largest source of *A. planipennis* larval mortality in both 2006 and 2007. Woodpeckers preferred 4th instars and prepupas, probably because these stages are always more than three times the size of smaller instars. A much larger proportion of the total 4th instars were predated compared with prepupae, so it appeared that woodpeckers focused on 4th instars compared with prepupae. Predation increased after the month of November, and continued through the winter. Because all trees were felled by March and February in 2006 and 2007, respectively, additional predation by woodpeckers could have occurred before adults emerged in the spring. Cappaert et al. (2005b) also found that woodpecker predation caused higher *A. planipennis* larval mortality than any other factor. Data collected from 24 sites in MI showed that woodpecker predation ranged from 9% to 95% of all larvae, however, no reason was given for this variation (Cappaert et al. 2005b). Lindell et al. (2008) found that woodpeckers predated about 30% of all larvae when comparing woodpecker attack sites and *A. planipennis* exit holes on ash trees.

The rate of woodpecker predation was significantly related larval density. A higher proportion of larvae were predated by woodpeckers in 2007 (30.1% of

all larvae) than in 2006 (9.9% of all larvae), coinciding with a five-fold increase in larval density. Similar correlations between the woodpecker predation rates and larval density were reported in previous studies on woodboring insects (Koplin and Baldwin 1970, Lindell et al. 2008). In a meta-analysis of predation of spruce bark beetle larvae (*Dendroctonus sp.*) by downy woodpeckers (*Picoides pubescens*), a common woodpecker in our study site, predation did not vary between high density epidemic populations and low density non-epidemic populations (Fayt et al. 2005).

A larger proportion of larvae were predated near the top (5-8 m) of the tree compared with the rest of the tree. This pattern is different the distribution of larvae found in the tree, with larval density highest between 3-4 m (Chapter 1). This pattern suggests that woodpeckers base feeding preferences on height, and do not focus their attention solely on larval density within an individual tree.

Canopy exposure rank did not affect rates of woodpecker predation at this site and Lindell et al. (2008) reported a similar result. They found a significant negative correlation between the proportion of forest cover and predation rates, but forest cover explained little of the variation in woodpecker predation (R^2=0.024).

Multi-year development did not enable to larvae to avoid woodpecker predation. About 50% of larvae that had begun feeding the previous year were attacked by woodpeckers, as prepupae, compared with about 25% of the prepupal larvae that began feeding in the current year.

Mortality from unknown causes was recorded infrequently in both 2006 (6.9% of all larvae) and 2007 (3.3% of all larvae) and most of this mortality occurred below the girdle on girdled trees and a higher proportion of early instars died than late instars. This was probably because the phloem below the girdle was dying while larvae were feeding in mid-late summer. Barter (1957) found higher mortality of *Agrilus anxius* (bronze birch borer) larvae from unknown causes on browning phloem tissue and early instars died at higher rates due to unknown causes than later instars.

The reason that early instars die at higher rates than late instars is still poorly understood. A meta-study by Zalucki et al. (2002) found that the most commonly reported cause of mortality of early Lepidoptera instars is unknown factors. Because the exact cause of mortality is unknown, further research is required to determine why earlier instars die at higher rates than later instars. However, the fact that more larvae die below the girdle on girdled trees than above the girdle creates a starting point for new studies. Moisture, nutritional, or defensive compound content of phloem may affect early instars more than late instars. Early instars are probably more susceptible to the inner host environment because they are small, and hence more susceptible to smaller amounts of defensive compounds, or less robust when encountering poor levels of moisture or nutrition within the phloem.

While there was a difference in mortality from unknown causes below and above the girdle, no significant difference in mortality from unknown causes occurred between larvae on control and methyl jasmonate trees. Methyl

jasmonate has been known to induce defense responses in other tree species, including *Picea abies* (norway spruce) (Franceshi et al. 2002) and *Pseudotsuga menziesii* (douglas fir) and *Sequoiadendron griganteum* (giant redwood) (Hudgins and Franceshi 2004). Methyl jasmonate is also known to induce stress volatile emissions in cotton plants (Rodriguez-Saona et al. 2001) and ash seedlings in a laboratory setting (Poland et al. 2006, Rodriguez-Saona et al. 2006). While ash species native to North America have no known constitutive or inducible defenses against *A. planipennis*, *Fraxinus mandshurica* (Manchurian ash) in China experiences little *A. planipennis* – induced mortality (Rebek et al. 2007). A total of 11 potential defensive allelochemicals were found in higher rates in *F. mandshurica* than in *F. americana* and *F. pennsylvanica* (Eyles et al. 2007). The lack of allelochemicals found in American ash trees may explain the lack of response to methyl jasmonate and the low rates of mortality from unknown causes.

Cannibalism occurred at very low levels in both 2006 (0.3%) and 2007 (0.8%). This was different then the rates of cannibalism that occur in cerambycids. Ware and Stephen (2006) studied *Enaphalodes rufulus* (red oak borer) in the laboratory with small phloem arenas; 84% of larvae exhibited cannibalistic behavior, and cannibals gained five times the weight of non-cannibals. However, my results are consistent with my laboratory studies of larvae placed in small phloem arenas; 1.5% of *A. planipennis* larvae died due to cannibalism (Chapter 4). Previous studies of buprestids and cerambycids have found that cannibalism generally occurs in high density populations. Cannibalism

was previously observed in high densities of *A. planipennis* larvae (Bauer et al. 2004), but the exact proportions and densities were not reported. Additionally, cannibalism was observed in high density populations of cerambycid *Phoracantha semipunctata* (Hanks et al. 2005), but exact proportions and were not reported.

A. planipennis cannibalism appears to be accidental, and is determined by the probability that larvae will come in contact with each other. The rate of cannibalism in my study had a weak positive relationship to larval density, with larvae having more contacts with other larvae in high population densities. Additionally, galleries formed by larvae were larger below the girdle than above (Chapter 1), leading to more contacts and significantly higher proportions of cannibalism occurring below the girdle than above the girdle on girdled trees in both 2006 and 2007. Rates of cannibalism are higher below the girdle than above even though larval population densities were smaller below the girdle than above the girdle (Chapter 1). However, the rates of cannibalism that I am reporting may be underestimated at very high larval densities because individual galleries became indistinguishable from each other, and therefore the presence of cannibalism was harder to detect.

No parasitism was observed in this study. However, parasitoids are known to attack the native bruprestid *A. anxius* (Barter 1957). Some of these native parasitoids of buprestids, such as *Heterospilus sp.* and *Atanycolus sp.* (Braconidae), *Phasgonophora sulcata* (Chalcidae), *Balcha sp.* and *Eupelmis sp.* (Eupelmidae), and three ichneumonids, attacked less than 1% of *A. planipennis*

larvae (Bauer et al. 2004). Recently *Atanycolus sp.*, was reported to parasitize *A. planipennis* larvae at much higher levels; in one site the average parasitism rate ranged from 2% to 73% per tree (Cappaert and McCullough 2008). This and other native parasitoids may become more important mortality factors for *A. planipennis* in the future. If parasitoids become an important source of mortality, additional research will be required to assess how parasitoids will interact and compete with woodpeckers.

Table 2.1 Age specific life table of *A. planipennis* larvae for 2006

	Populaton reduction factor	No. living at beginning	No. dying	Apparent loss[1]	Real loss[1]
1st/2nd Instar		4621			
	Woodpecker predation		8	0.6%	0.2%
	Unknown causes		111	7.8%	2.4%
	Cannibalism		18	1.3%	0.4%
	Delayed development		1285	90.4%	27.8%
	Subtotal		**1422**		**30.8%**
3rd Instar		3199			
	Woodpecker predation		185	23.0%	4.0%
	Unknown causes		95	11.8%	2.1%
	Cannibalism		17	2.1%	0.4%
	Delayed development		506	63.0%	11.0%
	Subtotal		**803**		**17.4%**
4th Instar		2396			
	Woodpecker predation		404	60.6%	8.7%
	Unknown causes		63	9.4%	1.4%
	Cannibalism		11	1.6%	0.2%
	Subtotal		**478**		**10.3%**
Prepupa		1918			
	Woodpecker predation		508	29.4%	11.0%
	Unknown causes		18	1.0%	0.4%
					11.4%
	Prepupae from 2yr larvae		-81		-1.8%
	Woodpecker predation 2yr larvae		55	67.9%	1.2%
	Subtotal		**500**		**10.8%**
	Total	**1418 (30.7%)**	**3203**		**69.3%**

[1]Apparent loss represents stage-specific mortality while real loss is the percentage of total larvae killed in that stage by that factor

Table 2.2 Age specific life table of *A. planipennis* larvae for 2007

	Populaton reduction factor	No. living at beginning	No. dying	Apparent loss[1]	Real loss[1]
1st/2nd Instar		18891			
	Woodpecker predation		12	2.8%	0.1%
	Unknown causes		89	20.6%	0.5%
	Cannibalism		93	21.5%	0.5%
	Delayed development		239	55.2%	1.3%
	Subtotal		**433**		**2.3%**
3rd Instar		18458			
	Woodpecker predation		703	46.4%	3.7%
	Unknown causes		221	14.6%	1.2%
	Cannibalism		132	8.7%	0.7%
	Delayed development		458	30.3%	2.4%
	Subtotal		**1514**		**8.0%**
4th Instar		16944			
	Woodpecker predation		2250	78.6%	11.9%
	Unknown causes		214	7.5%	1.1%
	Cannibalism		55	1.9%	0.3%
	Subtotal		**2519**		**13.4%**
Prepupa		14425			
	Woodpecker predation		2923	20.8%	15.5%
	Unknown causes		46	0.3%	0.2%
	Prepupa from 2yr larvae		-710		-3.8%
	Woodpecker predation 2yr larvae		308	43.4%	1.6%
	Subtotal		**2567**		**13.5%**
	Total	**11858 (62.8%)**	**7378**		**37.2%**

[1]Apparent loss represents stage-specific mortality while real loss is the percentage of total larvae killed in that stage by that factor

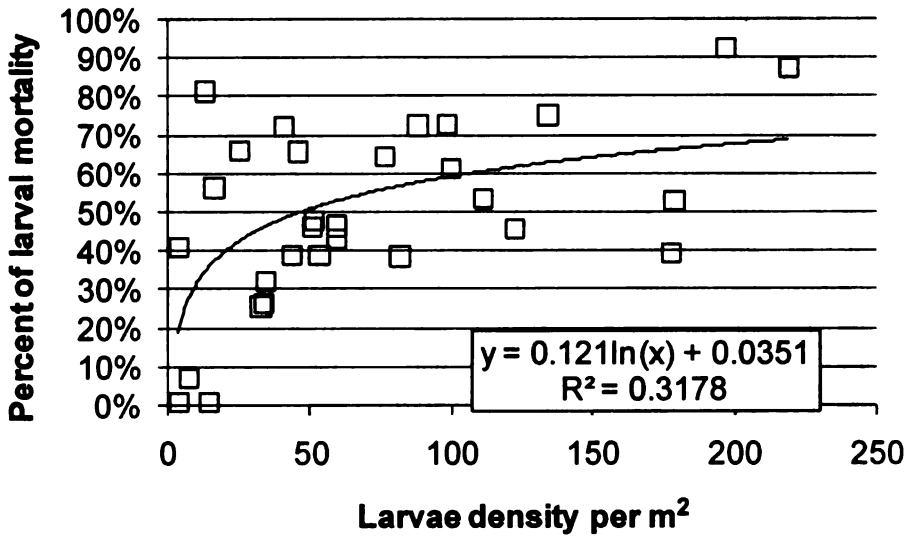


Fig. 2.1 Regression of *A. planipennis* larval mortality by woodpecker predation versus larval density in 2006. $Log_{10}(x+1)$ model is significant (GLM; $P < 0.05$).

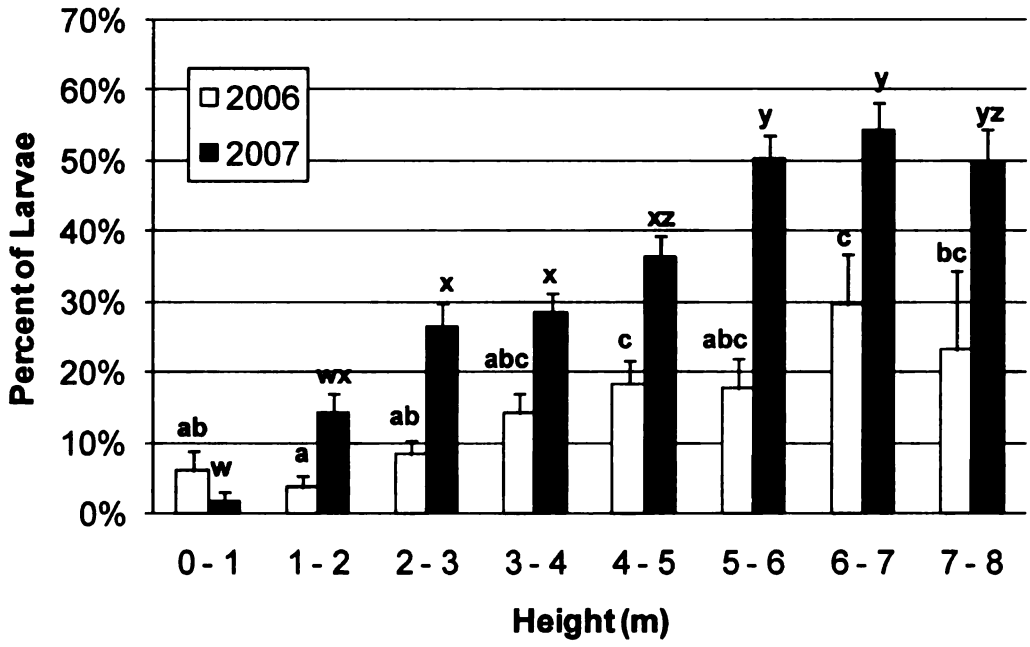


Fig. 2.2 Mean (± SE) proportion of *A. planipennis* larvae killed by woodpecker predation per m^2 by log height up to 8 m above ground in 2006 and 2007. Within year means with the same letter are not significantly different (Tukey protected LSD test; $P<0.05$).

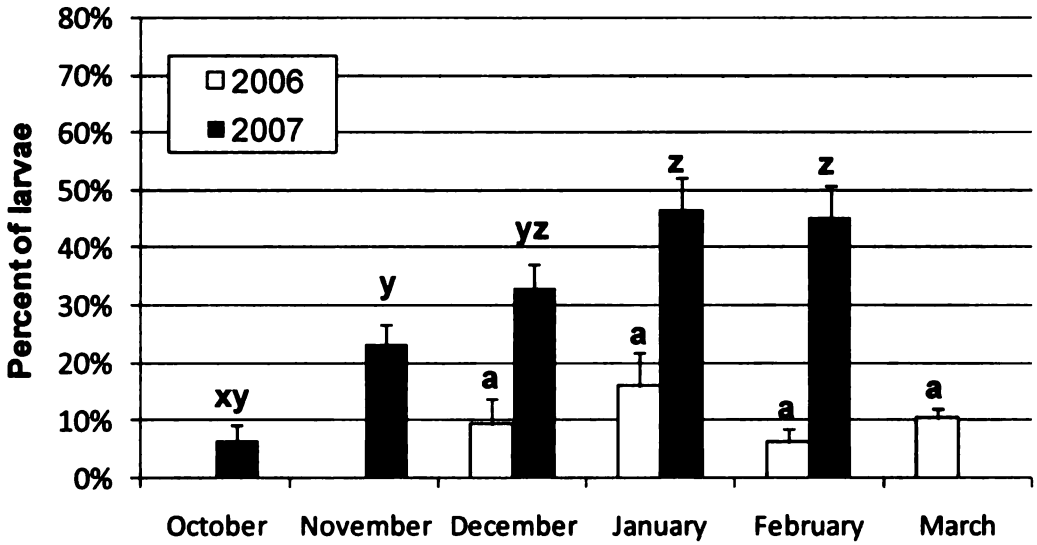


Fig. 2.3 Mean (± SE) proportion of *A. planipennis* larvae killed by woodpecker predation per m^2 in the month from October through March in 2006 and 2007. Within year means with the same letter are not significantly different (Tukey protected LSD test; $P<0.05$). Trees were not felled until December in 2006. Tree felling was completed by late February in 2007.

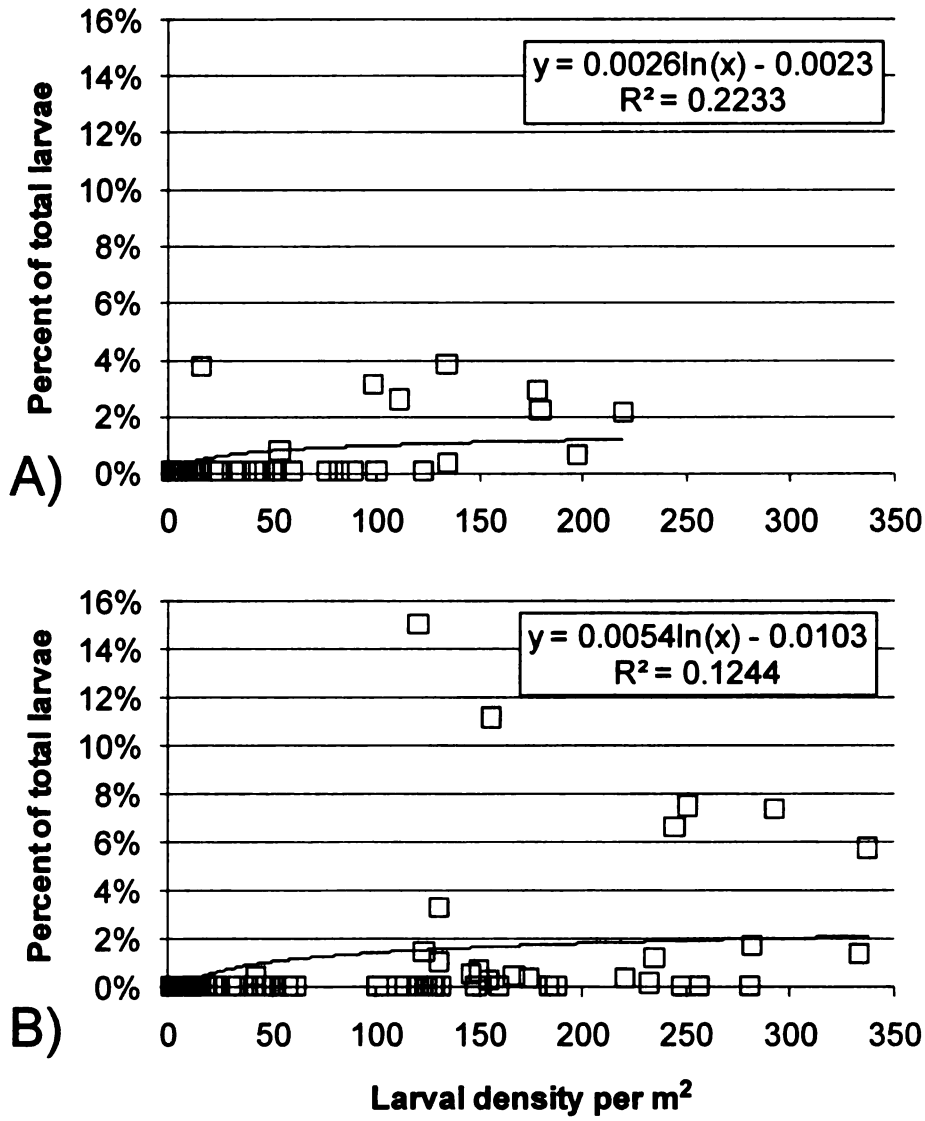


Fig. 2.4 Regression of *A. planipennis* larvae cannibalized by larval density in 2006 (A) and 2007 (B). $Log_{10}(x+1)$ models are significant (GLM; $P < 0.05$).

CHAPTER 3

Development of *Agrilus planipennis* larvae (Coleoptera: Buprestidae) on stressed and healthy *Fraxinus pennsylvanica* trees

INTRODUCTION

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), was initially thought to have a univoltine life cycle in North America (Cappaert et al. 2005a). However, recent studies have indicated that some larvae feed for two summers (Cappaert et al. 2005b), a pattern most common in trees with low densities of *A. planipennis*. For example, in an outlier site with a one-year old infestation, only 18% of 282 larvae developed in one year (Cappaert et al. 2005b). In a second, newly established outlier site, tree vigor appeared to play an important role in determining larval development time. When trees were debarked in February, the first winter after the initial infestation, the 36 larvae on a stressed tree were all 4th instars or prepupae. They would have emerged after a single summer of feeding. Only 23% of the larvae feeding on vigorous trees at the site were 4th instars or prepupae. The rest would likely have fed for an additional summer to complete development, emerging after two years (Cappaert et al. 2005b).

In this study, I determined the effects of host vigor and larval population density on the rate of larval development. I hypothesized that larvae feeding on stressed trees develop faster than healthy trees. I also hypothesized that larvae in a high density population develop faster than larvae feeding in a low density population. I monitored the relationship of development of larvae produced by adults caged on trees with varying densities of *A. planipennis* larvae produced by wild adults.

MATERIALS AND METHODS

2006 Study

For this study we used the same green ash, *Fraxinus pennsylvanica* Marshall (Oleaceae), trees described in Chapter 1 and Chapter 2. From the 90 trees in the 2006 study and the 90 trees in the 2007 study, we randomly selected two trees per treatment in each of the ten blocks. Thus, a total of 60 trees were used for this study including 20 trees girdled, 20 trees exposed to the stress-elicitor methyl jasmonate, or 20 trees left as untreated controls. The trees used in the 2006 study and the 2007 study were felled and debarked the following winter as described in Chapter 1.

Adult *A. planipennis* were reared from heavily infested bolts of ash trees collected from sites in Washtenaw County, MI from October through November 2005. The trees were bucked into logs, each 0.5 m long (approximately16 cm diameter) and placed in cold storage at 38° F. Logs were pulled from cold storage in early May 2006 and placed in 25 cm diameter cardboard tubes with screened caps in a room maintained at 26.7° F, 50% RH. Adult beetles began to emerge from logs three weeks later. After emergence, adult beetles were sexed and placed in clear plastic 120 ml cups in groups of six adult beetles (three males and three females) per cup for a two week period. Sex was determined by examining the beetles under a stereoscope. Males were identified by the first and second abdominal sections, which are narrower than those of females. Males also have a visible layer of setae on the ventral side of the thorax which females lack. Leaves of *F. pennsylvanica*, collected from the field in Ingham

County, were provided to the beetles and replaced twice a week. Cups with beetles were kept in growth chambers kept at 24° C, 75% RH and 16:8 light:dark photoperiod. After about a week, we observed adult beetles beginning to mate. Adult female beetles were assumed to have mated after fertile eggs appeared in the cups, typically two to three weeks after emergance. Eggs are fertile when they change from creamy white to reddish-brown.

On 13 June, we attempted to establish high and low densities of larvae on our study trees by caging adult *A. planipennis* on the trunks. Cages consisted of Medegen Gent-L-Kare® (Medegen Medical Products, Gallaway, TN) 4 oz. specimen containers painted white. The bottom was cut off and covered with plastic mesh attached with hot-glue. A 5 cm diameter hole was cut in the lid, leaving only the threads. Cages were attached to the bark of the trees at a height of 1.5 m. Lids were attached to the bark using GE Silicone II* Window & Door (Clear) (GE Sealants & Adhesives, Huntersville, NC) with the threads facing outwards. After two days, the silicone had set and the cage was screwed onto the lid.

To generate locally high larval densities, we placed four cages on each of ten trees per stress treatment. Cages were placed at the same height and spaced 2 cm apart, around the trunk. To generate low larval densities, we placed one cage at the same height on the north aspect on each of ten trees per stress treatment. In other words, there were 30 trees with four cages each, on ten control, ten methyl jasmonate, and ten girdled trees and 30 trees with one cage each, on ten control, ten methyl jasmonate, and ten girdled trees (total of 60

trees). When a trunk branched below 1.5 m, an additional set of cages were placed on the second trunk as above. The results from the two trunks were averaged for that tree. Petioles of *F. pennsylvanica* leaves were pushed into water-filled microcentrifuge tubes and placed into each cage. Foliage was replaced twice a week. One male and one female beetle that were at least two weeks old were placed in each cage. Using the same criteria as Anulewicz (2006), if a female beetle died during the first three days of the study, she was replaced with another female beetle to ensure adequate time for egg laying.

I waited an average of 74.9 ± 1.0 days (range 66 to 87 days) after adults were placed on trees before peeling so larvae could feed and develop. Between 18 August and 8 September, we peeled the bark under the cages with a drawknife. A mean area of 0.13 ± 0.01 m^2 was debarked under each cage. We recorded the number, life stage, and mortality of larvae in each window. Instars were identified by head capsule width (Cappaert et al. 2005b). As described in Chapter 1, we assumed that larvae found as 1^{st}, 2^{nd}, or 3^{rd} instars would probably not reach the prepupal stage by spring, and would require another summer of feeding before pupating (Cappaert et al. 2005a).

Although we removed 11 trees that had external symptoms of *A. planipennis* infestation in May 2006, a population of *A. planipennis* persisted in the plantation and surrounding area (Chapter 1 and Chapter 2). These wild beetles laid eggs on the trees. The 90 trees in this study were felled and debarked from December 2006 through March 2007 as described in Chapter 1. Larvae associated with the wild *A. planipennis* population, found when study

trees were debarked. Larval diversity was standardized per m^2 of exposed area on each tree.

2007 Study

We replicated the study in 2007 with a few modifications. Ten blocks of nine trees were selected using the same parameters as in 2006 for a total of 90 trees. Trees were girdled on 10 May. A line with ten methyl jasmonate bubble caps spaced 20 cm apart was suspended through the canopies on 25 May, and a second line with 10 methyl jasmonate bubble caps was suspended through the canopies on 25 June as described in Chapter 1.

Adult *A. planipennis* in 2007 were reared in a similar way as in 2006 with the exception that *Fraxinus udhei* (Wenzig) leaves, a tropical species, were provided in cups instead of *F. pennsylvanica* foliage.

F. udhei foliage was also placed in the cages on trees. To assure a higher rate of successful gallery formation, we placed two cages on each of two trees per treatment in randomly selected blocks (total of 60 trees) (20 trees per treatment). One cage was attached on the north aspect and the other on the south aspect. Adult beetles were caged on 26 June.

In 2007, we waited an average of 80 ± 1 days (range 76 to 101 days) after adult beetles were caged before beginning to debark areas under the cages. Between 10 September and 5 October, we debarked an average of 0.12 ± 0.003 m^2 under each cage. Larval galleries were traced onto transparencies, which were then scanned. The area of phloem consumed per larva was calculated

using the computer program Winfolia™ (Winfolia 2004a). Trees were then felled during between October 2007 and February 2008, as in the 2006 study.

Phloem Disruption - Pilot Study

In 2007, we initiated a pilot study with 10 additional trees in the same plantation. On 10 June, a 2 cm wide horizontal band of phloem was removed from half of the circumference of the trees at 1.38 m above ground on the south aspect. On 18 June, we selected 20 female and 20 male adult beetles that were field collected from a heavily infested site in Ingham country, MI. These beetles laid fertile eggs in a related study (Anulewicz et al. 2008) and were re-used for this pilot study. On each of the 10 trees, we placed one cage 4 cm above the phloem disruption and one cage 4 cm below the phloem disruption, directly in line with each other. Areas under the cages were debarked an average of 84 ± 1 days (range 80 to 86 days) after the adult beetles were caged. On average, 0.14 ± 0.005 m^2 were debarked per cage. We recorded the number, instar and mortality of larvae produced by caged females and standardized larval density per m^2 of exposed area. Galleries were traced onto transparencies which were scanned and the area of phloem consumed per larva calculated as described above.

Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. We evaluated three response variables, including

larval density from caged adults, the proportion of those larvae that reached at least the 4th instar, and the amount of phloem consumed per larva by instar in 2007. Larval density and phloem consumption were log10(x+1) transformed to normalize the data. Proportion of larvae that reached at least the 4th instar required no transformations. The same response variables were tested in the phloem disruption pilot study, following transformations to normalize the data.

All response variables were analyzed using the GLIMMIX procedure for mixed models in SAS statistical software (PROC GLIMMIX; SAS Institute 2003). Effects of three factors: tree vigor (control, methyl jasmonate, or girdled), density treatment (high density with four cages per tree and low density with one cage per tree) in 2006 only, and canopy exposure with five levels (1, 2, 3, 4, 5) were analyzed. Block and block crossed with treatment were used as random factors. Differences among treatment means were tested as unplanned comparisons and multiple comparison tests were applied only when overall analysis of variance (ANOVA) was significant (P<0.05). When significant differences among levels occurred, the Tukey protected least significant difference (LSD) test was used to evaluate those differences (Ott and Longnecker 2001). The $\log_{10}(x+1)$ relationship between density of the wild *A. planipennis* population and the proportion of one year larvae and the log10(x+1) relationship between density of the caged *A. planipennis* population and the proportion of larvae under cages that had reached at least 4th instar was analyzed using a general linear model with a $\log_{10}(+1)$ transformation (PROC GLM, SAS institute 2003).

RESULTS

2006 Study

Overall, a total of 174 larvae were found under the 171 cages in 2006. Adult female beetles survived in cages for an average of 9.5 ± 0.3 days. At least one larva was found on 31 of the 60 trees that had one or more cages. Larvae were associated with 56 of the total 171 cages (Table 3.1). Twice as many larval galleries were associated with cages on girdled trees compared with cages on control and methyl jasmonate trees (Table 3.1). There was a significantly higher number of galleries on trees with four cages compared to trees with a single cage, with an average of 12.3 ± 2.5 larvae per m^2 compared with 3.2 ± 1.3 larvae per m^2, respectively ($F_{1,23}$=15.98, P<0.001). Significantly more larvae were produced by beetles caged on girdled trees than control or methyl jasmonate trees ($F_{2,18}$=3.65, P=0.047). There were three times as many larvae on girdled trees compared with control and methyl jasmonate trees (Fig. 3.1). Larval densities were not significantly different between control and methyl jasmonate trees (Fig. 3.1). The interaction between larval density and treatment was not significant ($F_{2,23}$=2.01, P=0.156). Canopy exposure ($F_{3,23}$=2.09, P=0.130) did not significantly affect larval density below cages.

On average, 16.5% ± 3.1% of larvae found below the cages had reached the 4^{th} instar or were prepupal larvae at the time of debarking. Larvae developed significantly faster on girdled trees than on control or methyl jasmonate trees ($F_{2,11}$=7.99, P=0.007) (Fig. 3.2). Development of larvae on trees with four cages

and larvae on trees with one cage did not differ significantly ($F_{1,1}$=0.83, P=0.529). An average of 19.5% ± 5.4% and 8.8% ± 6.3% of larvae reached the fourth instar on trees with four cages and larvae on trees with one cage, respectively. The interaction of stress treatment and density was not significant ($F_{2,1}$=0.80, p=0.621). Density of larvae from the wild *A. planipennis* population did not significantly affect development rates of larvae ($F_{1,1}$=8.91, P=0.206). Larval development was not significantly affected by canopy exposure rating ($F_{3,1}$=1.61, P=0.512).

Mortality of larvae under cages was very rare. Only three, one, and two 1st instars died on control, methyl jasmonate and girdled trees, respectively, all from unknown causes. Cannibalism, woodpecker predation or pathogens were not observed. All late instar larvae were alive when the area under the cages was debarked.

2007 Study

Overall, a total of 278 larvae were found under the 106 cages in 2007. Adult females survived for an average of 10.7 ± 0.5 days. At least one larva was found on 32 of the 53 trees that had cages and larvae were associated with 50 of the 106 cages (Table 3.1). Larval galleries were associated with three times as many cages on girdled trees compared with control and methyl jasmonate trees (Table 3.1). There was no significant difference in the number of cages associated with larvae on control and methyl jasmonate trees (Table 3.1). Average larval density was significantly higher when beetles were caged on

girdled trees than on control or methyl jasmonate treatments ($F_{2,18}$=3.77, P=0.043). Six times as many larvae were found under cages on girdled trees compared with cages on control and methyl jasmonate trees (Fig. 3.1). Larval density did not vary significantly between control and methyl jasmonate trees (Fig. 3.1). Additionally, larval densities from beetles caged on control and methyl jasmonate trees in 2007 were similar to the larval densities on control and methyl jasmonate trees in the 2006 study (Fig. 3.1). Larval densities from beetles caged on girdled trees were twice as high in 2007 as they were on girdled trees in 2006. Larval density was not significantly affected by canopy exposure ($F_{4, 50}$=1.39, P=0.249).

Overall, an average of 56.5% ± 7.7% of larvae had reached 4th instar or the prepupal stage when debarking occurred. The proportion of larvae that had reached 4th instar or the prepupal stage did not vary significantly among treatments ($F_{2,12}$=1.23, P=0.327) (Fig 3.2) or by canopy exposure rating ($F_{4,4}$=3.22, P=0.142). No significant relationship existed between the density of larvae from wild populations and the development of larvae produced by caged adults ($F_{1,4}$=1.25, P=0.326).

Mortality of larvae under cages in 2007 was again rare. Only one 1st instar, one 1st and two 2nd instars, and one 2nd, two 3rd, and one 4th instar died on control, methyl jasmonate, and girdled trees, respectively, all from unknown causes. Cannibalism, woodpecker predation or pathogens were not observed.

The area of phloem consumed per larva per instar averaged 1.18 ± 0.23 cm^2, 2.11 ± 0.44 cm^2, 3.20 ± 0.33 cm^2, 7.56 ± 0.52 cm^2, and 12.45 ± 1.10 cm^2 for 1st, 2nd, 3rd, and 4th instar or prepupae, respectively. Treatment ($F_{2,10}$=1.28, P=0.319) and canopy exposure ($F_{4,89}$=2.02, P=0.099) did not significantly affect phloem consumption. Most phloem consumption occurred in later stages of development, with feeding by 4th instars and prepupal larvae accounting for about 75% of all phloem consumed.

Phloem Disruption - Pilot Study

Adult females caged above and below the thin strip of removed phloem survived for a mean of 14.6 ± 1.9 days. Galleries were found beneath only two of the ten cages above the phloem disruption for a total of six larvae, with a mean of 0.7 ± 0.4 larvae per tree. Seven of the ten cages below the phloem disruption produced 45 larvae, with a mean of 5.0 ± 1.4 larvae per tree. No larvae above the phloem disruption had reached the 4th instar by the time bark was removed in September 2007. Below the phloem disruption, a mean of 43.1% ± 12.7% of larvae were 4th instars or prepupae. The mean phloem consumed per larva per instar, derived mostly from larvae below the phloem disruption, was 1.34 ± 0.04 cm^2, 2.09 ± 0.14 cm^2, 4.82 ± 0.72 cm^2, 5.03 ± 2.14 cm^2, and 12.39 cm^2 for 1st instar, 2nd instar, 3rd instar, 4th instar, and prepupae, respectively, similar to the consumption rates recorded for larvae under the cages in the main study.

DISCUSSION

In both 2006 and 2007, larvae developed faster on girdled trees than on non-girdled trees, and while tree exposure to methyl jasmonate had no effect on larval development. Density of larvae produced by wild *A. planipennis* beetles was also higher on girdled trees but we found no significant relationship between density of wild larvae and development rate of larvae from caged beetles.

Interestingly, larvae were found more frequently and at higher densities under cages on girdled trees compared with control and methyl jasmonate trees. This occurred even though the same number of adult female *A. planipennis* was caged for the same amount of time on trees of all treatments. Cages on girdled trees had two to three times as many larvae associated with them compared with control and methyl jasmonate trees in 2006. In 2007, cages on girdled trees had three to five times as many larvae associated with them compared with control and methyl jasmonate trees. The population density of wild *A. planipennis* larvae was also higher on girdled trees. This suggests that both larval density and development rates are affected by host vigor and that larval development is not strongly affected by larval density. Additionally in the phloem disruption pilot study, more larvae were associated with cages below than above the phloem disruption.

In similar studies with native *Agrilus sp.*, larvae associated with caged adults were found in higher densities on girdled trees compared with non-girdled trees. When adult *Agrilus anxius* (bronze birch borer) were caged on girdled trees and on non-girdled trees, 30% and 82% of cages yielded larvae on non-

girdled and girdled trees, respectively (Nash et al. 1941). Barter (1957) did not report the exact number of larvae produced by adults in a four-year study using caged adults. His qualitative results, however, indicate that the larval population under the cages increased in density each year as tree vigor declined due to girdling from the larval feeding.

At least two factors may explain the difference in larval density among cages on girdled, control, and methyl jasmonate trees. First, adult *A. planipennis* and *A. anxius* may be making a choice about whether to lay or not to lay eggs depending on host suitability. Alternatively, more neonates may have died on control and methyl jasmonate trees without leaving detectable galleries. I was very careful in dissecting the bark and phloem, and it is very unlikely that neonate galleries were missed, so the second hypothesis can probably be rejected. Other reports suggest *A. planipennis* adults may be selective in where they choose to lay their eggs. Anulewicz et al. (2008a) found that when wild populations of adult *A. planipennis* were presented with logs from host species, non-host species and black plastic drain pipes, significantly more eggs were laid on ash logs than non-ash logs, and no eggs were found on plastic drain pipes.

Host selection by *A. planipennis* beetles likely involves adult attraction to host volatiles. Host selection is important because wood-boring larvae rely on the oviposition choices of adult females and larvae are unable to disperse to more suitable host (Hanks et al. 1993). Studies performed in Chapter 1 reported that wild adult beetles were captured more commonly on girdled trees than control and methyl jasmonate trees. Stressed trees are known to produce foliar

volatiles that are attractive to *A. planipennis* adults in laboratory tests (Rodriguez-Saona et al. 2001, Poland et al. 2006). Adult *A. planipennis* in cages were not directly exposed to foliar volatiles, but volatiles produced by bark from trees stressed by girdling can also produce antennal responses from *A. planipennis* adults (Crook et al. 2008). Some of the volatiles produced by bark on trees stressed by girdling are also found in Manuka oil distilled from the New Zealand tea tree (*Leptospermum scoparium* Myrtaceae). Field studies have found that traps baited with Manuka oil attract *A. planipennis* adults at significantly higher rates than unbaited traps (Crook et al. 2006, Poland and McCullough 2007a, Poland et al. 2007b, Crook et al. 2007, Anulewicz et al. 2007, McCullough et al 2008, Crook et al. 2008a, Crook et al. 2008b).

The hypothesis that female beetles lay few eggs on unsuitable hosts is supported by no-choice laboratory bioassays with *A. planipennis*. Anulewicz et al. (2006) reported that *A. planipennis* eggs were associated with *Fraxinus spp.* at four to ten times the rate compared with non-host species. Still, some eggs were laid on unsuitable hosts, including the sides of the cages. These eggs were laid at far lower rates compared with the number of eggs laid on the host, suggesting that another factor, albeit with less influence, also induces oviposition behavior.

Girdling affected larval development as well as ovipostion. Larvae from caged adults developed faster on girdled trees compared to larvae found on control and methyl jasmonate trees in 2006 but not in 2007. Buprestids generally develop faster and have a higher rate of developmental success on host trees

that are stressed by environmental factors such as drought or artificially by girdling (Koricheva and Larsson 1998). Data from *A. planipennis* outlier sites similarly showed larvae on stressed trees developed faster than larvae on non-stressed trees (Cappaert et al. 2005b, Tluczek et al. 2008).

While the exact mechanism for how girdling or phloem disruption affects larval development is still unknown, we can speculate on possible mechanisms. Girdling may affect the nutritional content, the defensive chemical response, moisture levels, or the ability of the tree to produce callus tissue. Available carbohydrates are known to increase in phloem tissue above branch-girdles on fruit trees by growers attempting to increase their yield (Jordan and Habib 1995). We did not compare the nutritional or chemical defenses on girdled with non-girdled trees, but future studies should evaluate them.

Girdling may also affect water pressure in the phloem. Water pressure is an important component of cell division and growth in trees, with a specific pressure required for continued cell growth (Boyer and Silk 2004). The level of moisture in an environment has been found to influence the development of *Phoracantha semipunctata* (Fabricius)., a cerambycid in eucalyptus (*Eucalyptus rudis* Endlicher) (Hanks et al. 1999). A similar process might be affecting the development of *A. planipennis*. Damage from girdling or feeding can disrupt phloem tissue not directly affected by the injury. This is because phloem tissues are elastic and adjust to pressure changes caused by disruptions in the phloem (Lee 1981a). While phloem and xylem water-potential has been shown to be highly correlated and water can flow between the phloem and xylem laterally

(Lee 1981b), girdling does not affect the xylem and therefore probably has minimal, direct impact on moisture levels. Additionally, anecdotal observations in the field found that moisture levels under the bark were still quite high on girdled trees, so moisture variation may not result from phloem girdling.

Fierke and Stephen (2008) reported that the ability of a tree to produce callus tissue was correlated with the level of attack the tree sustained from cerambycids. Trees that could not produce callus tissue were successfully attacked at higher rates than trees that could produce callus tissue. However, no callus tissue was observed around galleries formed by *A. planipennis* larvae under the cages on any tree, so tissue callus is probably not a contributing factor to larval development in this study.

Methyl jasmonate had no effect on population density, development, or mortality of larvae associated with larvae in this study. *A. planipennis* adults have been shown to be attracted to volatiles produced by ash seedlings exposed to methyl jasmonate (Rodriguez-Saona et al. 2006, Poland et al. 2006). Similarly, McCullough et al. (2006) found trees exposed to methyl jasmonate were no more attractive than untreated trees to wild adult beetles. Development of larvae produced by wild beetles on methyl jasmonate trees did not significantly vary from controls as well (Chapter 1). We suspect that that methyl jasmonate had no effect on our trees either because they were too large and mature, or we did not use enough methyl jasmonate to elicit a stress response. Leaves from trees in this study were clipped then aerated, but no volatiles were collected. Future studies should include measuring volatiles directly from trees.

Development of larvae from caged adults was not significantly related to the density of larvae produced by the wild *A. planipennis* population. The average larval density associated with cages did not change on control and methyl jasmonate trees from 2006 to 2007. However, the proportion of larvae that had reached the 4^{th} instar or prepupal stage in 2007 was two to eight times the proportion found in 2006. Larval development was also not significantly related to the population density of larvae produced by wild beetles when comparing individual trees. However, if you compare the average larval development at the study site with population density between 2006 and 2007, there appears to be a relationship. This was similar to the results reported in Chapter 1.

In both the caged study and the phloem pilot disruption study, larvae consumed a total of about 12 cm^2 of phloem before developing into a prepupa, regardless of treatment. Using this we can calculate that the total potential yield of *A. planipennis* is about 833 adults per m^2 of phloem. This supports the findings by McCullough et al. (2007) who observed densities of young larvae in heavily infested logs ranging from 300 to 1000 larvae, but produced an average of 89 adult beetles per m^2 of surface area after high rates of mortality occurred. The population density of larvae on girdled trees produced by wild adults averaged 71 larvae per m^2 in 2006 and 175 larvae per m^2 in 2007 (Chapter 1). This matches observations in the field that unutilized phloem was still available. About 75% of the phloem was consumed by later instars. This is relevant for

insecticide control because if feeding can be halted before larvae develop into late instars, feeding damage could be minimized.

Table 3.1 Mean (± SE) tree diameter at breast height (DBH), height, wild larval density and cages, trees, and *A. planipennis* larvae under cages from adults caged on *Fraxinus pennsylvanica* in 2006 and 2007

	Control	Methyl Jasmonate	Girdled
2006			
No. trees	20	20	20
Mean DBH (cm)	12.2 ± 0.6a	12.4 ± 0.4a	12.3 ± 0.5a
Mean tree height (m)	11.1 ± 0.3a	10.8 ± 0.3a	10.6 ± 0.3a
Mean wild larval density (m^2)	4.34 ± 1.09a	9.00 ± 6.69a	74.43 ± 13.35b
No. Cages	58	59	54
No. trees with more than 0 larvae from caged adults	8	11	13
No. cages with larvae	12	15	29
2007			
No. trees	18	17	18
Mean DBH (cm)	12.1 ± 0.5a	12.7 ± 0.7a	13.0 ± 0.5a
Mean tree height (m)	10.7 ± 0.3a	10.5 ± 0.4a	10.9 ± 0.3a
Mean wild larval density (m^2)	21.67 ± 10.03a	11.43 ± 3.39a	166.82 ± 15.79b
No. Cages	40	40	40
No. trees with more than 0 larvae from caged adults	8	8	16
No. cages with larvae	8	9	28

Within year means with the same letter are not significantly different among treatments (Tukey protected LSD Test; $P<0.0.5$)

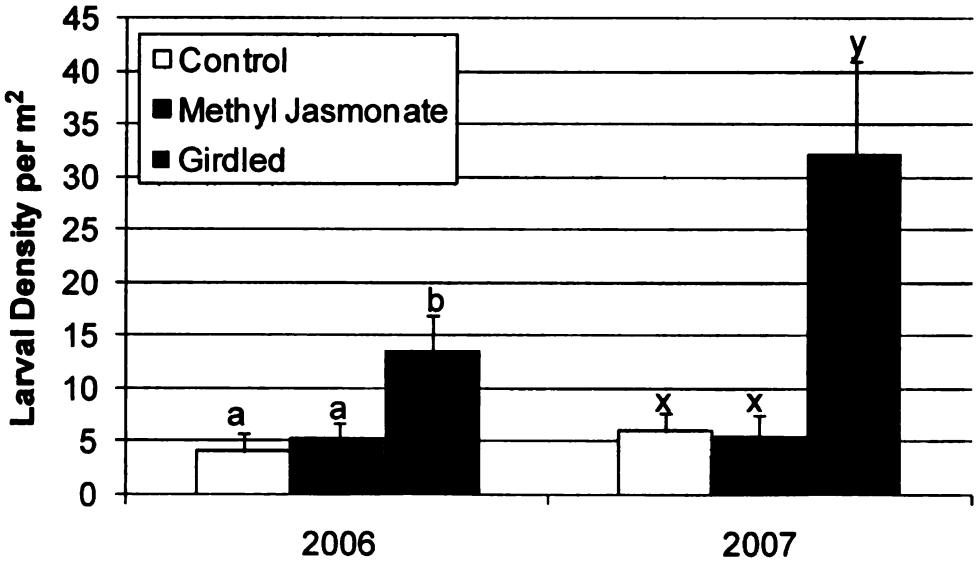


Fig. 3.1 Mean (± SE) density of *A. planipennis* larvae per m^2 produced by caged beetles on untreated control trees, trees exposed to methyl jasmonate, and girdled trees in 2006 and 2007. Within each year, means with the same letter are not significantly different (Tukey protected LSD test; $P < 0.05$).

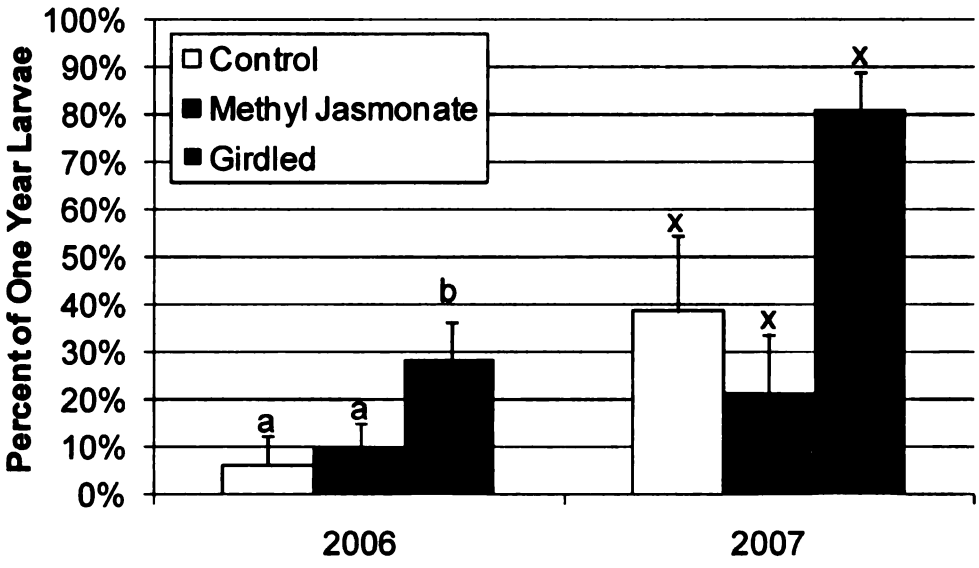


Fig. 3.2 Mean (± SE) percentage of *A. planipennis* larvae produced by cages per m^2 that reached 4^{th} instar or prepupal stage on untreated control trees, trees exposed to methyl jasmonate, and girdled trees in 2006 and 2007. Within each year, means with the same letter are not significantly different (Tukey protected LSD test; $P < 0.05$).

CHAPTER 4

Development, survival and phloem consumption of *Agrilus planipennis* Fairmaire larvae phloem arenas and potted ash sections

INTRODUCTION

After the discovery of emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in southeast Michigan in 2002, the life cycle of *A. planipennis* larvae was found to vary from one to two years in North America (Cappaert et al. 2005b). However, factors determining the rate of larval development remain unknown (Cappaert et al. 2005b). One possible mechanism may be that at higher densities intraspecific cannibalism provides more nutrients leading to faster larval development and a univoltine life cycle. When *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae), the red oak borer larvae cannibalize conspecific larvae, they grew at nearly five times the rate of larvae developing only on phloem and wood (Ware and Stephen 2006). Cannibalism was observed in high density populations of *A. planipennis* (Bauer et al. 2004) and larvae in high density sites generally develop in a single year. (Cappaert et al. 2005b). If cannibalism is common when *A. planipennis* larvae are at high densities, it may partially explain why larvae develop in a single year in high density environments, but require two years in trees with low densities.

The main goal of this study was to determine if *A. planipennis* larval cannibalism was related to larval density. If cannibalism occurred frequently, I hypothesized that, *A. planipennis* larval development rates would increase, similar to *E. rufulus.* I conducted two experiments. The first experiment involved

rearing larvae at different densities on phloem arenas, consisting of small pieces of ash phloem sandwiched between Plexiglas. Specifically, I expected development of *A. planipennis* larvae that cannibalized other larvae to be enhanced and cannibalism to be common in high density settings. In the second experiment, I attempted to establish varying densities of *A. planipennis* larvae on small sections of potted ash in a greenhouse.

METHODS

Phloem Arenas

On 17 July 2006, I collected 200 *A. planipennis* 1st instars from a white ash tree, *Fraxinus americana* L. (Oleaceae), in Jackson County, MI by chiseling the underlying cambium and sapwood, creating a wood chip that held a larva. Wood chips were placed in a 25.4 cm Petri dish lined with a damp paper towel and returned to the laboratory. Larvae from the wood chips were randomly assigned to either a low density or high density treatment.

On 17 July 2006, a 7.6 cm diameter key-hole saw on a 19 volt cordless drill was used to remove circular sections of bark with intact cambium and phloem from a single, *F. Americana* tree. Each piece of phloem was placed in a separate plastic bag in a cooler and transported back to the lab. The pieces of bark and phloem were sandwiched between two pieces of 5 cm by 5 cm Plexiglas (0.25 cm thick) with the outer bark facing down and the cambium facing up.

We randomly assigned 15 phloem arenas to high density treatments and 15 to low density treatments. Each high density phloem arena recieved eight 1st instars and each low density arena recieved one 1st instar. Larvae were placed in 0.3 cm deep grooves carved with a tapering 5-mm-wide metal spatula. High density arenas had eight grooves in two rows of four spaced 3 mm apart in the center of the arena, while the low density arenas had one groove in the center of the phloem arena. The two pieces of Plexiglas were then bolted together using four 5-cm-long, 0.8 cm bolt inserted in holes in the corners of the plexiglas and

secured with a wing-nut over a locking washer. Once secured, a layer of parafilm was wrapped around the edge of the arena to seal it. The arenas were placed in a growth chamber and kept at 24 °C and 75% RH in the dark.

Once a week, fresh phloem was harvested from a new *F. americana* tree. Larvae were extracted from the phloem arenas and moved onto the fresh phloem. The Plexiglas and all equipment were sterilized with 95% ethanol. Developmental instar, phloem consumption, and mortality were recorded each time larvae were transfered, but individual larvae could not be marked or distinguished. Larval instar was determined by measuring the width of the head capsule (Cappaert et al. 2005b). To measure phloem consumption, we traced the galleries onto transparencies, which were scanned and the area of phloem consumed was calculated using the computer program Winfolia™ (Winfolia 2004a). The area of phloem consumed per larva per week was calculated by dividing the total area of phloem consumed in an arena by the number of live larvae feeding that week on each arena. Some larvae burrowed below the surface of the top of the phloem arena. When I moved these larvae to a new phloem arena, they were dug out of the old arena using a knife. Galleries below the surface were traced onto the transparency to ensure all feeding was included.

Causes of larval mortality included handling, cannibalism, and mold. Handling mortality occurred when larvae were killed as they were transferred to a fresh phloem arena or if the larva was not placed properly into the new arena and failed to feed. Cannibalism was defined by Elgar and Crespi (1992) as the killing

and consumption of intraspecific individuals. We recorded a cannibalism event when we found the remnants of a larva where two galleries intersected, or when one gallery ended at another gallery and there was no trace of one of the associated larvae. Mold was recorded when fruiting bodies were observed on a larval cadaver. Some larvae were missing. Missing larvae may have been a result of cannibalism where the surviving larva completely consumed the missing larva and took over its gallery.

Larvae that died during the experiment were not replaced, and as time went on, the number of phloem arenas with live larvae decreased (Table 4.1). By 14 August, intact pieces of phloem could no longer be removed from trees, because trees were preparing for dormancy. Larvae remained in arenas for two final weeks of feeding before the last measurements were recorded.

Potted *Fraxinus americana* Sections

In December 2006, two uninfested *F. americana* trees (DBH 10 cm) were felled and trimmed in Riley Township, Clinton County, MI. Branches were removed and the trunks were waxed on both ends, then placed into cold storage. In January 2007, we cut the trees into 40 sections, each with a mean length of 29.4 ± 0.2 cm and diameter of 5.0 ± 0.2 cm. One end of each ash section was treated with the plant hormone Rootone® (Garden Tech™, Lexington KY) following methods of Herard et al. (2004). The other end and any bark wounds were waxed. The ash sections were then planted at a depth of 15 cm in Fafard 2 Mix (Conrad Fafard Inc., Agawam MA) potting soil in seven l pots made of thick

black plastic with an upper diameter of 22.9 cm and a height of 21.6 cm, filled to 3 cm from the top with soil (P. Bloese, MSU Tree Research Center, pers. comm. 2005). The pots were placed in pairs in plastic trays with 5 cm of water. Trays were refilled once or twice and were allowed to dry completely twice a month. The trays were kept in a greenhouse at the Tree Research Center at Michigan State University with an average temperature of 19°C, yielding 63 degree days per week (base 10°C). Two weeks after planting, callus tissue began to form along the top of the section and a variable number of sprouts formed on most stems.

Adult *A. planipennis* were reared from heavily infested bolts of ash trees collected from sites in Washtenaw County, MI from October through November of 2005. The trees were bucked into pieces that were each 0.5 m long (16 cm diameter) and placed in cold storage at 38° F. Sections were pulled from cold storage in early May 2006 and placed in 25 cm diameter cardboard tubes with screened caps in a room maintained at 26.7° F, 50% RH. Adult beetles began to emerge from logs three weeks later. After emergence, adult beetles were sexed by examining the beetles under a binocular dissecting microscope as described in Chapter 1. Groups of six adult beetles (three males and three females) were placed in clear plastic 120 ml cups with a snap-on lid. Leaves of *Fraxinus udhei* (evergreen ash), a tropical ash, were provided to beetles for feeding and were replaced twice a week. Cups were kept in growth chambers at 24° C, 75% RH and 16:8 light:dark photoperiod. After about a week, adult beetles began to mate. Adult females were considered to be mated after fertile eggs were laid in

the cups. Eggs are known to be fertile when they change from a cream to a red-brown.

On 7 March, we attempted to establish high and low densities of larvae by caging adult beetles that were at least two weeks old on our cut sections. Before cages were attached, a one cm wide strip of plastic tree wrap was wrapped around the stem to facilitate egg-laying. Cages consisted of Medegen Gent-L-Kare® (Medegen Medical Products, Gallaway, TN) 4 oz. specimen containers painted white. The bottom was cut off and covered with plastic mesh attached with hot-glue. A 5 cm diameter hole was cut in the lid, leaving only the threads. The lid was attached to the sections using clear window silicone caulk. Petioles of *F. udhei* were inserted into water-filled micro-centrifuge tubes. Foliage was replaced twice a week. One reared male and female that were at least two weeks old were placed in each cage. If a female beetle died during the first three days of the study, she was replaced with another female beetle to ensure adequate time for egg laying, following methods of Anulewicz et al. (2006).

Cages were attached to the bark of the cut sections. Lids were attached to the bark using GE Silicone II* Window & Door (Clear) (GE Sealants & Adhesives, Huntersville, NC) with the threads facing outwards. After two days, the silicone had set and the cage was screwed onto the lid. To generate high larval densities, we placed three cages on each of 20 ash sections. The cages were spaced evenly around the stem and staggered in height from the top to the bottom. To generate low larval densities, I placed one cage on each of 20 ash sections at the same height on a south aspect. Initially, a large number of adults

died before they could be placed in cages. Only 32 sections, 16 high density and 16 low density treatments, had female adult *A. planipennis* that survived for 4 days. On 10 April, additional egg-laying adults became available and were placed on the remaining eight ash sections (four high density and four low density sections).

Larvae were allowed to develop for two weeks after all adult *A. planipennis* died. At that time, two high density and two low density sections were randomly selected and debarked. This was repeated once a week for ten weeks. A mean of 464.3 ± 13.8 cm^2 was debarked per ash section. We recorded the number, life stage, mortality, and phloem consumption of larvae on sections as described for the phloem arenas. Phloem consumption, after tracing larval galleries onto transparencies, was calculated per larva. Because, the ash sections began to die during the study, the area of dead phloem was also measured on each section.

RESULTS

Phloem Arenas

A total of 90.0%, or 108 out of 120 larvae, died on high density phloem arenas after six weeks (Fig. 4.1A). Handling was the greatest cause of larval mortality, accounting for over half of all larvae (Fig. 4.1A). Ten to 25% of larvae were missing after 6 weeks (Fig. 4.1A). Cannibalism and mold were rare on high density arenas, with two and five larvae dying from cannibalism and mold, respectively (Fig. 4.1A). Most mortality occurred in the first two weeks of the experiment, with 24.2% of all larvae dying after one week, and 53.3% of all larvae by the second week (Fig. 1A).

A total of 80.0%, or 12 out of 15 larvae, died on low density phloem arenas (Fig. 4.1B) and all mortality was attributed to handling. As with the high density arenas, most mortality occurred in the first two weeks of the experiment. Overall, 13.3% of larvae died after one week and 46.7% of larvae were dead by the second week (Fig. 1B).

After six weeks, the 15 surviving larva on both high density and low density arenas had reached the 4^{th} instar. There was little variation between the total percentages of larvae that had reached the 4^{th} instar on high and low density arenas each week (Fig. 4.2). A total of 588 degree days (base 10 ^{o}C) were accumulated during the six weeks of the experiment (Table 4.1). Eight out of the 120 larvae on high density arenas had reached the 4^{th} instar by the second week (Table. 4.1).

Most larvae remained near the top of the phloem arena and were visible through the Plexiglas. When larvae were moved to a fresh arena, an average 72.6% ± 3.8% were feeding on the surface of the arena, while the rest of the larvae were burrowed below the surface. On average, surviving larvae consumed a total of 24.5 ± 5.5 cm^2 and 22.1 ± 3.0 cm^2 of phloem per larva over the course of six weeks for low density and high density treatments, respectively (Table 4.1).

Potted *Fraxinus americana* Sections

A. planipennis adults survived in cages for an average of 4.5 ± 1.3 days. A total of 40 and 23 larvae were recorded on seven high density and seven low density sections, respectively. Larval density on all stems, regardless of date peeled, averaged 2.0 ± 0.9 and 1.2 ± 0.6 larvae per section on high and low density stems, respectively. Therefore, no statistical comparisons of larval density could be made. Little larval mortality occurred on the ash sections; a total of six larvae died on three cut sections. Five early instars died when they tried to feed on dead and dessicated tissue. One second instar was cannibalized on a stem with a total of eight larvae.

Larvae on ash sections debarked three and four weeks after adults were placed on cages were all 2nd instars (Fig. 4.3). Most larvae recovered six to eight weeks after adult beetles were placed in cages were 3rd instars (Fig. 4.3). Ten weeks after adult beetles were placed in cages, all recovered larvae were either 4th instars or had reached the prepupal stage (Fig. 4.3). No larvae were found on

sections debarked on weeks 5, 9, 12, or 13 (Fig. 4.3). A total of 126 degree days (base 10 °C) accumulated between the time when *A. planipennis* adults were first caged and when 1st instars were first recovered. This increased to 443 accumulated degree days before 2nd and 3rd instars were recovered and to 630 degree days before 4th instars and prepupae were recovered.

Larvae consumed roughly 2 cm^2 of phloem per instar through the third instar, then consumed about 4 cm^2 of phloem to reach fourth instar and the prepupal stage, for a total mean of 9.9 ± 1.4 cm^2 of phloem consumed per larva (Fig. 4.4). Interestingly, the maximum horizontal dimension of galleries did not vary significantly between early and late instars ($F_{4,2}$=10.16, P<0.092) (Fig. 4.4). Larval gallery areas averaged 2.49 ± 0.24 cm^2, 5.38 ± 1.23 cm^2, 5.88 ± 0.45 cm^2, 6.55 ± 0.84 cm^2, and 9.23 ± 1.22 cm^2 for galleries formed by 1st, 2nd, 3rd, 4th instars and prepupae, respectively.

DISCUSSION

Phloem Arenas

Little variation existed between larval density and the rate at which larvae developed. These results are similar to the data reported in Chapters 1 and 3, which also showed that larval density did not affect the rate of larval development. Interestingly, *A. planipennis* larvae in phloem arenas consumed twice the area of phloem as larvae feeding on sections of *F. americana* or larvae feeding on live trees (Chapter 3). There may be two possible reasons for this. The nutritional quality of the phloem may have been poorer in the phloem arenas compared with living trees or in the cut sections of ash, so that larvae had to consume more tissue to fully develop. Another reason could be that the phloem in the arenas may have been missing a key developmental trigger that would cause the larva to stop feeding and begin to pupate. Additional studies on the nutritional and chemical composition of ash phloem and their effects on *A. planipennis* larvae are needed.

Cannibalism occurred at very low levels on phloem arenas, similar to results of studies of low levels in other *A. planipennis* populations in the field (Chapter 2, Bauer et al. 2004). Cannibalism has been observed at higher rates in other wood boring insects. For example, Ware and Stephen (2006) found that cannibalism in *E. rufulus* occurred about 50% of the time when larvae were placed in phloem arenas. Additionally, *E. rufulus* larvae grew at five times the normal rate after cannibalizing another larva. *A. planipennis* larvae did not, however, experience a similar increase in growth or in development rate.

The phloem arena design was a modification of the design used by Dodds (2001) and Ware and Stephen (2006) to study *E. rufulus* (Haldeman). The arenas used to study the *E. rufulus* had a large hole drilled into them for larval placement (Dodds 2001, Ware and Stephen 2006). This did not work for *A. planipennis* larvae because they need to push ventrally and dorsally against a substrate to move and feed. Arenas for *Dendroctonus frontalis* Zimmerman, the southern pine beetle, had holes to allow adult beetles to enter the arena and form nuptial galleries (Grosman 1992, Taylor 1992). The larvae were placed into the phloem in the arenas used in this study by hand, and while adult *A. planipennis* might have laid eggs through the holes on the bark, fresh phloem was needed weekly and eggs would not have had time to develop.

Most mortality occurred when larvae were handled. If phloem arenas are used in the future to study *A. planipennis*, then the size of the phloem arena should be increased so larvae will not have to be transferred weekly to fresh phloem. Care must be taken to ensure that the phloem is solid so that *A. planipennis* larvae can move and feed. We have observed that larvae must press against the substrate within a tight space to move efficiently. Even a small depression in the phloem may cause a larva to stop feeding and die.

Potted *Fraxinus americana* Sections

Overall, 4th instars and prepupal larvae were not found on ash sections until ten weeks after the adult beetles were caged. This coincided with development times observed in field populations (Chapter 3). Compared with

phloem arenas, larvae on cut sections developed much more slowly. Larvae that reached the 4th instar or prepupal stage consumed about the same area of phloem as larvae in Chapter 3, or about 10 cm^2 per larva.

Larval mortality was low on the cut sections and mostly occurred where portions of the stems had desiccated. As in other studies (phloem arenas, Chapter 2 and Chapter 3), cannibalism was very rare, with only one of 63 larvae being cannibalized.

I could not determine in this study if the difference in larval development rates between phloem arenas and ash sections was caused by degree day accumulation. Larvae used in the phloem arena experiment were collected in the field, we could not determine the initial age of the larvae.

Moisture levels may affect larval development of *A. planipennis* and other buprestids. Buprestid larvae on stressed trees perform better than on vigorous trees (Koricheva 1998), with examples including *Agrilus bilineatus* (Weber) (two-lined chestnut borer) on oaks (Haack 1982, Dunn 1986a), *Agrilus burkei* Fisher (flatheaded borer) on alders (Svihra 1993), *Agrilus anxius* Gory (bronze birch borer) on birch (Barter 1957) and on *A. planipennis* in its native range in China (Akiyama and Ohmomo 2000, Schaefer 2005, Williams 2005, 2006). *A. planipennis* larvae developed faster when feeding in areas under girdled phloem (Chapter 1 and Chapter 3) and on stressed compared to non-stressed trees (Siegert et al. 2007). All surviving larvae in this study would have likely developed into adults in one year. Both the phloem arenas and the cut sections created environments that were relatively dry compared to phloem found in living

trees. Further studies are needed to determine the optimum moisture level for larval development.

Table 4.1. Summary of variables recorded on phloem arenas with *A. planipennis* larvae and mean (± SE) number of larvae per arena, and percentage of larvae that were 4th instar

	Week				
	1	2	3	4	6
High density arenas					
No. arenas with live larvae	15	15	10	6	6
Mean No. larvae per arena	6.13 ± 0.34	3.60 ± 0.40	2.40 ± 0.40	1.83 ± 0.31	1.43 ± 0.32
Percentage of surviving larvae reaching 4th instar	0%	12.7%	68.3%	86.1%	100%
Mean phloem consumed per larva per arena (cm^2)	0.6 ± 0.1	2.1 ± 0.2	2.9 ± 0.5	4.3 ± 1.1	12.1 ± 1.1
Accumulated degree days (Base 10 °C)	98	196	294	392	588
Low density arenas					
No. arenas with larvae	13	8	6	3	3
Mean No. larvae per arena	1	1	1	1	1
Percentage of surviving larvae reaching 4th instar	0%	0%	33.3%	66.7%	100%
Mean phloem consumed per larva per arena(cm^2)	0.75 ± 0.18	1.88 ± 0.50	1.98 ± 0.38	10.49 ± 3.77	9.44 ± 0.70
Accumulated degree days (Base 10 °C)	98	196	294	392	588

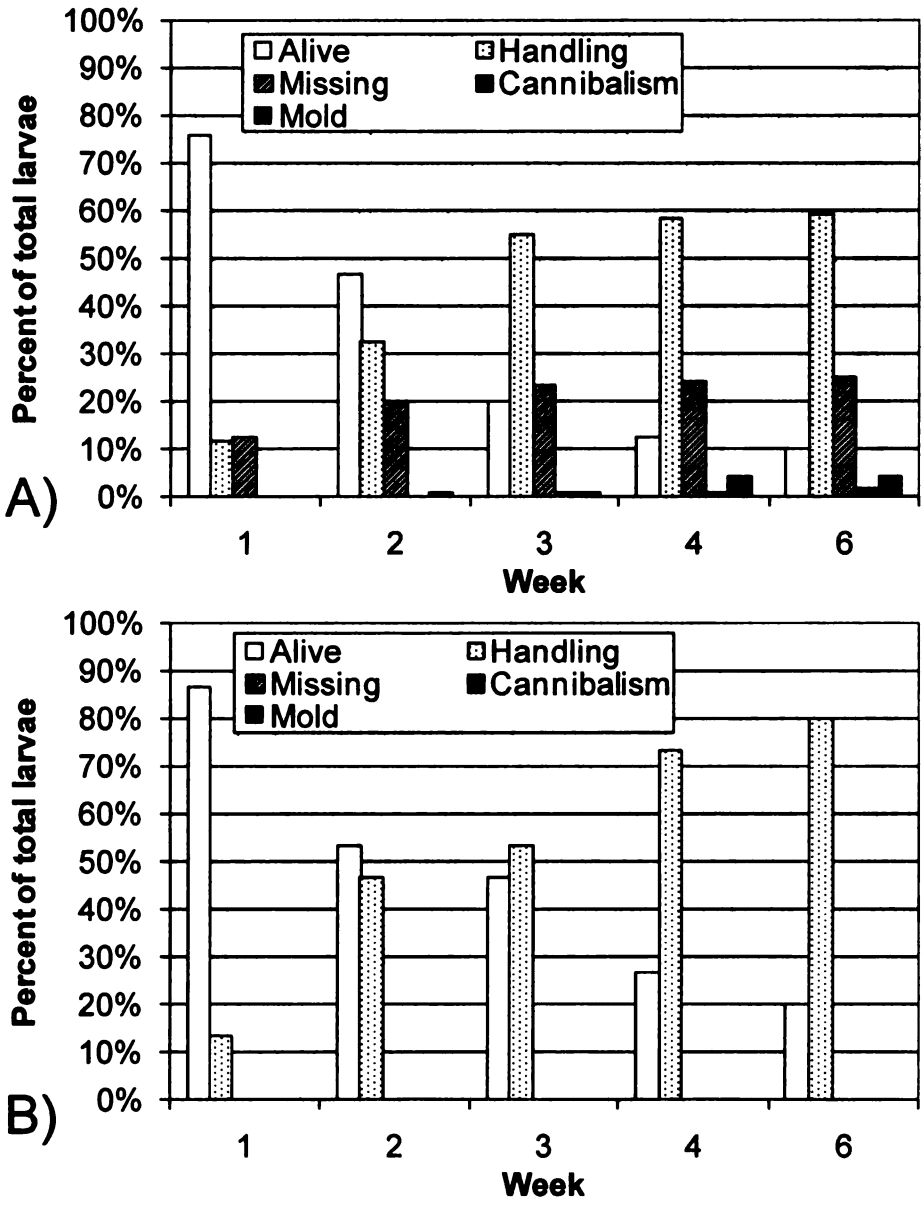


Fig. 4.1. Accumulated mortality of *A. planipennis* larvae on phloem arenas by week for A) high density phloem arenas (n=120 larvae) and B) low density phloem arenas (n=15 larvae).

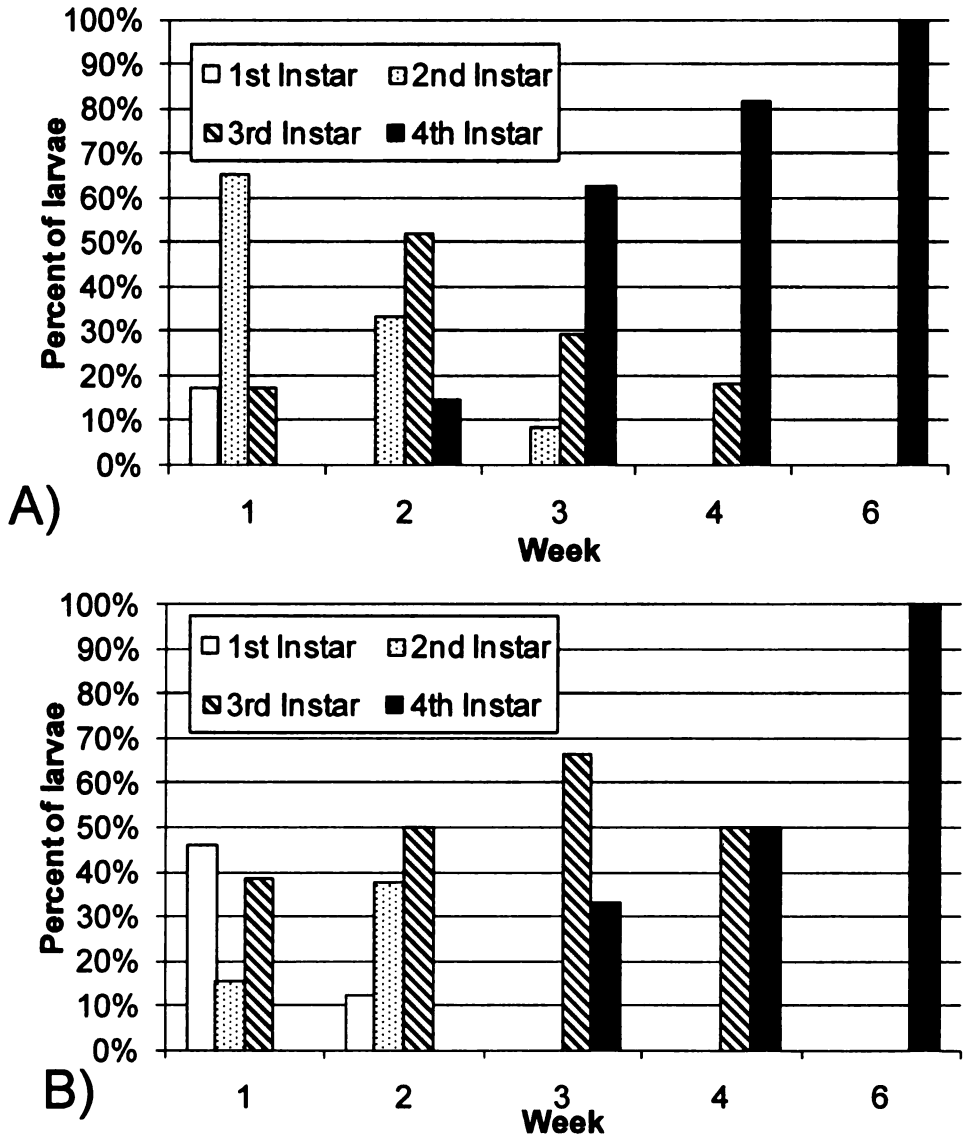


Fig. 4.2. Percentage of surviving larvae by instar by week for A) high density phloem arenas (n=120 larvae) and B) low density phloem arenas (n=15 larvae).

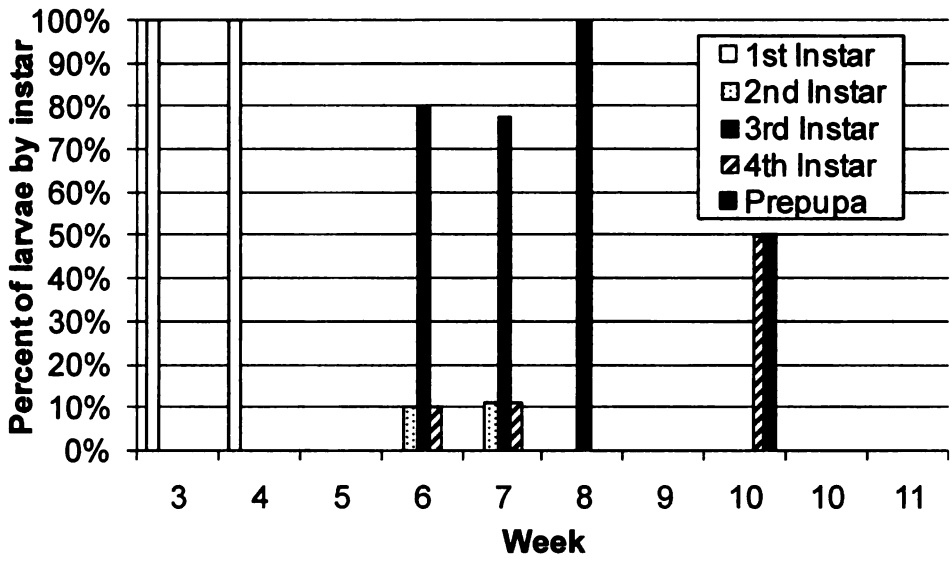


Fig. 4.3. Percentage of *A. planipennis* larvae by instar on potted *F. americana* sections debarked three to 11 weeks after adult beetles were placed in cages. n=13 larvae (Week 3), n=14 larvae (Week 4), n=13 larvae (Week 6), n=12 larvae (Week 7), n=2 larvae (Week 8), and n=9 larvae (Week 10).

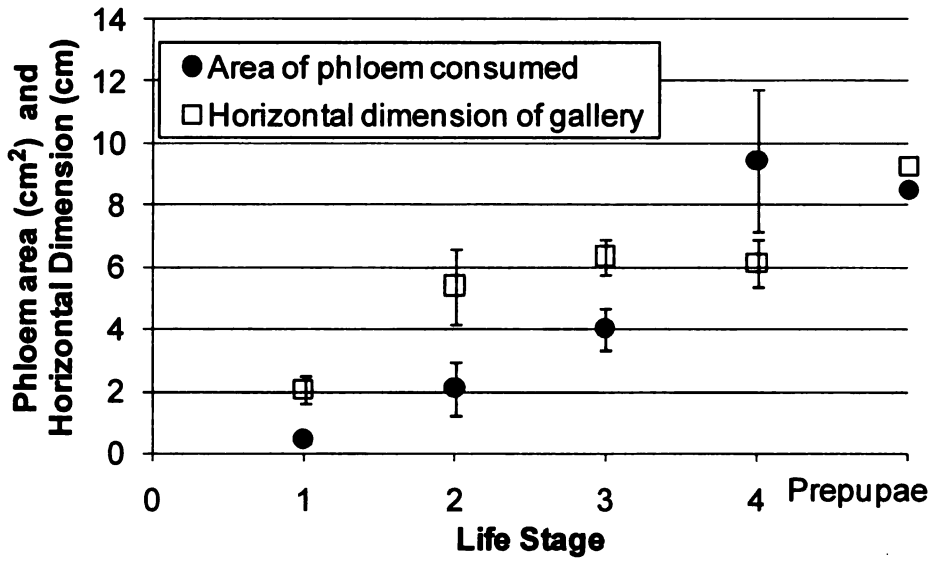


Fig. 4.4: Mean (± SE) area phloem consumed and mean horizontal gallery dimension of *A. planipennis* larvae by instar on potted *F. americana* sections.

APPENDICES

Appendix 1

Record of Deposition of Voucher Specimens*

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

Voucher No.: 2008-12

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

Influence of host vigor on larval distribution, development, and mortality of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America as influenced by host vigor

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

Entomology Museum, Michigan State University (MSU)

Investigator's Name(s) (typed)
Andrew Tluczek

Date March-5-2009

*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 Page

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	Emerged from logs July-2008. Jackson Co., Waterloo					10	10		MSU
Agrilus planipennis	Collected from logs August-2008, Ingham Co.		10						MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Andrew Tluczek

Date March-5-2009

Voucher No. 2008-12

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 3/13/2009 Date

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LITERATURE CITED

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