

EVALUATION OF EMERALD ASH BORER POPULATIONS AND THE ASH RESOURCE AT THREE
STAGES OF THE INVASION WAVE

By

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ABSTRACT

EVALUATION OF EMERALD ASH BORER POPULATIONS AND THE ASH RESOURCE AT THREE STAGES OF THE INVASION WAVE

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Questions consistently arise as to the status of *Agrilus planipennis* populations and the persistence of ash in forested settings, particularly in the original core of the *A. planipennis* infestation in southeast Michigan. I sampled *A. planipennis* populations in 24 green ash (*Fraxinus pennsylvanica*) sites, each 1 ha in size. Eight sites were located in each of three areas of southern Michigan representing (1) the original *A. planipennis* Core; (2) the invasion Crest, in south central Michigan, where *A. planipennis* populations were currently peaking, and (3) the invasion Cusp, in southwest Michigan where *A. planipennis* had recently become established.

Adult *A. planipennis* were captured in all 24 sites in both years of the study. No larvae were present in the two most westerly Cusp sites in 2010, but larvae were present in all 24 sites in 2011. Despite the depletion of overstory ash in southeast Michigan, *A. planipennis* continues to infest and kill trees in Core sites. Beetle populations were highest in south central Michigan and are rising in southwestern Michigan. Mortality of overstory ash decreased across an east to west gradient across southern Michigan. Green ash was abundant in the understory of all sites including Core sites. There was no evidence of current year ash germination in Core sites in southeast Michigan. If *A. planipennis* persists in these areas, regenerating ash will continue to become infested and killed, and potentially eliminating ash from these systems.

DEDICATION

I would like to dedicate this thesis to my grandmother, Margret Hansen (Gee), who passed away while I was working on this project, as well as my other grandparents Ronald Hansen (Bumpa), Frances Burr (Grandpa), and Ruth Burr (Grandma), and my great-grandparents Lewis Bauser (Opa), and Ottomine Hansen (Grandma Minnie). They watched over me as a child, and their strength of spirit is with me to this day.

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PREFACE

Emerald ash borer (*Agrilus planipennis*) was discovered in southeast Michigan in June of 2002 (Cappaert et al. 2005), but is believed to have entered North America six to ten years prior to its discovery. Since its arrival, *A. planipennis* has killed tens of millions of ash trees in Michigan alone, and is currently established in 15 states and two provinces of Canada.

Initial research conducted in North America focused on *A. planipennis* biology, host range, potential impact, and optimal trapping techniques. Researchers in Michigan, the surrounding states, and Canada have worked to develop better methods for detection and monitoring, and reducing the impact of this invasive pest.

This project focused on evaluating *A. planipennis* populations at three stages of the *A. planipennis* invasion wave, and the impact *A. planipennis* on the overstory, and potential regeneration of green ash (*Fraxinus pennsylvanica*). Previous literature has reported nearly 100% mortality of all *Fraxinus spp.* within infested stands (Gandhi et al. 2008). In Chapter 1, I address adult trapping and larval surveys that were conducted to assess beetle populations behind, within, and in front of the advancing *A. planipennis* invasion wave. Ash overstory trees were assessed in fixed radius plots and belt-transects to determine ash basal area, mortality, and canopy decline at all three stages of the invasion.

Studies reported in Chapter 2 focused on impacts of *A. planipennis* on overstory ash, and three stages of ash regeneration. All species of overstory trees, recruits, saplings, and seedlings were assessed in fixed radius plots and belt-transects to assess condition of overstory trees, and understory species composition to determine what species are likely to replace ash

in these systems. Readings were taken in the understory to measure available photosynthetically active radiation (PAR), and sapling growth associated with gaps formed by overstory ash mortality. My goal was to better understand *A. planipennis* population densities across southern Michigan, and *A. planipennis* impacts on overstory green ash, potential regeneration, and likely changes to ash stands in southern Michigan. Each chapter of the thesis is intended to be prepared as a manuscript for publication.

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CHAPTER 1

Emerald Ash Borer Populations and the Condition of the Green Ash Overstory at Three Stages of the Invasion Wave

INTRODUCTION

Emerald ash borer (*Agilus planipennis* Fairmaire) (Coleoptera: Buprestidae), is a phloem-feeding beetle native to Asia originally discovered in Detroit, MI and Windsor, Ontario and identified in July 2002 (Cappaert et al. 2005). Dendrochronological studies indicate *A. planipennis* entered North America at least six to ten years prior to its discovery (Siegert et al. 2007). Since its arrival, *A. planipennis* has attacked ash (*Fraxinus spp.*) trees in forest, rural, and urban settings, with mortality rates approaching 100% (Gandhi et al. 2008). Unlike many native phloem-feeding *Agilus spp.* which predominantly infest stressed trees (Balch and Prebble 1940; Barter 1965; Dunn et al. 1986; Muzika et al. 2000), *A. planipennis* can attack healthy ash trees (Poland and McCullough 2006). As ash resources are depleted in local areas, beetle populations disperse and colonize new locations.

The resulting *A. planipennis* invasion wave is expanding in all directions from its origin. In addition to natural dispersal, transportation of infested logs, nursery trees, and firewood has resulted in satellite populations of *A. planipennis* established miles from the original infestation sites (Cappaert et al. 2005), increasing the overall rate of expansion (Liebhold and Tobin 2010; Mercader et al. 2011). Presently, *A. planipennis* is established in 16 states and two provinces of Canada (EAB.info 2012).

Adult *A. planipennis* emerge in May and June from pupation cells in the outer sapwood or outer bark (Cappaert et al. 2005). Adults feed on ash foliage for 5 to 7 days before mating, and females feed for an additional 1 to 2 weeks before oviposition begins (Cappaert et al. 2005). Throughout July and August females deposit eggs beneath rough edges of bark or within cracks in the outer bark of ash trees. Larvae hatch within two weeks, chew into the phloem and begin feeding. Larvae overwinter in the thick outer bark or outer sapwood of trees, pupating the following spring. In high density infestations, *A. planipennis* complete their life cycle in a single year, but in healthy trees with low larval densities, most larvae require two years to develop (Cappaert et al. 2005; Siegert et al. 2010; Tluczek et al. 2011). Prolonged development of *A. planipennis* larvae may reflect host resistance or host quality (Poland and McCullough 2006; Tluczek et al. 2011).

As larval densities increase, feeding disrupts translocation of nutrients in trees, causing canopy decline, and eventually death of the host. External signs of *A. planipennis* become apparent, include D-shaped exit holes left by emerging *A. planipennis* adults, holes left by woodpecker predation on late instar larvae, and frequent formation of epicormic shoots and stump sprouts. As the ash component of a stand is depleted, *A. planipennis* disperse and colonize new locations. Stands behind the invasion wave typically have few remaining live overstory ash (Ghandi et al. 2008).

The long-term repercussions of the *A. planipennis* invasion on green ash in North America are currently unknown. *Agrilus planipennis* is known to attack and kill all *Fraxinus* spp. in North America, but preferentially infests green ash (*Fraxinus pennsylvanica* Marsh) (Anulewicz et al. 2007, 2008; Rebek et al. 2008). Green ash is the most widely distributed

species of ash in North America, ranging from Nova Scotia to Saskatchewan in the north and from Texas to Florida in the south (Wright 1959; Kennedy 2009). Green ash is found primarily on alluvial soils in riparian zones, but is capable of surviving on upland sites (Wright 1959; Kennedy 2009). It can grow in sites ranging from clay soils subject to frequent flooding to sandy silt soils where water can be limited (Burns and Honkala, 1990). Green ash is a common species in Michigan forests (Barnes and Wagner 1981). Millions of green ash have been killed in Michigan by *A. planipennis* (Poland 2007; EAB.info 2012) and millions more are at risk (FIA database 2012). Ecological impacts ensuing from widespread ash mortality could have cascading effects throughout systems where ash is a dominant overstory species (MacFarlane and Meyer 2005; Gandhi and Herms 2010).

In this study, I assessed *A. planipennis* population levels behind, within, and in front of the expanding invasion wave in green ash sites located along an east-west gradient across southern Michigan. The first invasion stage, classified as the “Core,” represents the original infestation area where most ash trees have been killed, and *A. planipennis* density has presumably dropped with the reduction of available resources. The second stage, designated as the “Crest,” represents sites where *A. planipennis* populations are near peak densities, and ash decline and mortality are moderate to high. The third stage of the invasion wave was designated as the “Cusp.” Ash trees in Cusp sites appeared healthy with few external signs of *A. planipennis* infestation, indicating *A. planipennis* populations had not yet built to damaging levels capable of adversely impacting overstory ash trees. Adult *A. planipennis* populations were assessed by capturing beetles on purple double-decker traps (McCullough and Poland 2009; McCullough et al. 2011; Poland et al. 2011) and sticky bands placed on girdled ash trees

and uninfested nursery ash trees planted in each site. Stressed ash trees attract *A. planipennis* (McCullough et al. 2009a, 2009b; Siegert et al. 2010; Tluczek et al. 2011), at least partly due to changes in volatiles emitted from foliage (Rodriguez-Soana et al. 2006; de Groot et al. 2008; Grant et al. 2010) and bark (Crook et al. 2008). Sticky bands were also placed on ungirdled “control” ash trees which were neither stressed nor baited, enabling me to characterize *A. planipennis* activity uninfluenced by lures or visual stimuli. I assessed larval density by felling and debarking control, girdled, and planted trees. I measured the condition of overstory ash to evaluate how *A. planipennis* will affect stand with a substantial green ash component. This study will provide insight into *A. planipennis* populations along the *A. planipennis* invasion wave and the impact of *A. planipennis* on green ash stands. Results will be useful to *A. planipennis* programs and resource managers as they attempt to manage this invasive pest.

MATERIALS & METHODS

Study sites: Twenty-four sites, each one ha, located on an east to west gradient across southern Michigan, were selected in July and August 2009 (Fig. 1.1). Sites were on state, county, and city property, and were second growth stands with trees between 60 and 90 years of age (Michigan.gov 2012). The overstory at each site was comprised of a minimum of 20% green ash, based upon available inventory data provided by managers, previous studies conducted in these locations, and on site visits. Eight sites were selected to represent each of three invasion stages designated as Core, Crest, and Cusp. Core sites were located in southeast Michigan (Fig 1.1,) in areas where the majority of ash trees were dead, and beetle populations were believed to have dropped with the reduction of available resources. To ensure ash mortality was the result of *A. planipennis*, trees in Core sites were examined for D-shaped exit holes in the bark left by emerging beetles, and bark was removed from several trees to determine if *A. planipennis* galleries were present. Crest sites in south central Michigan (Fig 1.1) were characterized by ash trees in various stages of decline. Roughly half of the ash trees in Crest sites were alive, and of those, most trees were obviously infested by *A. planipennis*. Exit holes and serpentine galleries beneath the bark were found on sampled trees. Many trees in Crest sites also had epicormic shoots, stump sprouts, and signs of woodpecker predation on larvae. In Cusp sites in southwest Michigan (Fig 1.1), ash trees showed little evidence of infestation. Exit holes and galleries were observed in some sites, but infrequently.

Adult *A. planipennis* populations: Adult *A. planipennis* populations were assessed in 2010 with a variety of trapping methods, including double-decker traps and sticky bands on girdled,

planted, and control trap trees. Traps and sticky bands were deployed at sites beginning on 10 May and remained in place until 16 August.

Two purple double-decker panel traps were placed at each site, in full sun when possible (McCullough and Poland 2009; McCullough et al. 2011; Poland et al. 2011). Traps consisted of a 3-m-tall polyvinyl chloride (PVC) pipe (10 cm diameter), and two three-sided prism panels constructed from purple corrugated plastic (Harbor Sales Inc., Sudlersville, MD). Each panel was 4 mm thick, 60 cm in height and 40 cm in width. The first panel was attached to the top of the PVC pipe using cable ties, and the second panel, similarly attached, was placed 60 cm below the top panel. Clear Pestick (Hummert international, Earth City, MO), was applied to both panels. The top panel was baited with two bubble caps of *cis*-3-hexenol (combined volatilized release rates of 7.4 mg/d determined in the laboratory at 20°C; Contech Enterprises, Inc., formerly Phero Tech, Inc., Delta, BC, Canada). The bottom panel was baited with an 80:20 blend of Manuka oil and Phoebe oil (release rate of 50 mg/d determined in the laboratory at 20°C; Synergy Semiochemicals Corp., Burnaby, BC, Canada). Double-decker traps were placed over 1.5 m tall T-posts set into the ground for support.

Three uninfested, green ash nursery trees, 3.8-6.4 cm DBH, (Bailey Nursery, Newport, MN) were planted at each site, typically in full sun along edges or in gaps to optimize beetle captures (Yu 1992; McCullough et al. 2009a, 2009b). I assumed transplant shock would be adequate to elicit the chemical stress responses attractive to *A. planipennis* (McCullough et al. 2009a, 2009b; Tluczek et al. 2011). A band of clear plastic wrap, 30 cm wide and coated in Tanglefoot (Contech Enterprises, Inc., formerly Phero Tech, Inc. Delta BC, Canada), was wrapped around each planted tree, 1 m above the base to capture adult *A. planipennis*.

Two naturally regenerated ash trees in each site were girdled. Trees growing in gaps with increased sun exposure were selected to optimize adult captures (Yu 1992; McCullough et al. 2009a, 2009b), but were also selected to be representative of ash trees within each site. To girdle trees, drawknives and handsaws were used to remove a 15 cm wide band of outer bark and phloem, 1 m above the base of the tree. A band of clear plastic wrap, 30 cm wide, was placed immediately above the girdle of each tree and coated with Tanglefoot.

Two additional ash trees growing on each site were selected using the same criteria described for girdled trees. These trees were not stressed or baited, and were designated as “control” trees. Sticky bands coated with Tanglefoot were applied 1 m above the base of the tree.

Girdled and control trees, double-decker traps, and planted trees were a minimum of 10 m apart. Trap trees and traps of the same type (i.e. control and control, double-decker and double-decker) were a minimum of 20 m apart.

Traps were checked biweekly. Adult *A. planipennis* were collected from each trap, counted, and returned to the laboratory, where beetles were soaked in Histo-Clear II (National Diagnostics, Atlanta GA) for two weeks to remove Pestick and Tanglefoot. Insects were then examined to confirm identification. To estimate densities of *A. planipennis* adults on traps and sticky bands, captures were standardized per m² of trap surface area.

Adult *A. planipennis* trapping was repeated in 2011 with the following modifications. Traps and sticky bands were placed in sites beginning on 9 May and remained in place until 12 August. Double-decker traps and planted trap trees were placed in roughly the same locations as the previous year. Trees selected for girdling and controls were as near as possible to the

location of trees in 2010. Three planted trap trees were obtained from Law's Nursery Inc. in Hastings MN. Lures with *cis*-3-hexenol were again used on the top panel of double-decker traps in 2011. Lures on the lower panel, however, were baited with manuka oil only, because Phoebe oil was unavailable in 2011.

Larval *A. planipennis* densities: Densities of *A. planipennis* larvae were evaluated on trap trees from October to December in 2010. All trees < 10 cm DBH, including planted trap trees and small control and girdled trees, were debarked from the base to roughly 2 m above ground. Stem diameters and tree height were measured prior to debarking. Because stem diameter was different on the top and bottom of trap trees, the equation for a conical frustum was used to determine m² of exposed surface area. Trees ≥ 10 cm DBH were felled and bucked into 1 m sections beginning just above the sticky bands. An area equal to 1/2 m in length and 1/2 the circumference of the upper half of each log was debarked on alternate sections. Sections were rolled to ensure all sides of the trees were sampled. Length and width of sections were measured to calculate larvae per m² of exposed phloem surface area.

Parasitoids found either as pupae in *A. planipennis* larval galleries, or as small larvae attached to the larger *A. planipennis* larvae, were recorded. Logs with *A. planipennis* parasitoids were returned to the laboratory and parasitoids were allowed to develop, and emerge as adults. Representative adult parasitoids from each site where they were collected were identified by Dr. John S. Strazanac from the University of West Virginia in Morgantown WV, as *Atanycolus cappaerti* Marsh and Strazanac (Hymenoptera: Braconidae). The proportion

of *A. planipennis* larvae parasitized and parasitoid densities per m² of exposed surface area were calculated per tree.

Larval density was surveyed in 2011 using methods from 2010 but with two modifications. Trap trees ≥ 10 cm were felled and cut into 1 m sections above the sticky bands, and an area 1 m in length and by 1/2 the circumference of the log was debarked on alternate sections. All trap trees were cut down and debarked in October and November.

Voucher specimens of ten larval *A. planipennis*, ten male and ten female adult *A. planipennis*, and ten male and ten female adult *A. cappaerti* were submitted to the Albert J. Cook Arthropod Research Collection at Michigan State University in East Lansing, Michigan. Specimens were submitted in March 2012.

Overstory: Ash and non-ash trees (DBH ≥ 10 cm) were counted, recorded as alive or dead, and DBH was measured in two belt-transects and four fixed radius plots. Belt-transects were 150 m x 2 m, running diagonally across each site in an X-formation. Trees within 10 m of the belt-transect intersections were marked to ensure individual trees were not measured more than once. Belt-transects divided sites into four quadrats and one macro-plot (18 m radius) was placed in the center of each quadrat. Basal area of all trees was calculated in cm and converted to m² using the formula $DBH^2 \times 7.854 \times 10^{-5}$ (VanderSchaaf 2008). Basal area of all trees was summed and divided by the area measured to calculate m² per hectare. Trees were grouped into five diameter classes (10 cm increments) for analysis. Basal area was calculated for all species combined, and for live ash, dead ash, and total ash for each site. Dead ash trees were assumed to be killed by *A. planipennis* if evidence such as holes left from woodpecker predation

of larvae, and *A. planipennis* exit holes were present. Sections of bark were removed from dead ash trees if no external signs of *A. planipennis* infestation were apparent to confirm presence of larval galleries. Dead ash trees without *A. planipennis* galleries were excluded from *A. planipennis* mortality estimates.

Overstory assessments were repeated in 2011 with one modification. Basal area of non-ash trees was assumed to remain relatively constant, so only ash trees were measured.

External signs of *A. planipennis*: Woodpeckers preying on late instar larvae and stump sprouts growing from exposed roots at the base of trees were assessed on live and dead ash trees.

Woodpecker predation on ash trees was visually estimated as absent, low (1 to 6 woodpecker attacks), and high (> 6 woodpecker attacks). Date of woodpecker predation cannot be determined with visual surveys, so estimates represented cumulative woodpecker predation.

Stump sprouts were visually estimated as absent, low (1 to 4 stump sprouts), and high (> 4 stump sprouts).

On live trees, epicormic shoots and canopy dieback were also recorded. Epicormic shoots growing on the trunk or branches were visually estimated as absent, low (1 to 4 epicormic shoots), and high (> 4 epicormic shoots). Canopy dieback was visually estimated in 10% increments, where 0% indicates a full canopy, and 90% indicates a nearly complete absence of leaves (Zarnoch et al. 2004). Dieback was assessed from 21 June to 23 July, after trees were fully flushed but before current-year larvae began feeding.

I estimated the external signs of *A. planipennis* again in 2011 with the following modifications. Canopy dieback of ash was assessed from 18 June to 20 July. Canopy dieback of

non-ash trees was assumed to remain relatively constant, so non-ash trees were not assessed in 2011.

Statistical analysis: Data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Adult *A. planipennis* captures, larval densities, and basal area were normalized by $\log_{10}(x + 1)$ transformations. Adult *A. planipennis* captures, larval densities, and basal area were tested as unplanned comparisons to assess differences among the three invasion stages. Tukey's honestly significant difference (HSD) multiple comparison procedure was applied if the overall analysis of variance (ANOVA) was significant ($P < 0.05$). Two-way ANOVA was used to evaluate main effects of trap type, invasion stage and the interaction of the two factors on adult captures and larval densities (PROC GLM; SAS Institute 2003) (Ott and Longnecker 2001). Estimates of trap surface area, trap tree DBH, the surface area debarked, parasitoid densities, canopy dieback, and tree size class were not normalized by transformations. Friedman's two-way nonparametric test was therefore used to evaluate differences among the different types of trap trees (i.e. control, girdled, and planted), the three invasion stages, and the interaction between the two factors (PROC RANK; SAS Institute 2003) (Friedman 1937). Friedman's two-way nonparametric test was also used to evaluate effects of invasion stage and diameter class on ash mortality, canopy dieback, and abundance of epicormic shoots, stump sprouts, and woodpecker attacks (PROC RANK; SAS Institute 2003) (Friedman 1937). When results for nonparametric tests were significant ($P < 0.05$), nonparametric multiple comparisons were applied (Zar 1984). All analyses were conducted at $P < 0.05$ level of significance using SAS statistical software (SAS Institute 2003).

RESULTS

Adult *A. planipennis* captures: In 2010, 2600 adult *A. planipennis* were captured on traps, including 338, 1960, and 302 beetles in Core, Crest, and Cusp sites, respectively. The proportions of total beetle captures were 13%, 75%, and 12% in Core, Crest, and Cusp sites, respectively. Adults were captured in all 24 study sites. Beetle captures peaked from 21 June to 2 July, when 1142 *A. planipennis* were captured, representing 44% of the years' total. Captures per m² of *A. planipennis* adults in Crest sites were 5-fold higher than in Core sites, and 9-fold higher than in Cusp sites ($F = 47.94$; $df = 2, 211$; $P < 0.001$) (Fig. 1.2A). More *A. planipennis* were captured in Core sites compared to Cusp sites, but the difference was not significant.

Trap type affected adult *A. planipennis* captures in 2010. Double-decker traps accounted for 75 to 80% of the beetles captured in all sites. Double-decker traps captured at least one beetle on 100%, 100%, and 88% of traps in Core, Crest, and Cusp sites, respectively. Sticky bands on girdled trees captured 12%, 17%, and 17% of the adults in Core, Crest, and Cusp sites, respectively. At least one *A. planipennis* was captured on 69%, 94%, and 44% of sticky bands in Core, Crest, and Cusp sites, respectively. Sticky bands on control and planted trees captured < 10% of the adults. Sticky bands captured at least one beetle on 25%, 69%, and 27% of control trees in Core, Crest, and Cusp sites, respectively, while sticky bands on 21%, 79%, and 6% of planted trees were positive in Core, Crest, and Cusp sites, respectively. The interaction between trap surface area and invasion stage was not significant ($P = 0.99$). Surface area differed among trap types ($H = 28.38$; $df = 3, 216$; $P < 0.001$) (Table 1.1). Area of double-decker panels was 15-fold greater than the surface area of sticky bands on girdled and control trees,

and 25-fold greater than sticky bands on planted trees. The area of sticky bands on girdled and control trees was nearly 2-fold greater than the area of sticky bands on planted trees. Surface area of sticky bands on girdled and control trees were similar. Sticky bands on girdled trap trees captured more *A. planipennis* per m² than double-decker traps and sticky bands on control and planted trees. ($F = 12.69$; $df = 3, 210$; $P < 0.001$) (Table 1.1). Differences in *A. planipennis* captures per m² between double-decker traps and the sticky bands on control and planted trees were not significant. The interaction between the three invasion stages and trap type on adult captures per m² was not significant ($P = 0.22$).

In 2011, 2504 adults were captured on traps, including 319, 1498, 687 beetles in the Core, Crest and Cusp sites, respectively. Beetle activity peaked later in 2011 than in 2010. Beetle captures peaked from 5 July to 15 July, when 1116 *A. planipennis* were collected, comprising 45% of the total captures. Captures of *A. planipennis* in Crest sites were 4-fold higher than in Core and Cusp sites ($F = 17.8$; $df, 2, 210$; $P < 0.001$) (Fig. 1.2A), where captures did not differ.

As in 2010, trap type affected adult *A. planipennis* captures in 2011. Double-decker traps accounted for 62%, 44%, and 75% of the adults captured in Core, Crest, and Cusp sites, respectively. At least one beetle was captured on 94%, 100%, and 94% of double-decker traps in Core, Crest, and Cusp sites, respectively. Sticky bands on girdled trees captured 27%, 42%, and 18% of adults in Core, Crest, and Cusp sites, respectively. Sticky bands captured at least one *A. planipennis* on 81%, 100%, and 69% of girdled trees in Core, Crest, and Cusp sites, respectively. Sticky bands on control and planted trees captured < 10% of the adults in all sites.

At least one beetle was captured on 31%, 62%, and 50% of control trees, and 33%, 71%, and 13% of planted trees in Core, Crest, and Cusp sites, respectively. Area of double-decker panels was 15-fold greater than surface area of sticky bands on girdled and control trees, 30-fold greater than sticky bands on planted trees, and the area of sticky bands on girdled and control trees was 2-fold greater than the area of sticky bands on planted trees ($H = 7.52$; $df = 3, 212$; $P < 0.001$) (Table 1.1). Surface area of sticky bands on girdled and control trees was similar. The interaction between trap type and invasion stage on trap surface area was not significant ($P = 0.98$) on an area basis. Sticky bands on girdled trees captured nearly 3-fold more adults per m^2 than sticky bands on planted trees, 4-fold more than sticky bands on control trees, and 6-fold more than panels on double-decker traps. Sticky bands on planted trees captured twice as many adults per m^2 as panels on double-decker traps ($F = 15.4$; $df = 3, 209$; $P < 0.001$) (Table 1.1). Differences in *A. planipennis* captures per m^2 among other trap types were not significant. The interaction between invasion stage and trap type did not affect captures of adults per m^2 ($P = 0.06$).

Larval *A. planipennis* densities: Rectangular areas of bark, hereafter referred to as “windows,” were debarked on girdled, control, and planted trees in fall 2010 to assess *A. planipennis* larval density. Area of exposed phloem per tree averaged 0.6 ± 0.04 , 0.6 ± 0.04 , and $0.9 \pm 0.2 m^2$ in Core, Crest, and Cusp sites, respectively, and did not differ among invasion stages ($P = 0.93$). Overall, 1818 larvae were recorded in windows debarked on trap trees in 2010, including 441, 902, and 475 larvae on trees in Core, Crest and Cusp sites, respectively. Larvae were found on

trees in 22 of the 24 study sites. No larvae were found on trees in the two most westerly sites in Ottawa County, MI. Larval densities differed among all three invasion stages ($F = 17.03$; $df = 2, 163$; $P < 0.001$) (Fig. 1.2B). Larval *A. planipennis* density was significantly related to adult *A. planipennis* density on traps when all 24 sites were pooled (Figure 1.3A), but relationships within each invasion stage were not significant [$(P = 0.26)$, $(P = 0.30)$, and $(P = 0.18)$ in Core, Crest and Cusp sites, respectively].

In 2010, girdled trap trees accounted for 68%, 69%, and 64% of all larvae recorded in Core, Crest, and Cusp sites, respectively, and at least one *A. planipennis* larva was found in 94%, 100%, and 60% of the girdled trees in Core, Crest, and Cusp sites, respectively. Larvae in control trees accounted for 22%, 18%, and 33% of all larvae recorded in Core, Crest, and Cusp sites, respectively, and 44%, 88%, and 40% of control trees had at least one *A. planipennis* larva in Core, Crest, and Cusp sites, respectively. Planted trap trees, accounted for only 10%, 13%, and 1% of all larvae in Core, Crest, and Cusp sites, respectively. One or more *A. planipennis* larvae were present on 54%, 63%, and 13% of trees in Core, Crest, and Cusp sites, respectively.

Average DBH of control and girdled trees was more than twice the DBH of planted trees ($H = 28.38$; $df = 2, 163$; $P < 0.001$) (Table 1.1). Trap tree DBH did not differ between girdled and control trees. Nor was the interaction between trap type and invasion stage significant ($P = 0.99$). Densities of larvae on girdled trees were twice as high as on control trees, and 11-fold higher than on planted trees. Larval densities on control trees were 5-fold higher than on planted trees ($F = 43.04$; $df = 2, 163$; $P < 0.001$) (Table 1.1). The interaction of trap type and invasion stage did not affect larval density ($P = 0.51$).

Area of phloem exposed in bark windows in 2011 to assess larval density averaged 0.8 ± 0.1 , 0.7 ± 0.04 , and $0.8 \pm 0.03 \text{ m}^2$ per tree in Core, Crest, and Cusp sites, respectively, and was not affected by invasion stage ($P = 0.85$). In 2011, 2895 larvae were recorded in all 24 sites, including 584, 1245, and 1066 on trees, in Core, Crest and Cusp sites, respectively. Trap trees in Crest sites had higher larval densities than those in Core and Crest sites ($F = 7.2$; $df = 2, 162$; $P = 0.001$) (Figure. 1.2B). Larval densities were higher in Cusp sites compared to Core sites, but the difference was not significant. Larval *A. planipennis* density was significantly related to adult *A. planipennis* density on traps when all sites were pooled (Figure 1.3B). Within each invasion stage, larval density was significantly related to adult captures per m^2 in Core sites ($R^2 = 0.71$; $P = 0.05$), but not in Crest ($P = 0.13$) or Cusp sites ($P = 0.19$).

Girdled trees accounted for 71% to 75% of all larvae in all sites, and at least one *A. planipennis* larva was found in 88%, 100%, and 88% of girdled trees in Core, Crest, and Cusp sites, respectively. Control trees accounted for 11%, 12%, and 31% of total larvae found in Core, Crest, and Cusp sites, respectively, and 69%, 81%, and 63% of control trees had at least one *A. planipennis* larva in Core, Crest, and Cusp sites, respectively. Larvae on planted trap trees accounted for 16%, 17%, and 4% of total larvae found in Core, Crest, and Cusp sites, respectively, and at least one *A. planipennis* larva was found on 57%, 75%, and 25% of trees in Core, Crest, and Cusp sites, respectively. Similar to 2010, DBH of control and girdled trees in 2011 was more than twice that of planted trees ($H = 7.52$; $df = 2; 162$; $P < 0.001$) (Table 1.1), but did not differ between girdled and control trees. Larval density on girdled trees was 4-fold greater than on control trees and 10-fold greater than planted trees ($F = 39.7$; $df = 2, 162$; $P <$

0.001) (Table 1.1). More larvae were found on control trees than on planted trees, but differences were not significant. The interaction between trap type and invasion stage on larval density was not significant ($P = 0.16$).

***Atanycolus cappaerti* parasitism:** In 2010, 283 *A. planipennis* larvae were parasitized by *A. cappaerti*, including 57, 223, and 3 larvae in Core, Crest, and Cusp sites, respectively. Parasitism rates by *A. cappaerti* averaged $12 \pm 4.0\%$, $27 \pm 6.0\%$, and $1.3 \pm 1.2\%$ in Core, Crest, and Cusp sites, respectively. Parasitism rates in Crest sites were higher than in Cusp sites ($H = 10.28$; $df = 2, 21$; $P = 0.005$), but other differences were not significant. Densities of parasitoids averaged 1.4 ± 0.6 , 7.2 ± 2.4 , and 0.1 ± 0.1 parasitoids per m^2 of exposed phloem in Core, Crest, and Cusp sites, respectively. Average densities of parasitoids recorded on *A. planipennis* larvae were higher in Crest sites than Core and Cusp sites ($H = 20.42$; $df = 2, 163$; $P < 0.001$), where densities did not differ.

Parasitoid density was higher on *A. planipennis* larvae in girdled trees than on planted and control trees, and higher on control trees compared to planted trees ($H = 42.65$; $df = 2, 163$; $P < 0.001$). Densities of *A. cappaerti* in girdled trees were lower in Cusp sites than girdled trees in Core and Crest sites (Friedman's $F = 9.99$; $df = 4, 160$; $P < 0.001$), where densities did not differ. Parasitoids density on *A. planipennis* larvae averaged 4.7 ± 1.8 , 22.2 ± 7.3 , and 0.3 ± 0.2 parasitoids per tree on girdled trees in Core, Crest, and Cusp sites, respectively, compared to 0.6 ± 0.4 , 3.1 ± 1.3 , and 0.2 ± 0.2 parasitoids in control trees in Core, Crest, and Cusp sites, respectively. No parasitoids were observed on planted trap trees in 2010.

In 2011, 145 *A. cappaerti* parasitizing *A. planipennis* larvae were recorded which was approximately half as many as had been recorded in 2010, this including 28, 84, and 33

parasitoids in Core, Crest, and Cusp sites, respectively. Parasitism rates by *A. cappaerti* averaged $5.3 \pm 1.2\%$, $7.7 \pm 3.5\%$, and $2.3 \pm 1.1\%$ *A. planipennis* larvae in Core, Crest, and Cusp sites, respectively, and did not differ significantly among stages ($P = 0.21$). Difference in parasitoid densities on *A. planipennis* larvae among the three invasion stages were not significant ($P = 0.11$), and averaged 0.7 ± 0.2 , 2.4 ± 1 , 0.5 ± 0.3 parasitoids per m^2 in Core, Crest and Cusp sites, respectively. Girdled trees had significantly higher average densities of parasitoids on *A. planipennis* larvae than control and planted trees ($H = 17.66$; $df = 2, 161$; $P < 0.001$). Average densities of *A. cappaerti* were 1.3 ± 0.9 , 2.3 ± 0.8 , and 0.4 ± 0.2 parasitoids on control trap trees, girdled trap trees, and planted trap trees, respectively. The interaction between trap type and invasion stage on parasitoid densities was not significant ($P = 0.89$).

Overstory: In 2010, 3353 trees (DBH ≥ 10 cm) were measured, including 1015, 924, and 1414 trees representing 37 species in Core, Crest, and Cusp sites, respectively. Total basal area averaged 17.3 ± 3.8 , 11.1 ± 2.4 , and $16.9 \pm 1.3 m^2$ per ha in Core, Crest, and Cusp sites, respectively, and did not differ among the invasion stages ($P = 0.27$).

In 2010, 1035 green ash trees (DBH ≥ 10 cm) were recorded, including 216, 376, and 443 trees in Core, Crest and Cusp sites, respectively. Most ash trees were < 30 cm in DBH (88%, 95%, and 87% in Core, Crest, and Cusp sites, respectively). Ash mortality varied among the three invasion stages ($H = 290.77$; $df = 2, 1032$; $P < 0.001$) (Table 1.3), but not among diameter classes ($P = 0.53$). In three of the eight Core sites, 100% of the overstory ash had succumbed to *A. planipennis*. In contrast, 70% was the highest ash mortality recorded in any Crest site, and

34% was the highest mortality in Cusp sites. Ash killed by *A. planipennis* were present in all eight Core sites, six Crest sites, and four Cusp sites.

In 2011, 1054 ash trees were recorded in transects and plots, including 207, 404, and 443 trees in Core, Crest, and Cusp sites, respectively. As in 2010, 85% to 97% were < 30 cm in DBH. Ash mortality again varied among the three invasion stages ($H = 259.55$; $df = 2, 1,052$; $P < 0.001$) (Table 1.3), but not among diameter classes ($P = 0.44$). In three Core sites, all overstory ash was dead, as in 2010. The highest overstory ash mortality recorded was 100%, 85%, and 50% in Core, Crest, and Cusp sites, respectively. Ash trees killed by *A. planipennis* occurred in all Core sites, seven Crest sites, and seven Cusp sites.

Total ash basal area (dead and alive) in 2010 averaged 2.3 ± 0.5 , 2.7 ± 0.6 , and 5.2 ± 1 m^2 per ha in Core, Crest, and Cusp sites, respectively. Ash basal area was more than twice as high in Cusp sites compared to Core sites ($F = 4.37$; $df = 2, 21$; $P = 0.026$); other differences among stages were not significant. Live ash accounted for 21%, 63%, and 97% of total ash basal area in Core, Crest, and Cusp sites, respectively, and differed among invasion stages ($F = 21.09$; $df = 2, 21$; $P < 0.001$) (Fig. 1.4A). Most live ash were < 30 cm in DBH (87%, 97%, and 84% in Core, Crest, and Cusp sites, respectively). Basal area of dead ash in Core sites was more than 8-fold higher than in Cusp sites ($F = 4.64$; $df = 2, 21$; $P = 0.02$) (Fig. 1.4B), but other differences among stages were not significant. Most dead ash was < 30 cm in DBH (88%, 90%, and 98% in Core, Crest, and Cusp sites, respectively).

Total ash basal area in 2011 averaged 2.2 ± 0.4 , 2.7 ± 0.6 , and 5.5 ± 0.9 m^2 per ha, in Core, Crest, and Cusp sites, respectively, and was higher in Cusp sites than in Crest or Core sites

($F = 6.69$; $df = 2, 21$; $P = 0.006$), which did not differ significantly. The proportion of total ash basal area still alive was 14%, 44%, and 85% in Core, Crest, and Cusp sites, respectively, and differed among stages ($F = 20.72$; $df = 2, 21$; $P < 0.001$) (Fig. 1.4A). Most live ash (83 to 95%) were < 30 cm in DBH. Dead ash basal area did not differ among Core, Crest and Cusp sites in 2011 ($P = 0.16$) (Fig. 1.4B). Most dead ash was < 30 cm in DBH (83%, 96%, and 88% in Core, Crest, and Cusp sites, respectively).

External signs of *A. planipennis*: All sites had ash trees with some canopy dieback. Ash trees in Cusp sites had healthier canopies than ash trees in Core and Crest sites ($H = 112.3$; $df = 2, 713$; $P < 0.001$) (Table 1.2), where canopy dieback did not differ significantly. Ash canopy dieback did not differ significantly among diameter classes ($P = 0.31$). There was no interaction of invasion stage and diameter class on ash canopy dieback ($P = 0.91$).

Ash tree canopies in 2011 were again healthier in Cusp sites than Core and Crest sites ($H = 20.41$; $df = 2, 581$; $P < 0.001$) (Table 1.2), where canopy dieback did not differ significantly. The apparent reversal in ash canopy decline from 2010 to 2011 in Core and Crest sites reflected increased ash mortality in these sites in 2011. A number of trees with relatively high canopy dieback in 2010 did not survive, increasing the proportion of surviving trees with healthier canopies. Trees measuring 10 to 20 cm DBH had healthier canopies than trees with a DBH of 30.1 to 40 cm DBH ($H = 14.00$; $df = 2, 1,052$; $P = 0.007$), while other differences among size classes were not significant. There was no significant interaction between invasion stage and tree size class on ash canopy dieback ($P = 0.88$).

Fewer ash trees had epicormic shoots in 2010 in Cusp sites than in Core and Crest sites ($H = 81.28$; $df = 2, 710$; $P < 0.001$) (Table 1.2), which did not differ. Tree diameter class did not

affect epicormic shoot abundance ($P = 0.88$), and the interaction between invasion stage and tree diameter class was not significant ($P = 0.74$). In Crest sites, 40% of trees had high levels of epicormic shoots (> 4 shoots), compared to 29% and 11% of trees in Core and Cusp sites, respectively. Few trees had low levels of epicormic shoots (1 to 4 shoots) 11%, 6%, and 4% of trees in Core, Crest, and Cusp sites, respectively.

In 2011, the proportion of trees with epicormic shoots was higher in Core and Crest sites than in Cusp sites ($H = 24.23$; $df = 2$, 581; $P < 0.001$) (Table 1.2). Tree diameter class did not affect epicormic shoots ($P = 0.30$), and the interaction between invasion stage and tree diameter class was not significant ($P = 0.94$). Trees with high levels of stump sprouts comprised 58%, 27%, and 22% in Core, Crest, and Cusp sites, respectively. Few trees had low levels of epicormic shoots, including 5%, 9%, and 6% of trees in Core, Crest, and Cusp sites, respectively.

The proportion of live and dead ash trees in 2010 with stump sprouts was higher in Core and Crest sites than in Cusp sites ($H = 157.49$; $df = 2$, 958; $P < 0.001$) (Table 1.2). Tree diameter class did not affect stump sprout presence ($P = 0.97$), and the interaction between invasion stage and tree diameter class was not significant ($P = 0.62$). Trees with high levels of stump sprouts (> 4 sprouts) comprised 38%, 40%, and 6% of trees in Core, Crest, and Cusp sites, respectively. Trees with low levels of stump sprouts (1 to 4 sprouts) comprised 6%, 7%, and 3% of trees of trees in Core, Crest, and Cusp sites, respectively.

The proportion of live and dead ash trees in 2011 with stump sprouts was higher in Core and Crest sites than in Cusp sites ($H = 157.49$; $df = 2$, 977; $P < 0.001$) (Table 1.2). Tree diameter class did not affect stump sprout presence ($P = 0.93$), and the interaction between the three stages and tree diameter class was not significant ($P = 0.92$). Trees with high levels of stump

sprouts comprised 39%, 53%, and 12% of trees in Core, Crest, and Cusp sites, respectively. Few trees had low levels of stump sprouts (1 to 4 sprouts), including 9%, 8%, and 6% of trees in Core, Crest, and Cusp sites, respectively.

Live and dead ash trees with holes left by woodpeckers preying on late instar larvae in 2010 were more abundant in Core sites compared to Cusp sites ($H = 259.61$; $df = 2, 958$; $P < 0.001$) (Table 1.2), but all other differences were not significant. Tree diameter class was not related to woodpecker predation on late instar larvae ($P = 0.11$), and the interaction between the invasion stage and tree diameter class was not significant ($P = 0.10$). Trees with > 6 woodpecker attacks comprised 73%, 56%, and 13% of trees in Core, Crest, and Cusp sites, respectively. Trees with 1 to 6 woodpecker holes were rare, accounting for 4%, 6%, and 3% of trees in Core, Crest, and Cusp sites respectively.

The proportion of ash trees with woodpecker predation increased in all three stages from 2010 to 2011. Woodpecker attacks were higher in Core sites than Cusp sites ($H = 249.28$; $df = 2, 977$; $P < 0.001$) (Table 1.2), but other differences were not significant. Tree diameter class did not affect the abundance of woodpecker predation on late instar larvae ($P = 0.42$), and the interaction invasion stage and tree diameter class was not significant ($P = 0.99$). Trees with high levels of woodpecker predation comprised 88%, 75%, and 30% of trees in Core, Crest, and Cusp sites, respectively. Few trees had low levels of woodpecker predation, including 3%, 6%, and 7% of trees in Core, Crest, and Cusp sites, respectively.

DISCUSSION

Effects of *A. planipennis* in stands of green ash are similar to the effect of major forest pathogens such as *Cryphonectria parasitica* (formerly *Endothia parasitica*, formerly *Diaporthe parasitica*) which caused chestnut blight in American chestnut (*Castanea dentata*). Similarly, *Ophiostoma ulmi* (prior to 1940s) and *O. novo-ulmi* (post 1940s) vectored by both exotic and native bark beetles caused Dutch elm disease in American elm (*Ulmus americana*) (Braier and Buck 2001). Both chestnut blight and Dutch elm disease killed healthy trees, spread across the natural ranges of their host trees in a matter of decades, and have caused near 100% mortality of overstory American chestnut and American elm (Anagnostiakis 1987; Bey 1990; Anagnostiakis and Hillman 1992; Brasier 2000). Like these fungal pathogens, *A. planipennis* has become established in 16 states and two provinces of Canada (EAB.info 2012), attacks and kills healthy ash trees (Poland and McCullough 2006), and ash mortality rates approach 100% (Gandhi et al. 2008). By comparison, the invasive insect *Anoplophora glabripennis* (Motschulsky), while capable of killing healthy trees (Haack et al. 2010), has not spread nearly as quickly as *A. planipennis*. First discovered in New York in 1996, infestations of *A. glabripennis* are currently established in four additional states (National Invasive Species Information Center 2012), and multiple *A. glabripennis* infestations have been successfully eradicated (National Invasive Species Information Center 2012). Another invasive insect, *Adelges tsugae* (Annand), introduced into Virginia in the 1950s (Souto et al. 1996), has become a major pest of eastern hemlock trees (*Tsuga canadensis*) in the eastern United States. Mortality rates for eastern hemlock ranges from 0 to 95% of trees within stands, and are related to stand size, aspect, and local climate (Orwig and Foster 1998; Orwig et al. 2002). In contrast, mortality caused by *A.*

planipennis varies little among stands and approaches 100% of overstory trees (Gandhi et al. 2008; Knight et al. 2008).

My results indicate *A. planipennis* persists at low densities in Core sites. The number of live ash stems and basal area was significantly lower in Core sites than in Crest and Cusp. Reduced *A. planipennis* adult captures in Core sites compared to Crest sites presumably reflects reduced numbers of live overstory ash trees, causing *A. planipennis* populations to expand outward from the original infestation sites in southeast Michigan (Figure 1.5). Previous studies found *A. planipennis* did not develop on non-ash hosts in North America (Anulewicz et al. 2006; Anulewicz et al. 2008), so beetle populations will presumably decline as their host resource is depleted (Figure 1.5). Low density populations can be susceptible to Allee effects, which can lead to negative population growth rates (Courchamp et al. 1999) and eventual extinction (Liebhold and Tobin 2008). Mortality factors such as parasitism by *A. cappaerti*, and perhaps woodpecker predation, could further reduce local *A. planipennis* populations (Cappaert et al. 2005; Lindell et al. 2008; Tluczek 2009; Cappaert and McCullough 2009) (Figure 1.5). Ash trees in Core sites, however, are still becoming infested. It is likely *A. planipennis* will persist as long as live ash trees remain.

Despite the persistence of *A. planipennis*, and high levels of ash mortality, live overstory ash trees were still present in Core sites. It is unknown why some ash trees have survived, but the patchy distribution of *A. planipennis* colonization may reflect beetle dispersal patterns. *Agrilus planipennis* do not disperse randomly. Ash phloem abundance (Mercader et al. 2009; Siegert et al. 2010), and the presence of stressed ash trees (McCullough et al. 2009a, 2009b; Tluczek et al. 2011) affect *A. planipennis* dispersal in sites with low densities (Figure 1.5). Not every tree will

be colonized simultaneously. While *A. planipennis* populations are lower in Core sites compared to Crest sites, beetles were captured in every Core site, and ash canopy dieback did not differ between these two invasion stages. Ash canopy dieback does not become apparent until trees are moderately to heavily infested, and often culminate in death of the host (Poland and McCullough 2006). Future studies to monitor ash and *A. planipennis* populations over time are needed.

My results indicate *A. planipennis* populations were peaking in Crest sites in 2010 and 2011. Despite high densities of *A. planipennis*, on average > 50% of ash trees in Crest sites were still alive, so it is likely *A. planipennis* populations will remain near peak densities until ash populations are further depleted. Parasitism by *A. cappaerti*, and woodpecker predation on *A. planipennis* larvae, do not appear to reduce *A. planipennis* populations sufficiently to prevent new trees from being infested, so ash mortality is likely to rise in Crest sites. As ash mortality escalates, populations of *A. planipennis* and green ash will likely be reduced to levels currently observed in Core sites (Figure 1.5).

Given the abundance of available resources in Cusp sites, *A. planipennis* populations can be expected to grow (McCullough and Siegert 2007) (Figure 1.5). Adult captures and larval densities increased substantially in Cusp sites from 2010 to 2011. In five of the eight Cusp sites, more adult *A. planipennis* were captured on a single trap over a two week period in 2011 than were captured on all traps combined in those sites during the 2010 season. If no efforts are made to reduce *A. planipennis* population growth, patterns observed in southeast Michigan are likely to be repeated in Cusp sites, and across the range of green ash in the United States.

There are 56 known species of parasitoids In North America that attack *Agrilus* larvae (Taylor et al. 2012), but *Atanycolus cappaerti* is emerging as an important mortality factor for *A. planipennis* (Cappaert and McCullough 2009) (Figure 1.5). The mechanism(s) by which *A. cappaerti* locates *A. planipennis* larvae are unknown. Many parasitoids are capable of detecting plant volatiles produced as an induced response to insect attack (Stowe et al. 1995, Gols and Harvey 2009). Trees stressed from *A. planipennis* feeding also emit volatiles from their foliage (Rodriguez-Saona et al. 2006; de Groot et al. 2008; Grant et al. 2010) and bark (Crook et al. 2008) that are attractive to *A. planipennis* (McCullough et al. 2009a, 2009b; Siegert et al. 2010; Tluczek et al. 2011). It seems likely that *A. cappaerti*, which evolved to prey on native phloem-feeding insects, is able to detect volatiles emitted from ash trees stressed by feeding *A. planipennis* larvae. This is supported by my data, as the majority of parasitoids were found on girdled trees. Whether *A. cappaerti* parasitism rates will increase and what impact they may have on *A. planipennis* populations remains unknown, but I found no evidence in the literature of a phloem-feeding insect controlled by a parasitoid.

The decline in parasitism between 2010 and 2011 may have been caused by the removal of trap trees with parasitoids from the study sites. If *A. cappaerti* is attracted to girdled trees, whether to plant volatiles or an increase of *A. planipennis* larval density, felling and debarking trees in 2010 may have reduced their numbers. Future research on *A. cappaerti* is needed to document the biology of the parasitoid, parasitism rates on *A. planipennis* larvae, prey finding behavior of *A. cappaerti*, and options for possible augmentative biocontrol.

Woodpeckers are also continuing to cause mortality of *A. planipennis* larvae in southern Michigan (Figure 1.5). In my research, holes left by woodpecker predation on larvae were

found on most ash trees in Core and Crest sites, and nearly half of the ash trees in Cusp sites by 2011. The accumulation of holes left by woodpecker attacks suggests *A. planipennis* populations are being reduced by woodpeckers (Figure 1.5), but not every infested tree is being targeted. Studies have shown there is a positive correlation between *A. planipennis* larval density and woodpecker predation, but woodpecker predation can also be patchy (Cappaert et al. 2005; Lindell et al. 2008; Tluczek 2009). This is consistent with my findings that woodpecker attacks were more common on trees in Core sites where *A. planipennis* populations have been present longer than in Crest and Cusp sites, but were not present on all trees. If native woodpeckers are adapting to *A. planipennis*, and woodpecker populations increase due to capitalization of the resource, larval mortality rates caused by woodpecker predation may increase. However, to date, woodpecker predation on *A. planipennis* larvae has not stopped the spread of the beetle, nor has it sufficiently reduced *A. planipennis* populations in Core sites to prevent the infestation of remaining ash trees.

Effective methods to detect and monitor *A. planipennis* infestations are an important aspect of managing this invasive pest. My study suggests trap trees and traps are a better detection tool than visual surveys. Despite the increasing of *A. planipennis* populations in Cusp sites in 2011, most trees still appeared healthy with few sprouts or holes left by woodpecker predation. This is consistent with previous studies reporting external signs of *A. planipennis* rarely appear until a tree becomes heavily infested (Cappaert et al. 2005; Poland and McCullough 2006).

The question then arises as to what is the best trap for detecting *A. planipennis*? In my study, adult *A. planipennis* were more attracted to girdled trap trees than other trap types, in both 2010 and 2011. This is consistent with other studies conducted on *A. planipennis* in North

America which found girdled trees were more attractive than trees stressed by other injury or baited with attractive volatiles (McCullough et al. 2009a, 2009b; McCullough et al. 2011; Tluczek et al. 2011). Although higher densities of beetles were captured on sticky bands of girdled trees, double-decker traps consistently were more successful at capturing at least one beetle and had the highest total *A. planipennis* captures. These findings are consistent with a study evaluating various trap types for *A. planipennis* (McCullough et al. 2011). Based on surface area, girdled trees are more attractive, but girdling additional trees is destructive and wrapping a larger proportion of the trunk is not practical. Furthermore, in Cusp sites in 2010, when beetle populations were at their lowest density, at least one double-decker trap captured an adult *A. planipennis* in all eight sites, but larvae in girdled trees were only found in six sites, indicating that double-decker traps were better at detecting *A. planipennis* at low population densities than girdling and debarking trees. As a detection tool, it is not density of beetle captures, but rather the successful capture of at least one *A. planipennis* that is important. Since double-decker traps are larger, more apparent, and captured the most *A. planipennis* of all the traps used in this research, they have the highest probability of detection, and would be the optimal trap for detecting *A. planipennis* in front of the invasion wave.

The proportion of total beetles captured on double-decker traps in Core and Crest sites dropped substantially from 2010 to 2011. Reduced captures may be partially due to the change in lures from a Manuka/Phoebe oil blend in 2010 to Manuka oil alone in 2011. A previous study which traps baited with Manuka/Phoebe oil had significantly higher capture rates compared to traps baited with just Manuka oil (Crook et al. 2008). This does not explain, however, why the proportion of total beetle captures on double-decker traps in Cusp sites remained constant

between 2010 and 2011. The study comparing Manuka oil and Phoebe oil by Crook et al. (2008) was conducted on the edges of infested ash stands. Rodriguez-Saona et al. (2006) found that stressed ash emitted elevated levels of stress volatiles eliciting antennal responses and were attractive to *A. planipennis*. Volatiles emitted from an increased number of stressed trees may, however, mask lures on traps. In my study, few trees in Cusp sites were showing signs of stress, and *A. planipennis* populations were low. My findings indicate that at low *A. planipennis* densities, where few trees are emitting stress volatiles, traps baited with a Manuka/Phoebe oil blend may not have a higher capture rate than traps baited with Manuka oil alone. Phoebe oil contains the compound *7-epi-sesquithujene*, which Manuka oil lacks (Crook et al. 2008). The presence of *7-epi-sesquithujene* may reduce the degree to which volatiles emitted from stressed trees mask lures on artificial traps. Crest sites had the highest number of stressed trees, which could explain the drop in effectiveness of double-decker traps in these locations. Ash trees in Core sites are stressed, but few trees remain to emit stress volatiles compared to Crest sites. Further studies to determine if lures become less effective as more trees become stressed would be useful.

Natural enemies, such as woodpeckers and the braconid parasitoid *A. cappaerti* can cause substantial mortality of *A. planipennis* (Cappaert et al. 2005; Lindell et al. 2008; Cappaert and McCullough 2009; Tluczek 2009), but to date, have not prevented the spread and successful colonization of new ash stands. Infested trees decline rapidly as *A. planipennis* populations build; mortality rates, for example, nearly doubled in Cusp sites from 2010 to 2011. Therefore, trapping to detect low density populations in front of the leading edge of the invasion wave would be useful for timely implementation of control tactics to minimize ash

mortality. If *A. planipennis* populations are not reduced and prevented from colonizing new locations, then trends in ash mortality observed in Core sites will be repeated in Crest and Cusp sites, and in green ash stands across its range.

Table 1.1. Mean (\pm SE) trap tree DBH (diameter at breast height), total captures of adult and larval *A. planipennis*, adults and larvae per m², surface area of adult traps, and surface area examined for larvae in 2010 and 2011 in 24 sites in southern Michigan.

	Control trees	Girdled trees	Planted trees	Double-decker traps
2010				
Mean (\pm SE) DBH (cm)	13.1 \pm 1a	15.1 \pm 1.1a	6.4 \pm < 0.01b	*
Adults				
Mean (\pm SE) total captures	1.9 \pm 0.5c	8.9 \pm 2b	1.7 \pm 0.5c	41.1 \pm 9.7a
Mean (\pm SE) trap surface area (m ²)	0.1 \pm 0.01b	0.1 \pm 0.01b	0.06 \pm < 0.01b	1.5 \pm < 0.01a
Mean (\pm SE)E beetles per m ²	14.1 \pm 3.2b	62.4 \pm 13.9a	28.1 \pm 7.7b	27.6 \pm 6.6b
Larvae				
Mean (\pm SE) total larvae	12.2 \pm 3.1b	30.9 \pm 4.7a	2.3 \pm 0.5c	*
Mean (\pm SE) surface area examined per tree (m ²)	1.5 \pm 0.3a	1.5 \pm 0.4a	0.5 \pm 0.01a	*
Mean (\pm SE) larvae per m ²	28.2 \pm 4.1b	57.7 \pm 8.2a	5.1 \pm 1.1c	*
2011				
Mean (\pm SE) DBH (cm)	13.3 \pm 0.8a	14.5 \pm 0.7a	5 \pm 0.1b	*
Adults				
Mean (\pm SE) total captures	3.5 \pm 1.2c	17.3 \pm 4.2b	2.0 \pm 0.5c	28.5 \pm 4.2a
Mean (\pm SE) trap surface area (m ²)	0.1 \pm 0.01b	0.1 \pm 0.01b	0.05 \pm < 0.01c	1.5 \pm < 0.01a
Mean (\pm SE)E beetles per m ²	25.5 \pm 7.4bc	126.1 \pm 28.3a	43.2 \pm 12.4b	19.2 \pm 2.8c
Larvae				
Mean (\pm SE) total larvae	9.2 \pm 2.1b	44 \pm 6.1a	5 \pm 1b	*
Mean (\pm SE) surface area examined per tree (m ²)	0.7 \pm 0.04a	0.9 \pm 0.07a	0.7 \pm 0.03a	*
Mean (\pm SE) larvae per m ²	14 \pm 2.7b	63.3 \pm 9.4a	6.7 \pm 1.3b	*

Within rows, means followed by the same letter are not significantly different ($P < 0.05$)

Table 1.2. Total number of ash trees, and live ash trees, mean (\pm SE) percent of ash mortality, ash canopy dieback and proportion of ash with epicormic shoots, stump sprouts and woodpecker predation in 2010 and 2011 in 24 sites in southern Michigan. Canopy dieback and epicormic shoots were recorded on live ash trees, and stump sprouts and holes left by woodpeckers preying on *A. planipennis* larvae were recorded on all ash

	Total no. ash	Total no. live ash	Ash mortality (%)	Canopy dieback (%) ¹	Epicormic shoots (%) ²	Stump sprouts (%) ²	Woodpecker predation (%) ²
2010							
Core	216	55	67 \pm 11a	40 \pm 5a	38 \pm 6a	39 \pm 7a	76 \pm 6a
Crest	376	255	27 \pm 9b	34 \pm 2a	44 \pm 11a	37 \pm 10a	52 \pm 11ab
Cusp	443	403	11 \pm 4c	11 \pm 1b	22 \pm 7b	11 \pm 5b	20 \pm 6b
2011							
Core	207	40	79 \pm 10x	39 \pm 6y	56 \pm 14y	43 \pm 9y	93 \pm 4y
Crest	404	179	45 \pm 11y	28 \pm 3y	52 \pm 12y	51 \pm 10y	73 \pm 13yz
Cusp	443	365	20 \pm 7z	20 \pm 2z	36 \pm 8z	19 \pm 6z	41 \pm 10z

¹ 90% dieback = nearly a complete lack of leaves, \leq 20% dieback = healthy canopy

² Percentage of trees with at least one epicormic shoot, stump sprout, or hole left by woodpecker predation

³ Within columns, means followed by the same letter are not significantly different (a,b,and c for 2010; x, y, and z for 2011) ($P < 0.05$)

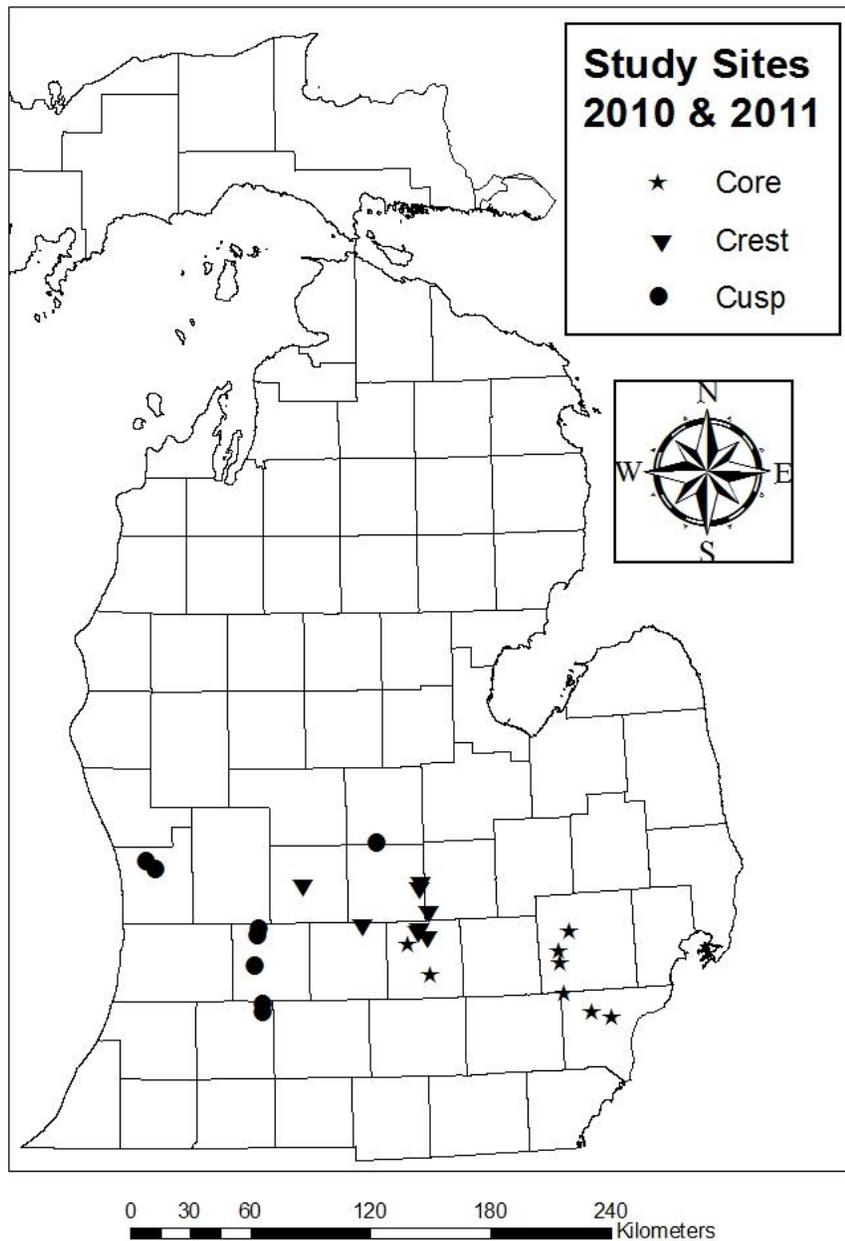


Figure 1.1. Locations of 24 green ash sites representing three stages of the *A. planipennis* invasion wave in 2010 and 2011. Core sites were characterized by low densities of *A. planipennis* and high ash mortality. Crest sites were characterized by high densities of *A. planipennis* and moderate to high ash mortality. Cusp sites were characterized by low densities of *A. planipennis* and low ash mortality.

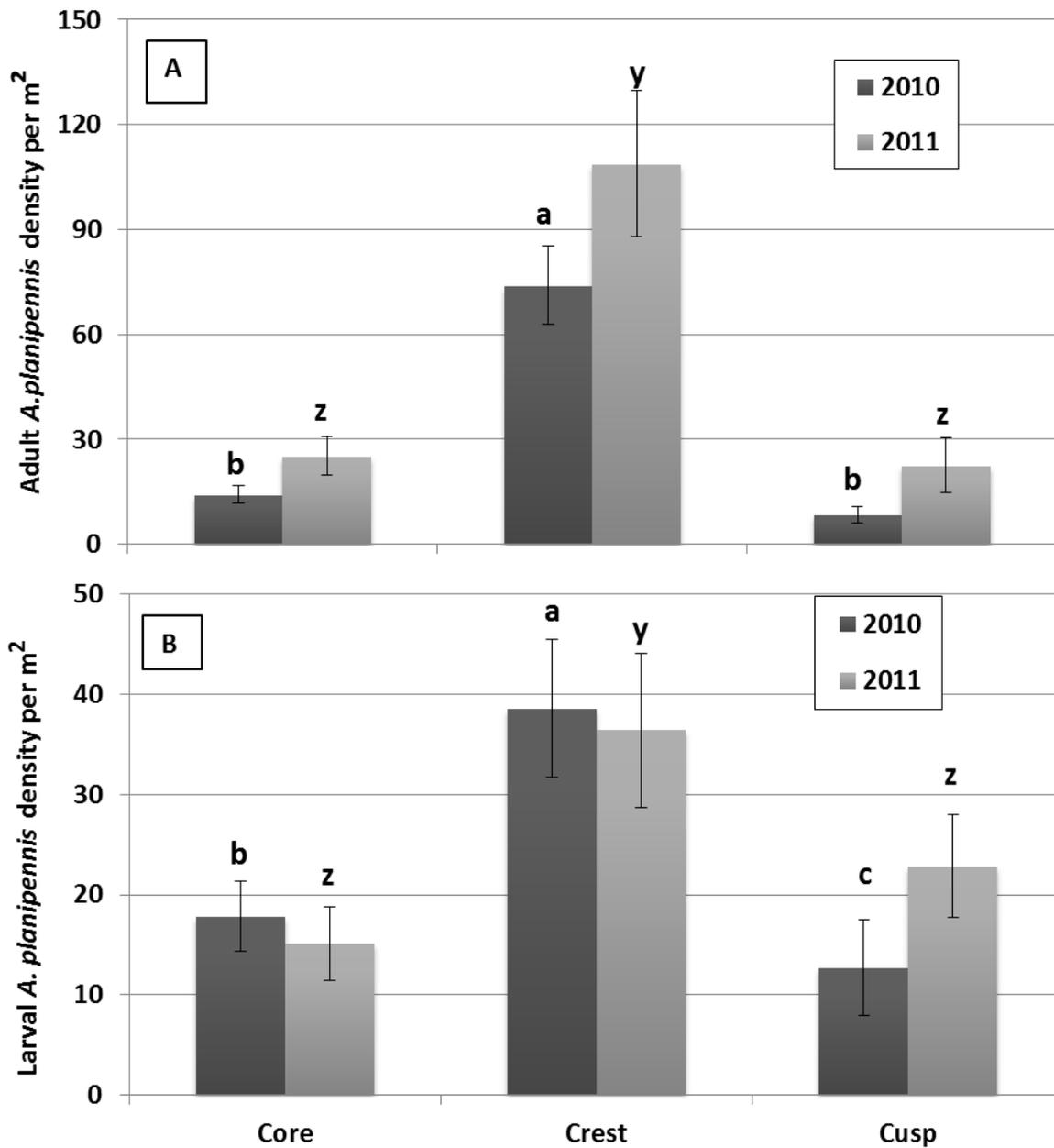


Figure 1.2. **A.** Mean (\pm SE) captures of *A. planipennis* adults per m² of trap area in Core, Crest, and Cusp sites in 2010 and 2011. **B.** Mean (\pm SE) density of *A. planipennis* larvae per m² of exposed ash phloem in Core, Crest, and Cusp sites in 2010 and 2011. Means with the same letter are not significantly different (Tukey's protected HSD test; $P < 0.05$). (a, b, and c for 2010; and y and z for 2011)

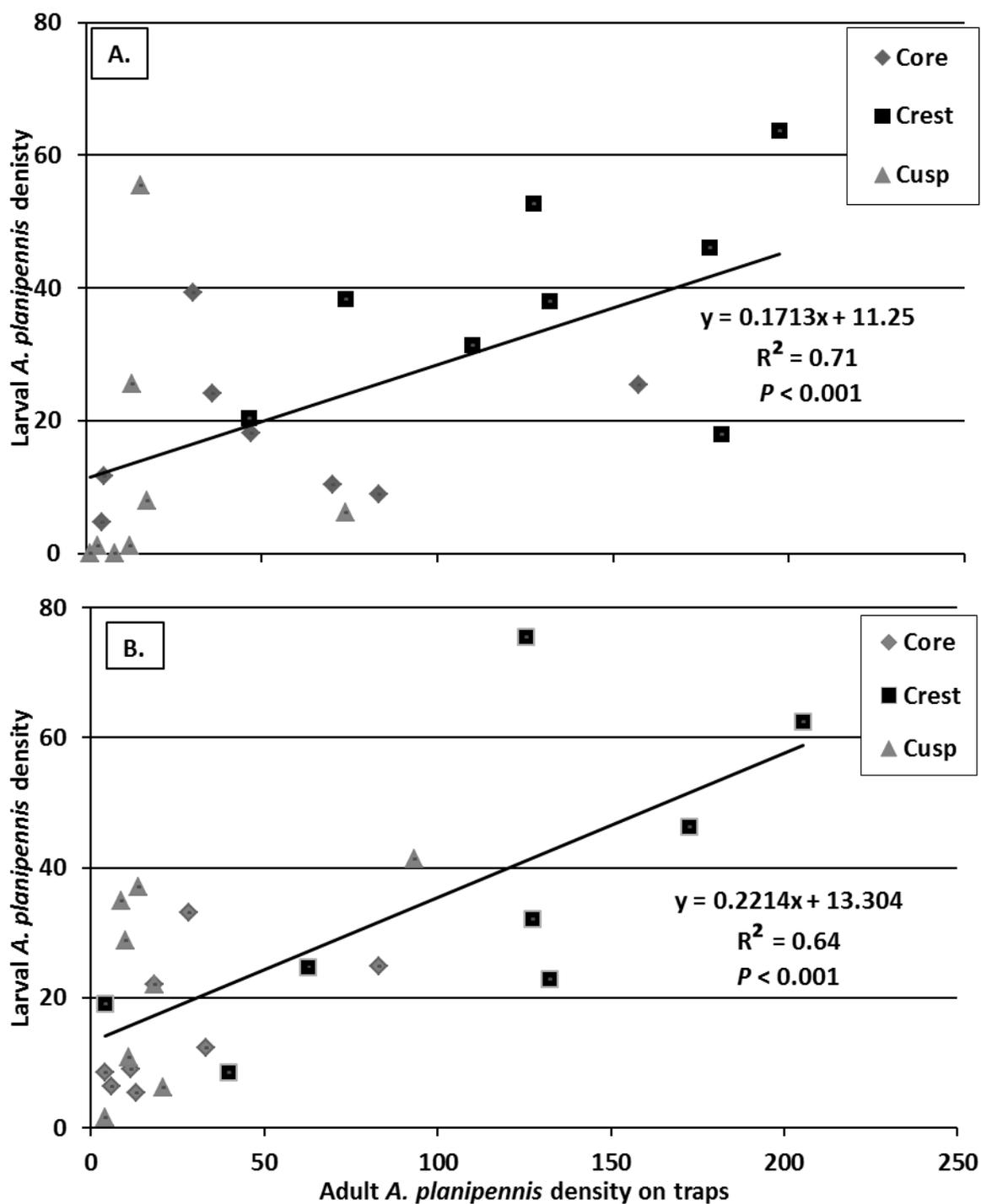


Figure 1.3 Linear regression of larval *A. planipennis* density per m² of exposed phloem and adult *A. planipennis* density per m² on traps in (A.) 2010 and (B.) 2011 at 24 sites in southern Michigan (N = 24).

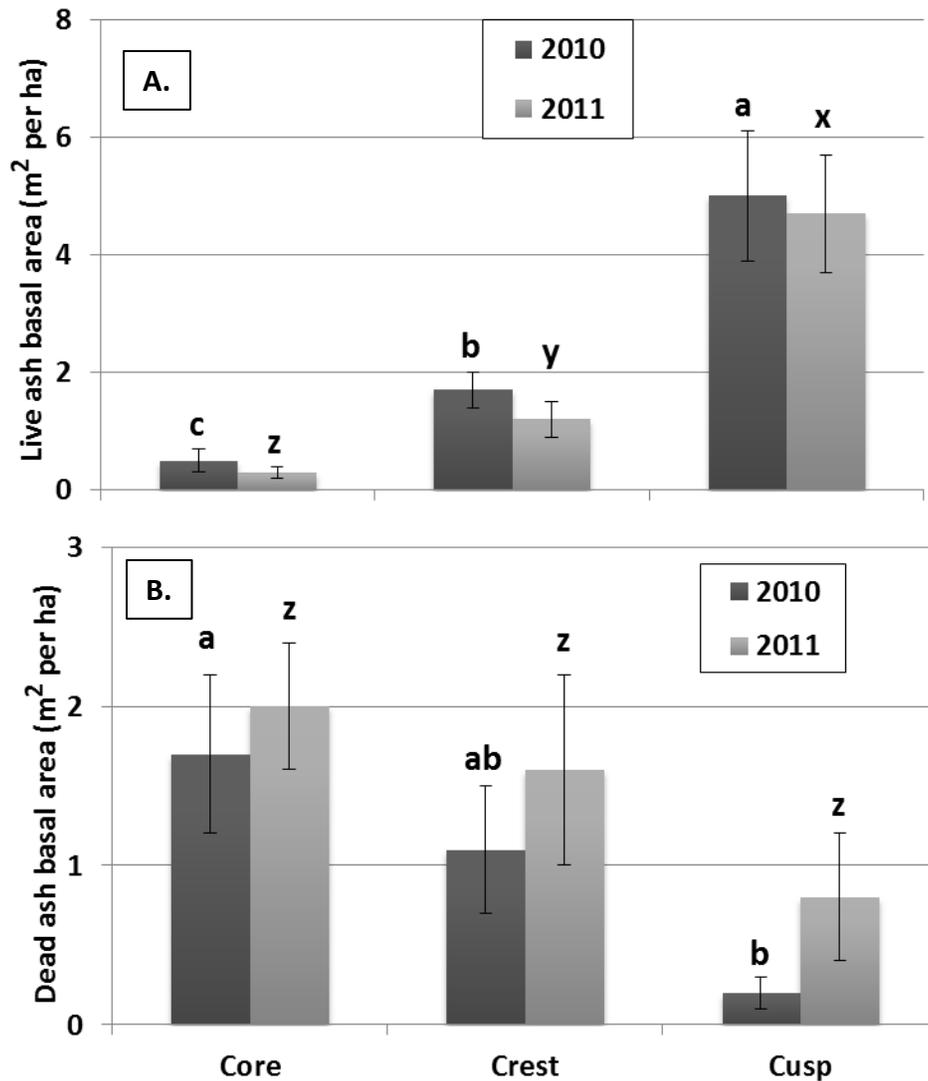


Figure 1.4. A. Mean (\pm SE) live basal area (m^2 per ha) of green ash in Core, Crest, and Cusp sites in 2010 and 2011. **B.** Mean (\pm SE) dead basal area (m^2 per ha) of green ash in Core, Crest, and Cusp sites in 2010 and 2011. Means with the same letter are not significantly different (Tukey's protected HSD test; $P < 0.05$) (a, b, and c for 2010; x, y, and z for 2011).

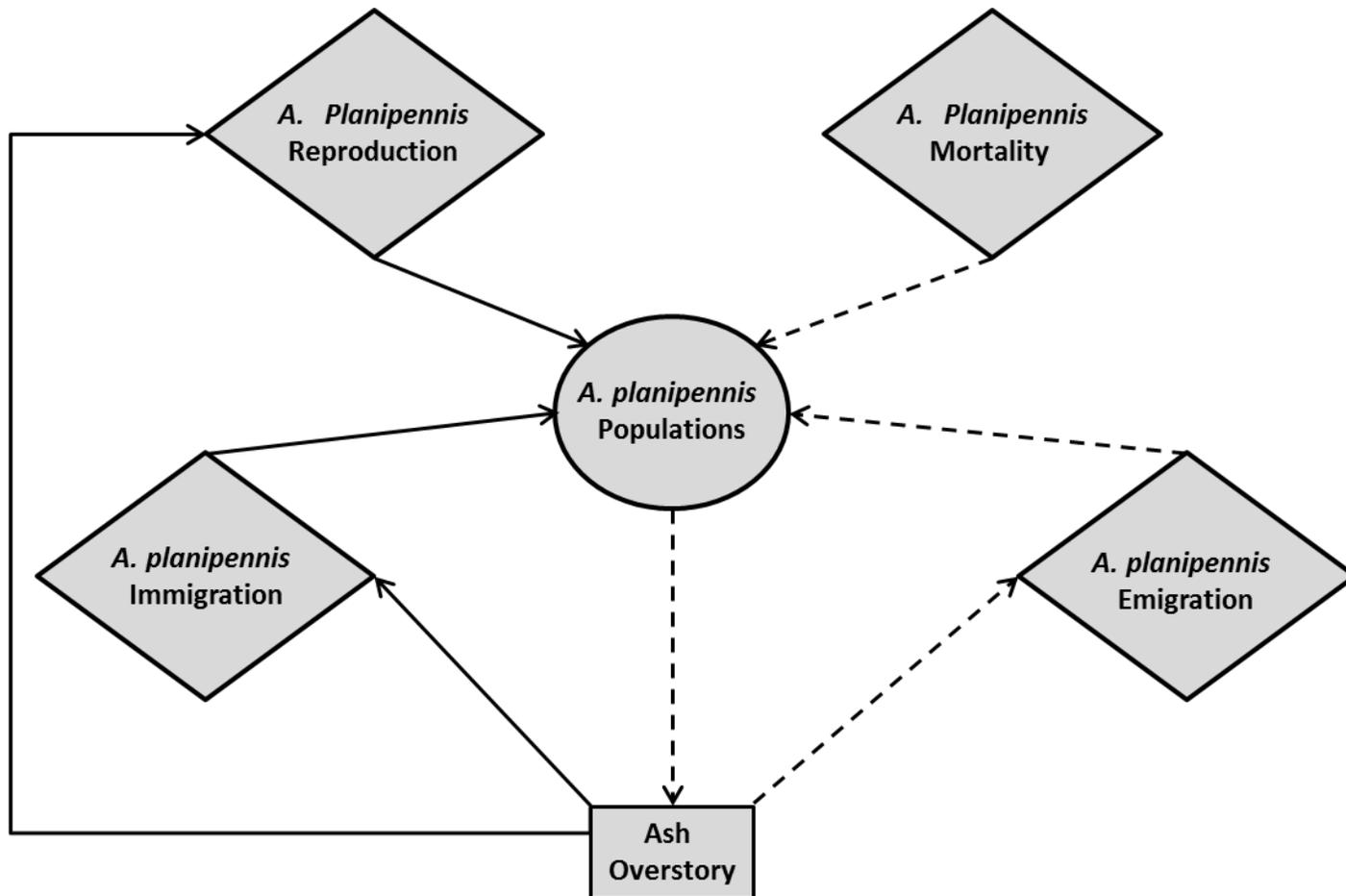


Figure 1.5. A flow diagram representing *A. planipennis* populations and the negative effects on overstory ash. The positive effects of overstory ash abundance on *A. planipennis* occurrences through reproduction and immigration. The negative effect of *A. planipennis* mortality factors such as parasitism and woodpecker predation on larvae, and effects of the reduction of ash overstory on *A. planipennis* populations through emigration. Positive relationships are represented by \longrightarrow and negative relationships are represented by $- - - \rightarrow$

Chapter 2

Green ash Overstory and Regeneration Ahead, Within, and Behind the Emerald

Ash Borer Invasion Wave

INTRODUCTION

Since its introduction in the early to mid-1990s (Siegert et al. 2007), emerald ash borer, (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) has killed tens of millions of ash trees (*Fraxinus spp.*) in the United States and Canada (EAB.info 2012). *A. planipennis* preferentially feeds on green ash (*Fraxinus pennsylvanica*) (Anulewicz et al. 2007; Rebek et al. 2008) and has caused widespread mortality of this species in eastern North America. Green ash, with the widest distribution of all North American *Fraxinus spp.*, is native to all states east of the Rocky Mountains, as well as seven provinces of Canada (Wright 1959; Kennedy 1990). Green ash is typically found in bottomlands, but can survive in upland sites (Wright 1959; Kennedy 1990). In southern Michigan, green ash is widespread, particularly in lowlands and riparian zones (Barnes and Wagner 1981), and is common in areas prone to frequent flooding (Kennedy 1990). With the introduction and spread of *A. planipennis*, the future of green ash within these systems is uncertain.

Ash comprises a substantial proportion of Michigan's hardwood forest resource. According to the USDA Forest Inventory and Analysis (FIA database 2012), there were 869 million *Fraxinus spp.* trees with diameter at breast height (DBH) ≥ 3 cm in Michigan as of 2011.

Female *A. planipennis* oviposit individual eggs in cracks in the outer bark, or beneath rough edges of ash bark from mid to late summer. Eggs hatch within two weeks, and larvae feed in the cambium and phloem, constructing serpentine galleries (Poland and McCullough 2006). Larval *A. planipennis* pass through four instars and typically complete feeding in late fall, then excavate pupal chambers in the outer sapwood or bark (Cappaert et al. 2005). Over time, as densities of larvae increase, feeding disrupts the translocation of nutrients, resulting in canopy thinning or dieback. Epicormic shoots may appear on branches or trunks, and stump sprouts may be produced by dying trees. As ash trees within stands die, beetles disperse and colonize new locations. The demise of ash could cascade through the ecosystem, affecting species in multiple trophic levels. To date, classical biocontrol, silvicultural techniques, and predation by native predators and parasitoids have failed to stop *A. planipennis* from killing tens of millions of ash trees.

Agrilus planipennis has the potential to drastically alter forest composition by virtually eliminating ash as a major overstory component, and allowing shade intolerant species to increase in the overstory. Classical theory on successional trajectories projects that in a system with minimal disturbances, shade-tolerant species will grow to dominate stands (Kobe et al. 1995; Catovsky and Bazzaz 2000). Large canopy gaps formed by mortality of overstory ash tree, however, result in increased light reaching the forest floor, providing an opportunity for shade intolerant species to compete with more tolerant species (Messier et al. 1999).

Many questions arise regarding the long-term impact of *A. planipennis* in stands. How does overstory and understory species composition vary with invasion stage? Does ash

continue to persist and regenerate in stands invaded by *A. planipennis*? What species will likely replace ash in the overstory?

The goal of this study was to characterize ash and other species in the overstory and understory in stands representing three stages of the *A. planipennis* invasion wave. The first stage, classified as the “Core”, includes stands within the original infestation zone in southeast Michigan. Most overstory ash trees in Core stands have succumbed, creating substantial canopy gaps. The second stage of the invasion wave, designated as the “Crest,” represents stands in south central Michigan where *A. planipennis* populations are near peak densities and ash trees are in various stages of decline. The third stage of the invasion wave, termed “Cusp,” includes stands in southwest Michigan where ash appear healthy with few external signs of *A. planipennis* infestation. Results should provide insight into 1) current green ash populations at three stages of the *A. planipennis* invasion wave; 2) the potential of green ash to persist in Michigan forests; and 3) potential future stand composition.

MATERIALS & METHODS

Study sites: Twenty-four sites, each 1 ha in size, were selected in July and August 2009. Sites were located on an east to west gradient across southern Michigan (Figure. 1.1) on state, county, and municipal property, and were second growth stands with dominant trees between 60 and 90 years of age (Michigan.gov 2012). The overstory in each site (pre-*A. planipennis*) was comprised of a minimum of 20% green ash. Sites were classified as Core, Crest, or Cusp sites based on ash tree condition at the time of selection. Eight Core sites, primarily in southeast Michigan were selected. In Core sites, most overstory ash were dead, creating large gaps. Lateral growth from neighboring non-ash trees had often extended into these gaps. To ensure *A. planipennis* was the cause of ash mortality, bark was removed from a subsample of dead ash to identify the characteristic serpentine galleries left by *A. planipennis* larvae. Eight Crest sites were selected in south central Michigan in areas where ash trees were in various stages of decline. Large gaps were present but neighboring trees had not extended their branches into the gaps. Dead ash trees in Crest sites were also examined to ensure mortality was caused by *A. planipennis*. Eight Cusp sites, primarily in southwest Michigan, were selected in areas where overstory ash had little or no signs of *A. planipennis* infestation, and no canopy gaps caused by ash mortality were present.

Overstory: All trees (DBH \geq 10 cm) were identified to species and DBH measured in June and July 2010 along two 150 x 2 m belt-transects that ran diagonally across each site in an X-formation. Trees within 10 m of the intersection of the belt-transect were marked to prevent multiple measurements of individual trees. The two belt-transects divided each site into four quadrats. One macro-plot (18 m radius) was established in each quadrat. Trees within macro-

plots were identified to species and DBH recorded. Canopy dieback was recorded to assess *A. planipennis*-related injury to ash trees, and to estimate the relative health of non-ash trees at the site. Visual estimates of dieback were recorded in increments of 10%; 0% indicated a full canopy and 100%, indicated the complete absence of live branches (Zarnoch et al. 2004). Dieback was assessed in early to mid-summer of each year, after trees were fully flushed but before current-year larval feeding could further affect ash overstories. Data from belt-transects and macro-plots were used to calculate relative importance values (RIV) for overstory trees in each site. The index is derived using relative frequency (representing the sum of occurrences within plots of a species as a proportion of an individual species related to all species), plus relative density (count of individuals of a species as a percentage of the total count of individuals of all species), plus relative dominance (basal area of a given species as a percentage of the total basal area of all species) (Kent and Coker 1992).

Overstory ash trees were assessed again in belt-transects and macro-plots in 2011. Non-ash density and basal area presumably remained relatively constant between years, and were not surveyed in 2011.

Recruits: Recruits included trees ≥ 3 cm and < 10 cm in diameter. Recruits were identified to species in subplots with a 7 m radius located at the center of each macro-plot in 2010. Total number of green ash recruit stems per ha were calculated. Green ash stem densities and mortality attributed to *A. planipennis* did not differ among invasion stages, so recruits were not resurveyed in 2011.

Saplings: Trees < 3 cm in diameter and ≥ 45 cm in height were classified as saplings. Sapling density by species was recorded in the same subplots (7 m radius) used for recruits. Total

number of stems and green ash stems per ha were calculated. Presence of current-year deer browse on green ash saplings was also recorded.

PAR and sapling growth: Ash saplings in subplots were counted again in 2011 and photosynthetically active radiation (PAR) associated with individual ash saplings was measured. Readings were recorded with a LI-COR 250A (LI-COR Inc. Lincoln, NE) in four randomly selected Core, Crest, and Cusp sites (12 sites total). Light readings were taken after canopies were fully flushed, but before injury caused by current-year larvae could further affect ash canopies. Readings were conducted in one site per day, three sites per week, beginning 20 June and concluding 15 July. A total of 37 readings over 1 to 2 m tall green ash saplings were collected systematically around fixed radius plots and belt-transects across each site. Growth of individual saplings was determined by measuring the distance between terminal bud scars of the top segment on the terminal leader of each plant. Sapling growth was measured beginning 12 June and concluded 17 June. If an ash sapling was not present within a 2.5 m^2 radius of a selected location, a PAR reading was collected without an associated sapling.

Light readings began at dawn and were completed by sunrise to reduce variability from random sunflecks penetrating the canopy. Adjustments for dawn and sunrise times by date and location were determined by GAISMA.com (2011). Readings were 15 second averages. While each reading was taken within the site, a paired reading was taken simultaneously in an adjacent open field (synchronized by two-way radios). Readings within sites were divided by the simultaneous open field reading to determine percentage of sun penetrating the canopy. If PAR in the open field was zero, the reading within the stand was dropped from analysis. All

light readings, with and without an associated ash sapling, were used to calculate average PAR levels in the understory of each site.

Seedlings: In 2010, seedlings < 45 cm in height were tallied by species in 1.8 m radius micro-plots, located at the center of each of the four subplots in each site. Total number of seedlings and green ash seedling per ha were calculated. Ash seedlings were examined for cotyledons to determine if they represented current-year germination. Ash seedlings, with and without cotyledons, were counted again in 2011.

Statistical analysis: Variables recorded in plots, transects, and PAR readings were used to assess species composition, basal areas, stems per ha, and sapling growth among the three stages of invasion. Data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Several variables, including total basal area, ash basal area, PAR, and green ash sapling growth were normalized by $\log_{10}(x + 1)$ transformations. Differences in basal area, PAR, and green ash sapling growth among the three invasion stages were tested as unplanned comparisons, and Tukey's honestly significant difference (HSD) multiple comparison test was applied if the overall analysis of variance (ANOVA) was significant ($P < 0.05$) (PROC GLM; SAS Institute 2003) (Ott and Longnecker 2001). Regression analysis was used to evaluate the linear relationship between PAR and green ash sapling growth (PROC CORR; SAS Institute 2003) (Ott and Longnecker 2001). Density of green ash recruits, saplings, seedlings, and ash seedlings with cotyledons were not normalized by transformations. The nonparametric Kruskal-Wallis statistic was applied to assess differences in these response variables among the three invasion stages (Kruskal and Wallis 1952) (PROC NPAR1WAY; SAS Institute 2003). When results were significant ($P < 0.05$), a nonparametric multiple comparison was applied (Zar 1984). The

five most abundant species of recruits, saplings, and seedlings based upon stems per ha were ranked in each of the three invasion stages. All analyses were conducted at $P < 0.05$ level of significance using SAS statistical software.

RESULTS

Overstory: In 2010, 3353 overstory trees (DBH \geq 10 cm) (all species) were measured in the 24 sites including 1015, 924, and 1414 trees in Core, Crest, and Cusp sites, respectively. Most trees were \leq 30 cm in DBH (71%, 83%, and 87% in Core, Crest, and Cusp sites, respectively). Total basal area averaged 17.3 ± 3.8 , 11.1 ± 2.4 , and 16.9 ± 1.3 m² per ha in Core, Crest, and Cusp sites, respectively. Live basal area of all species averaged 14.9 ± 3.4 , 9.6 ± 2.4 , and 16.2 ± 1.3 m² per ha in Core, Crest, and Cusp sites, respectively. Differences among the three invasion stages did not affect total basal area ($P = 0.26$), live basal area ($P = 0.18$), nor dead basal area ($P = 0.26$). Of the 3353 trees recorded across all sites, 377 were dead. Of the 377 dead trees, 67% were ash, 19% were American elm (*Ulmus americana*), and 14% were comprised of other species. Galleries of the native elm bark beetle (*Hylurgopinus rufipes* (Eichhoff)) and the smaller European elm bark beetle (*Scolytus multistriatus* (Marsham)) were found beneath the bark of dead elm trees.

Overall, 1035 green ash trees with DBH \geq 10 cm were recorded in 2010 including 216, 376, and 443 trees in Core, Crest, and Cusp sites, respectively, and 87% to 95% of ash in all sites were \leq 30 cm in DBH. Ash basal area (live plus dead ash) in 2010 averaged 2.3 ± 0.5 , 2.7 ± 0.6 , and 5.2 ± 1 m² per ha in Core, Crest, and Cusp sites, respectively, and differed among invasion stages ($F = 4.37$; $df = 2, 21$; $P = 0.03$). Total ash basal area was twice as high in Cusp sites compared to Core sites; other differences among stages were not significant. Live ash basal area in 2010 averaged 0.5 ± 0.2 , 1.7 ± 0.3 and 5.0 ± 1.1 m² per ha, in Core, Crest and Cusp sites, respectively. Live ash basal area in Cusp sites was 10-fold higher than in Core sites, and nearly

3-fold higher than in Crest sites, while live ash basal area in Crest sites was 3-fold higher than in Core sites ($F = 21.09$; $df = 2, 21$; $P < 0.001$). Ash mortality attributed to *A. planipennis* in 2010 averaged $66.7 \pm 11.0\%$, $26.7 \pm 8.7\%$, and $11.1 \pm 3.9\%$ percent in Core, Crest, and Cusp sites, respectively, and differed among invasion stages ($H = 290.77$; $df = 2, 1032$; $P < 0.001$). Dead ash basal area averaged 1.7 ± 0.5 , 1.1 ± 0.4 and $0.2 \pm 0.1 \text{ m}^2$ per ha, in Core, Crest and Cusp sites, respectively, and also differed among invasion stages ($F = 4.64$; $df = 2, 21$; $P = 0.02$). Dead ash basal area was 8-fold higher in Core sites compared to Cusp sites, but other differences among stages were not significant.

In 2011, 1054 overstory green ash trees were tallied when sites were re-surveyed, including 207, 404, and 443 trees in Core, Crest, and Cusp sites, respectively. As in 2010, 85% to 97% of ash trees were ≤ 30 cm in DBH. Total ash basal area in 2011 averaged 2.2 ± 0.4 , 2.7 ± 0.6 , and $5.5 \pm 0.9 \text{ m}^2$ per ha, in Core, Crest, and Cusp sites, respectively. Total ash basal area was more than twice as high in Cusp sites compared to Core and Crest sites ($F = 6.69$; $df = 2, 21$; $P = 0.006$), which did not differ. Live ash basal area averaged 0.3 ± 0.1 , 1.2 ± 0.3 and $4.7 \pm 1 \text{ m}^2$ per ha, in Core, Crest and Cusp sites, respectively. Live ash basal area in Cusp sites was 15-fold greater than in Core sites, nearly 4-fold greater than in Crest sites, while live ash basal area in Crest sites was 4-fold greater than in Core sites ($F = 20.72$; $df = 2, 21$; $P < 0.001$). Dead ash basal area averaged 2.0 ± 0.4 , 1.6 ± 0.5 and $0.8 \pm 0.4 \text{ m}^2$ per ha, in Core, Crest and Cusp sites, respectively, and did not differ among invasion stages ($P = 0.161$). In 2011, on average, $78.6 \pm 10.1\%$, $44.8 \pm 11.2\%$, and $19.8 \pm 7.3\%$ of overstory trees were killed by *A. planipennis* in Core,

Crest and Cusp sites, respectively, and ash mortality differed among all three invasion stages ($H = 259.55$; $df = 2, 1,051$; $P < 0.001$).

Canopy dieback: Canopy dieback of non-ash trees in 2010 was low in all sites, averaging $< 5\%$ in all three invasion stages. Ash trees, however, averaged $40.2 \pm 4.7\%$, $34 \pm 2.3\%$, and $11.0 \pm 1.2\%$ dieback in Core, Crest, and Cusp sites, respectively, and dieback differed among invasion stages ($H = 112.3$; $df = 2, 713$; $P < 0.001$). Ash canopy dieback in Core sites was nearly 4-fold greater than in Cusp sites, and 3-fold greater in Crest sites compared to Cusp sites. Differences in ash canopy dieback between Core and Crest sites were not significant.

Canopy dieback on ash trees in 2011 averaged $38.5 \pm 6.0\%$, $28.4 \pm 2.7\%$, and $19.9 \pm 1.7\%$ in Core, Crest, and Cusp sites, respectively, and again differed among invasion stages ($H = 20.41$; $df = 2, 581$; $P < 0.001$). Canopy dieback was nearly twice as high in Core sites compared to Cusp sites, and higher in Crest sites than in Cusp sites, but did not differ between Core and Crest sites.

A total of 37 overstory species were recorded in the 24 sites in 2010. The five most abundant species in the three invasion stages, based upon RIV, were similar in all sites (Table 2.1). Green ash, American elm, and black cherry (*Prunus serotina*) ranked in the top five in all three invasion stages (Table 2.1). Red oak (*Quercus rubra*) and black walnut (*Juglans nigra*) were relatively abundant in Cusp sites, and red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*) ranked highly in the Core and Crest sites.

Recruits: In 2010, 913 recruits representing 28 species were recorded, including 246, 321, and 346 recruits in Core, Crest, and Cusp sites, respectively. Densities of recruits averaged 499 ± 95 , 655 ± 160 , 702 ± 92 stems per ha in Core, Crest, and Cusp sites, respectively, and did not differ

among invasion stages ($P = 0.06$). The five most abundant recruit species based on stems per ha were similar to overstory species composition (Table 2.2). Red oak recruits, however, were only abundant in Core sites, were scarce in Crest sites, and were not observed in Cusp sites. Black walnut recruits were represented in Core and Crest sites, but not in Cusp sites, and red maple recruits were not observed in Crest sites.

Overall, 315 green ash recruits were tallied in 2010, including 63, 174, and 78 recruits comprising 34%, 64%, and 29% of all recruits in Core, Crest, and Cusp sites, respectively. Densities of total ash recruits averaged 120 ± 29 , 353 ± 90 , and 158 ± 38 stems per ha in Core, Crest, and Cusp sites, respectively, and did not differ among invasion stages ($P = 0.15$). Ash recruits were recorded in 23 of the 24 study sites, but were not encountered in one Cusp site (Barry County). Of the 315 green ash recruits, however, 63 were dead. On average, $29.2 \pm 16\%$, $21.9 \pm 9.5\%$, and $30.3 \pm 11.1\%$ of ash recruits in Core, Crest, and Cusp sites, respectively, were dead, and differences among invasion stages did not differ ($P = 0.98$). Only 31 of the 63 dead ash recruits had *A. planipennis* galleries beneath the bark. On average, $7.6 \pm 5.6\%$, $12.9 \pm 7.6\%$, and $9.7 \pm 8.3\%$ of ash recruits were killed by *A. planipennis* in Core, Crest, and Cusp sites, respectively, and this mortality did not differ among invasion stages ($P = 0.06$).

Saplings: Overall, 2931 saplings representing 25 species were counted in 2010, including 1337, 1112, and 474 saplings in Core, Crest, and Cusp sites, respectively. Sapling densities averaged 2693 ± 354 , 2273 ± 456 and 962 ± 235 stems per ha in Core, Crest, and Cusp sites, respectively, and differed among stages ($H = 19.37$; $df = 2, 93$; $P < 0.001$). Sapling densities were twice as high in Core and Crest sites compared to Cusp sites, but differences between Core and Crest sites were not significant.

Saplings were recorded in all subplots in Core sites, while 6% and 16% of the 32 subplots in each of the Crest and Cusp sites had no saplings, respectively. Species composition of saplings was similar to that of overstory trees and recruits (Table 2.2), except that red oak and black walnut saplings were only observed in Core sites. Red maple was abundant in Core and Cusp sites, but was infrequent in Crest sites.

In 2010, 2133 green ash saplings were tallied, including 967, 911, and 255 saplings accounting for 72%, 81%, and 54% of total sapling counts in Core, Crest, and Cusp sites, respectively. Ash saplings were recorded in subplots at 22 of the 24 study sites; none were recorded in two Cusp sites (Barry Co., Ottawa Co.). Densities of ash saplings were 3-fold greater in Core and Crest sites compared to Cusp sites, but did not differ between Core and Crest sites ($H = 23.62$; $df = 2, 93$; $P < 0.001$) (Figure 2.2). On average $14.4 \pm 3.7\%$, $6.4 \pm 2.5\%$, and $12.4 \pm 4.3\%$ of ash saplings had evidence of deer browse in Core, Crest, and Cusp sites, respectively, but deer browse did not differ among invasion stages ($P = 0.23$).

In 2011, 1905 green ash saplings were recorded, including 892, 855, and 158 saplings in Core, Crest, and Cusp sites, respectively. Ash saplings were again absent in the same two sites as in 2010. Ash sapling densities were 6-fold higher in Core and Crest sites compared to Cusp sites, but did not differ between Core and Crest sites ($H = 27.90$; $df = 2, 93$; $P < 0.001$) (Figure 2.2). Deer browsed on an average of $18.1 \pm 3.8\%$, $13.2 \pm 3.4\%$, of ash saplings in Core and Crest respectively, which was higher than Cusp sites where $2.6 \pm 1.5\%$ of ash saplings were browsed ($H = 20.81$; $df = 2, 29$; $P < 0.001$).

PAR and sapling growth: A total of 428 PAR readings were collected including 146, 144, and 138 readings in Core, Crest, and Cusp sites, respectively. In 2011, 237 green ash saplings were paired with PAR readings (115, 91, and 31 saplings in Core, Crest, and Cusp sites, respectively).

Available PAR was higher in Crest sites than in Core and Cusp sites ($F = 16.81$; 2, 425; $P < 0.001$), where readings did not differ (Figure 2.3A). Ash sapling growth was also highest in stands where *A. planipennis* had caused substantial ash mortality. Leader growth of ash saplings in Crest sites was 5-fold greater than Cusp sites, and double that recorded in Core sites, which was greater than Cusp sites ($F = 49$; $df = 2, 234$; $P < 0.001$) (Figure 2.3B). Annual growth of ash saplings was significantly and positively related to PAR (Figure 2.4).

Seedlings: In 2010, seedlings were present in 94% of the 32 micro-plots in Core sites, 78% in Crest sites, and 84% in Cusp sites. A total of 2249 seedlings, representing 22 species, were tallied, including 733, 692, and 824 seedlings in Core, Crest, and Cusp sites, respectively. Seedling densities averaged $25,219 \pm 3552$, $23,808 \pm 5536$, and $28,350 \pm 6886$ per ha in Core, Crest, and Cusp sites, respectively, and did not differ among invasion stages ($P = 0.46$). Red oak and black walnut seedlings were not observed in any site, while red maple seedlings were fairly abundant in Crest sites (Table 2.2).

In 2010, 1704 green ash seedlings were counted, including 386, 656, and 662 seedlings in Core, Crest, and Cusp sites, respectively. Green ash seedlings were recorded in every site. Densities of green ash seedlings averaged $13,280 \pm 2363$; $22,560 \pm 557$; and $22,776 \pm 5622$ stems per ha in Core, Crest, and Cusp sites, respectively, and did not differ among invasion stages ($P = 0.95$).

In 2011, 1,622 green ash seedlings were recorded in 23 of the 24 study sites, including 386, 467, and 769 seedlings in Core, Crest, and Cusp sites, respectively. Densities of ash seedlings averaged $13,280 \pm 2210$; $16,067 \pm 3519$; and $26,457 \pm 6930$ stems per ha in Core, Crest, and Cusp sites, respectively, and did not differ among invasion stages ($P = 0.87$).

In 2010, 102 ash seedlings had cotyledons, indicative of current-year germination. This included 44 seedlings in six Cusp sites, and 58 seedlings in four Crest sites. No ash seedlings with cotyledons occurred in micro-plots in Core sites. Extrapolated densities of ash seedlings with cotyledons averaged 1995 ± 664 and 1513 ± 571 per ha in Crest, and Cusp sites, respectively, and were significantly higher than Core sites, which had not ash seedlings with cotyledons ($H = 12.32$; $df = 2, 93$; $P = 0.002$). Crest and Cusp sites did not differ.

In 2011, 99% of the ash seedlings with cotyledons occurred in plots in four Cusp sites. Density of current-year ash seedlings averaged 34 ± 34 and 3371 ± 1610 seedlings per ha in Crest and Cusp sites, respectively, but none were found in Core sites. Average densities of ash seedlings with cotyledons were higher in Cusp sites ($H = 16.45$; $df 2, 93$; $P < 0.001$) than other sites.

DISCUSSION

Millions of overstory ash trees have been killed by *A. planipennis* in forested sites in Michigan and the surrounding states (Poland 2007; EAB.info.2012). Previous studies examining ash stands in southeast Michigan reported overstory ash mortality rates approaching 100% (Gandhi et al. 2008; Herms et al. 2009). In my study, 100% mortality of overstory ash occurred in three of eight Core sites in southeast Michigan, but live overstory ash remained in the other five sites. My objectives were to determine the potential for green ash to persist in Michigan forests and potential future stand composition. With high levels of ash mortality, what are the long term implications for green ash stands? Will overstory species composition be altered by this invasive pest? My results indicate *A. planipennis* will largely remove green ash as a functional component of stands in southern Michigan, but has yet to alter non-ash composition of the overstory.

High levels of ash mortality created sizeable gaps in the overstories of Core sites in southeast Michigan, but the percentage of PAR penetrating the canopy in Core sites was similar to that in Cusp sites, where overstory ash remains relatively healthy. These data indicate canopy gaps in Core sites have generally been closed by the lateral ingrowth of neighboring non-ash overstory trees, and growth of recruits. Studies conducted in Appalachian hardwood forests found crown expansion rates of overstory trees vary among and within species, but lateral ingrowth of overstory trees (≥ 25 cm in DBH) averaged 18 cm per year (Trimble and Tyron 1966; Runkle 1982; Runkle and Yetter 1987). In addition, recruits could have capitalized on increased PAR within affected stands, and will be growing into gaps. Most trees in Core sites were ≤ 30 DBH, indicating they were recruits at the time stands were originally infested by

A. planipennis. Similar size class distributions, however, were found in Crest and Cusp sites. If gaps had been filled by recruits capitalizing on available PAR levels, then I would have expected to see more live trees of the ≤ 30 cm DBH size class in Core sites compared to Crest and Cusp sites, but that was not the case. Although my data indicate green ash is the most prominent species at the recruit and sapling level in Core sites, ash has not reached the overstory. Based on these data, I conclude most gap closures are likely due to lateral ingrowth of non-ash overstory trees, rather than understory growth.

Ash seedlings with cotyledons were present in Crest and Cusp sites, but not in Core sites. Seedling abundance is dependent on seed production by overstory trees (Figure 2.6), but the surviving overstory ash trees in the Core sites are confronted with multiple barriers for successful reproduction. Green ash is a dioecious species (Wright 1959; Barnes and Wagner 1981; Kennedy 1990), so not only does an individual ash need to survive, but an additional ash of the opposite sex must also survive. Ash pollen is dispersed by wind over fairly short distances (60 to 90 m), and surviving trees must be in range for pollination to occur (Kennedy 1990). Green ash of both sexes can flower annually (Wright 1959; Kennedy 1990), but this does not guarantee reproduction will occur. If all other conditions are met (i.e. trees of both sexes survive and are within range for pollination), surviving trees could fail to flower simultaneously. The absence of newly germinated ash in Core sites is consistent with a previous study on ash regeneration in southeast Michigan which documented a lack of ash in the seed bank of stands where overstory ash were killed by *A. planipennis* (Herms et al. 2009). If ash is not reproducing in Core sites, then future overstory ash is dependent on ash currently growing in the understory

(Figure 2.6). Trends in ash germination observed in Core sites are likely to repeat in Crest and Cusp sites as overstory ash dies.

A study conducted in ash stands in southeast Michigan from 2007 to 2009 reported green ash was still abundant in the understory (Kashian and Witter 2011). This is consistent with my results; green ash was still the most prevalent species in the understory of Core and Crest sites. Density of ash saplings was highest in sites where overstory trees were dead or declining in the Core and Crest sites. In Core sites, the reduction of available PAR caused by lateral ingrowth of non-ash overstory trees, and the lack of newly germinated ash seedlings will likely reduce ash sapling densities over time (Figure 6.2). Current levels of PAR and vertical growth of ash saplings were high in Crest sites, suggesting, saplings may yet grow into gaps, however, there is no evidence that ash recruits and saplings in Core sites are successfully reaching reproductive age, making it unlikely that ash saplings in Crest sites will either. Similar patterns can be expected to occur in Cusp sites as *A. planipennis* populations continue to build.

Mortality of green ash recruits caused by *A. planipennis* was low in sites in all three invasion stages. Trees at the recruit stage are the most likely to replace overstory ash as it succumbs to *A. planipennis* (Figure 2.6). Green ash regeneration can survive in shaded conditions up to 15 years, and can often outcompete other species when released (Kennedy 1990). *Agrilus planipennis* is more attracted to trees exposed to sunlight (Yu 1992; McCullough et al. 2009a, 2009b), and recruits predominately grow in shaded conditions. Beetles are also attracted to stressed ash trees (McCullough et al. 2009a, 2009b; Siegert et al. 2010; Tluczek et al. 2011). Shade and stressed overstory trees may have allowed recruits to remain relatively undetected, even in Core sites. As more overstory trees succumb, the beetles will likely infest

recruits growing in the understory, preventing them from reaching reproductive age (8 to 10 cm DBH) (Kennedy 1990) (Figure 2.6).

Species composition of recruits and saplings were generally consistent among all sites, regardless of *A. planipennis* invasion stage. Large gaps in the overstory could provide an opportunity for more shade intolerant species to grow into the overstory (Messier et al. 1999). The prevalence of green ash in the understory at all stages of the invasion, however, may prevent the growth of less tolerant species. If overstory ash is killed, and understory ash fails to reach reproductive age, ash will eventually be lost from the understory. Based on this study, I predict few ash currently in the understory of Core sites will reach the overstory, and that if overstory trees survive, they will be too few and far between to successfully reproduce. If efforts are not made to substantially slow *A. planipennis* population growth, green ash will be functionally lost from these stands in the next 20 years. Furthermore, decimation of green ash by *A. planipennis* will be repeated in Crest and Cusp sites, and throughout the range of green ash.

Red maple and sugar maple are poised to succeed ash as dominant overstory species in most sites I surveyed. Neither species of maple was abundant in the overstories of Core and Crest sites, but sugar maple was common at all other stages of growth, and red maple was abundant in the understory of Core and Cusp sites (Tables 2.1, 2.2). Both species of maple can thrive on a variety of sites, within different communities, and have ecophysiological traits such as shade tolerance, abundant seed production and seed dissemination that make them strong competitors (Godman et al. 1990; Walters and Yawney 1990; Abrams 1998). American elm also had a strong presence at all three invasion stages (Tables 2.1, 2.2), but over time, may

become less important because of Dutch elm disease (Barnes and Wagner 1981, Bey 1990). Additionally, understory American elm often declines when suppressed by more shade tolerant sugar maple (Barnes and Wagner 1981, Bey 1990). If sugar maple becomes more prevalent, the ability of American elm to persist in the understory may decline. Black cherry is classified as shade intolerant (Marquis 1990), but was abundant in all four stages of growth in Core sites (Tables 2.1, 2.2,). In Crest and Cusp sites, black cherry was abundant in the overstory (Table 2.1), but sporadic and generally scarce in in the understory (Table 2.2). Black cherry may become more prominent in Crest and Cusp sites due to the increased availability of PAR in gaps formed by dead ash. Northern red oak and black walnut were common in the overstory in Core and Crest sites, but were infrequently encountered in lower canopies (Tables 2.1, 2.2). Red oak has poor seed survival, and typically requires the production of 500 acorns to produce one 1-year-old seedling (Sander 1990). Black walnut is shade intolerant and must be dominant or codominant in a stand to survive (Barnes and Wagner 1981; Williams 1990). If ash in the understory does not reach the overstory, my data suggests American elm, black cherry, red maple, and sugar maple will likely dominate these stands.

Table 2.1. Total number of trees (N), mean (\pm SE) diameter at breast height (DBH) and basal area, relative density, relative frequency, relative dominance, and relative importance values (RIV) of the most abundant tree species across 24 sites representing three *A. planipennis* invasion stages. Relative importance values rank species contribution to a stand's overstory. All measurements were taken in summer of 2010.

Species	N	Mean \pm SE DBH (cm)	Mean \pm SE basal area (m ² per ha)	Relative density	Relative frequency	Relative dominance	RIV ¹
Core sites							
Green Ash	216	20 \pm 0.6	2.3 \pm 0.5	27%	19.5	18.4	65.9
Red Oak	103	32.1 \pm 1.5	2.9 \pm 1.4	13%	17.1	23.7	53.8
Black Cherry	122	25 \pm 1.2	2.2 \pm 0.9	15%	19.5	18.4	52.9
American Elm	130	17.6 \pm 0.5	1.0 \pm 0.4	16%	14.6	8.3	39.0
Black Walnut	82	33.1 \pm 1.2	2.2 \pm 1.1	10%	7.3	18.5	35.8
Crest sites							
Green Ash	376	16.8 \pm 0.3	2.7 \pm 0.6	61%	25.0	40.7	126.7
American Elm	88	18.8 \pm 0.8	0.8 \pm 0.4	14%	18.8	12.0	44.7
Black Walnut	84	26.5 \pm 1.2	2.2 \pm 1.1	14%	6.3	23.4	43.7
Black Cherry	39	25.5 \pm 1.8	0.7 \pm 0.4	6%	15.6	10.2	31.8
Red Oak	24	22.9 \pm 2.2	0.3 \pm 0.2	4%	15.6	5.1	24.7
Cusp sites							
Green Ash	443	21 \pm 0.4	5.2 \pm 1.0	39%	15.1	37.1	91.2
Red Maple	198	17.8 \pm 0.5	1.6 \pm 1.1	17%	13.2	11.4	41.6
American Elm	174	17.8 \pm 0.6	1.5 \pm 0.4	15%	15.1	10.4	40.5
Black Cherry	118	19.5 \pm 0.8	1.2 \pm 0.4	10%	15.1	8.5	33.6
Sugar Maple	138	18.7 \pm 0.8	1.3 \pm 0.4	12%	11.3	9.5	32.8

¹ Relative importance values = relative density + relative frequency + relative dominance.

Table 2.2. Total count (N) and mean (\pm SE) stems per ha., of the five most abundant species of recruits, saplings, and seedlings in 2010, sampled at 24 sites in southern Michigan representing the three stages of the *A. planipennis* invasion wave.

Recruits ¹	N	Mean \pm SE stems per ha	Saplings ²	N	Mean \pm SE stems per ha	Seedlings ³	N	Mean \pm SE stems per ha
Core sites								
Green Ash	63	120 \pm 29	Green Ash	967	1962 \pm 331	Green Ash	386	13,281 \pm 2364
Red Maple	56	113 \pm 56	Black Cherry	98	199 \pm 71	Red Maple	123	4232 \pm 1895
Sugar Maple	28	56 \pm 30	Sugar Maple	65	132 \pm 59	Black Cherry	81	3131 \pm 963
Black Cherry	24	48 \pm 20	Red Maple	40	81 \pm 37	Sugar Maple	41	1411 \pm 249
Red Oak	16	32 \pm 27	American Elm	24	49 \pm 21	American Elm	19	654 \pm 346
Crest sites								
Green Ash	174	353 \pm 90	Green Ash	911	1849 \pm 443	Green Ash	656	22,570 \pm 5570
Basswood	47	95 \pm 78	Sugar Maple	46	93 \pm 51	Northern Hackberry	18	619 \pm 446
American Elm	20	41 \pm 20	Northern Hackberry	19	39 \pm 29	Sugar Maple	4	138 \pm 82
Sugar Maple	19	39 \pm 18	American Elm	18	37 \pm 26	Basswood	3	103 \pm 76
Shagbark Hickory	12	24 \pm 24	Black Cherry	15	30 \pm 26	American Elm	3	103 \pm 103
Cusp sites								
Sugar Maple	79	160 \pm 41	Green Ash	255	518 \pm 212	Green Ash	662	22,776 \pm 5623
Green Ash	78	158 \pm 38	Sugar Maple	47	95 \pm 47	Red Maple	92	3166 \pm 1926
American Elm	50	102 \pm 25	Black Cherry	11	22 \pm 10	Musclewood	16	551 \pm 360
Red Maple	46	93 \pm 43	American Elm	10	20 \pm 6	Bitternut	15	516 \pm 320
Black Cherry	13	26 \pm 9	Red Maple	10	20 \pm 10	Swamp White Oak	9	310 \pm 113

¹ Recruits (\geq 3 cm and $<$ 10 cm stem diameter).

² Saplings ($<$ 3 cm stem diameter and \geq 45 cm).

³ Seedlings ($<$ 45 cm in height).

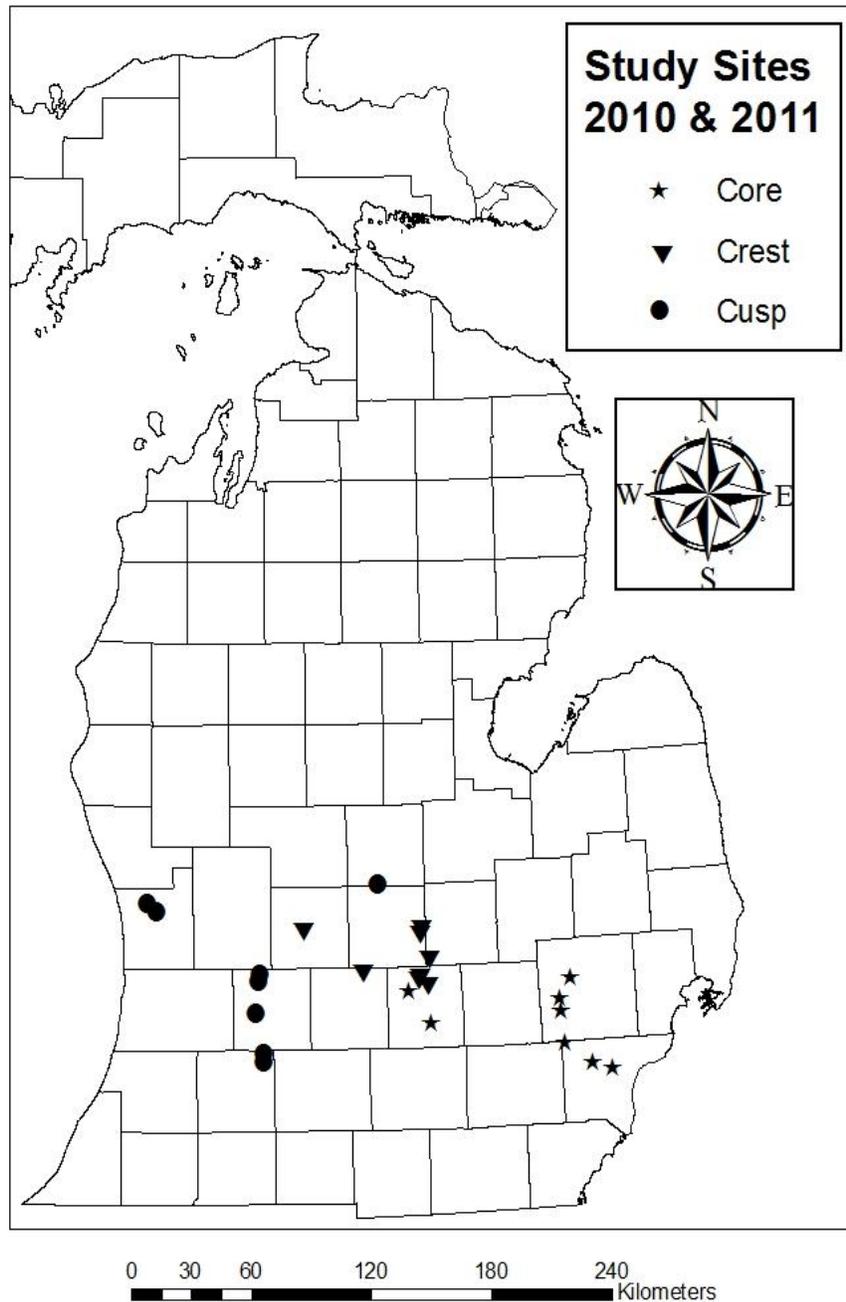


Figure 2.1. Locations of 24 green ash sites representing three stages of the *A. planipennis* invasion wave. Core sites were characterized by low densities of *A. planipennis* and high ash mortality. Crest sites were characterized by high densities of *A. planipennis* and moderate to high ash mortality. Cusp sites were characterized by low densities of *A. planipennis* and low ash mortality.

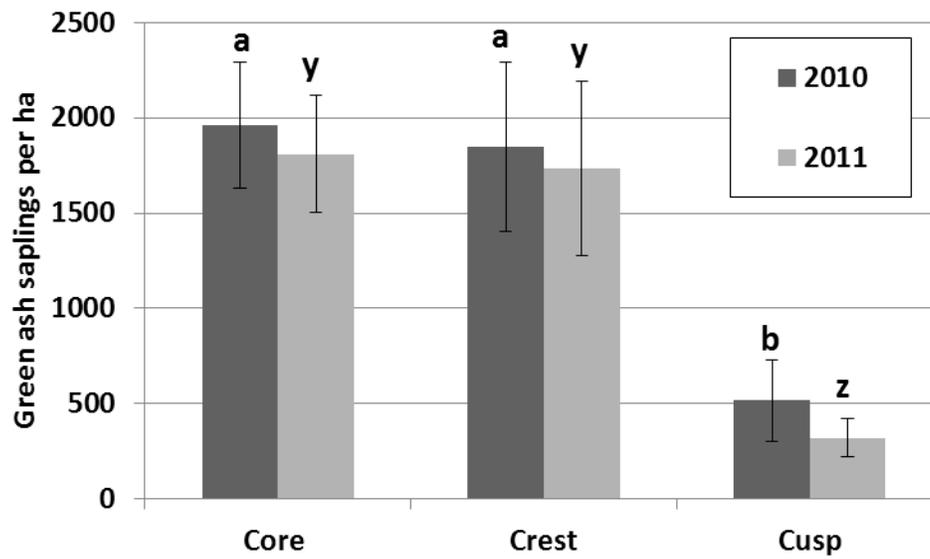


Figure 2.2. Mean (\pm SE) density of green ash saplings per ha in 2010 (N = 2133) and 2011 (N = 1905). Means with the same letter are not significantly different among the three invasion stages (a and b for 2010, y and z for 2011).

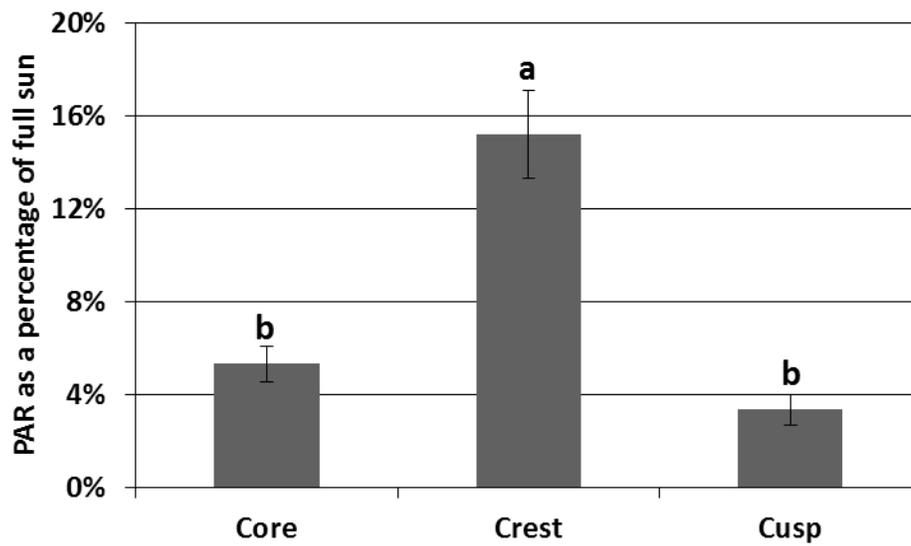


Figure 2.3. Mean (\pm SE) percentage of full sun represented by photosynthetically active radiation (PAR) measured after dawn and before sunrise in 12 sites between 20 June to 15 July 2011 (N = 428 readings). Means with the same letter are not significantly different (Tukey's protected HSD test; $P < 0.05$).

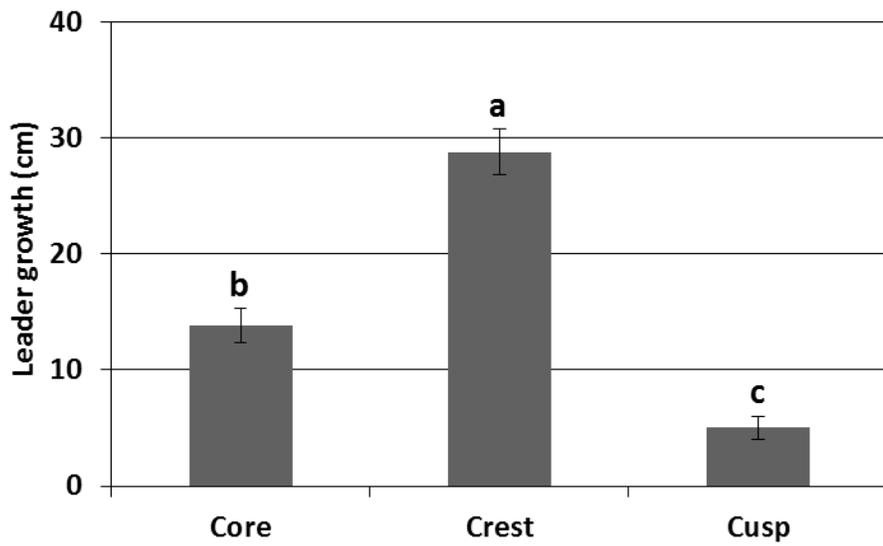


Figure 2.4. Mean (\pm SE) growth (cm) of leaders of green ash saplings measured in 12 sites between 12 June to 17 June 2011 at the three *A. planipennis* invasion stages (N = 237 saplings). Means with the same letter are not significantly different (Tukey's protected HSD test; $P < 0.05$).

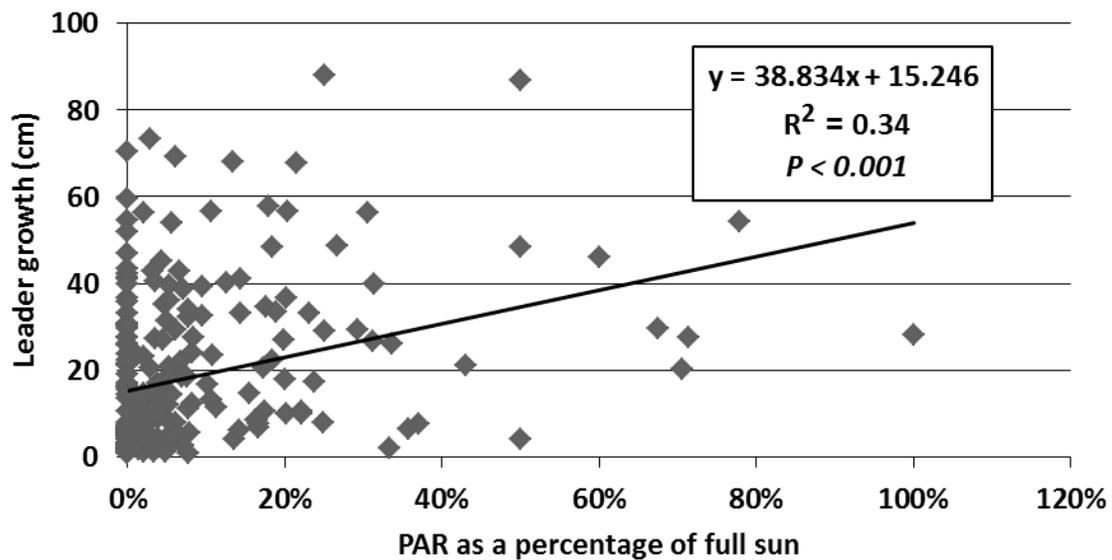


Figure 2.5. Linear regression of annual growth of terminal leaders of green ash saplings and percent of full sun represented by photosynthetically active radiation (PAR) measured in 12 sites in southern Michigan (N=237 paired measurements). Saplings were 1 to 2 m tall, but diameter and exact height of saplings were not measured ($P < 0.05$).

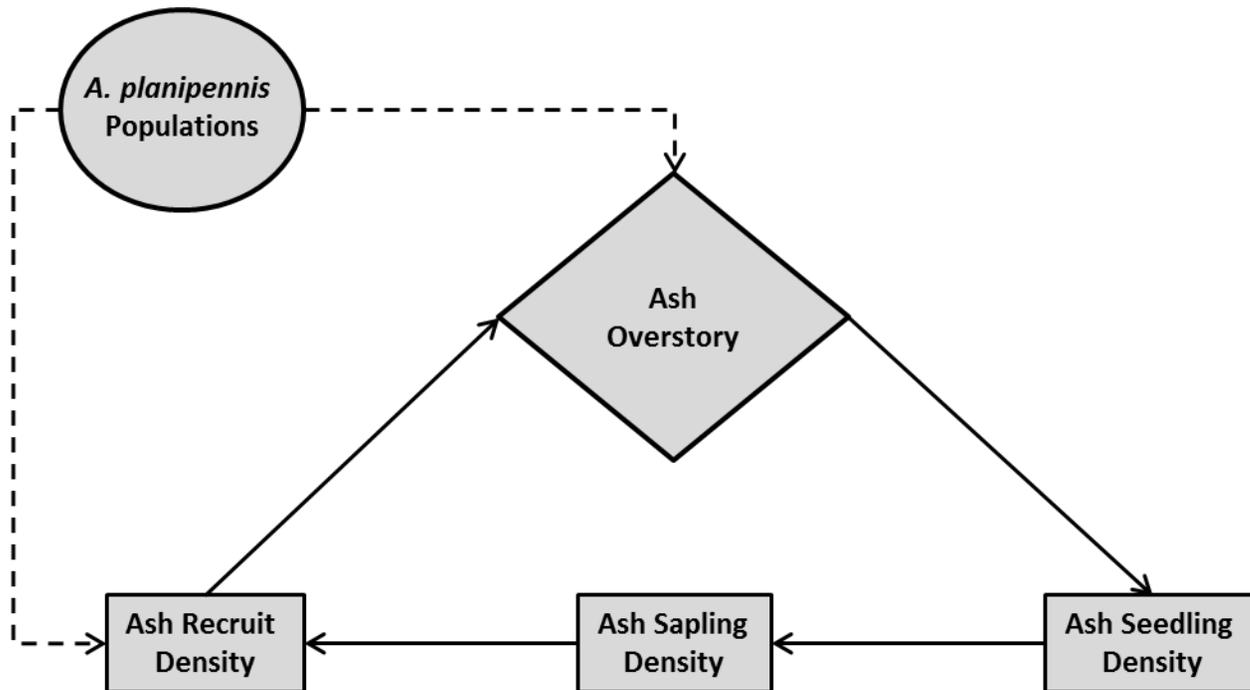


Figure 2.6. A flow diagram representing effect of *A. planipennis* populations on overstory ash and ash recruits, and the positive relationship of overstory ash abundance on seedlings, saplings, and recruits. Positive relationships are represented by \longrightarrow and negative relationships are represented by $- \rightarrow$

APPENDICES

Appendix A

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2012-03

Title of thesis:

Evaluation of Emerald Ash Borer Populations and the
Ash Resource at Three Stages of the Invasion Wave

Museum where deposited:

The Michigan State University (MSU) Albert J. Cook Arthropod Research Collection

Table A.1. Specimens submitted to the Michigan State University (MSU) Albert J. Cook Arthropod Research Collection

Family		Life Stage	Quantity	Preservation
Buprestidae	<i>Agrilus planipennis</i>	Larva	10	Ethanol
Buprestidae	<i>Agrilus planipennis</i>	Adult, Male	10	Pinned
Buprestidae	<i>Agrilus planipennis</i>	Adult, Female	10	Pinned
Braconidae	<i>Atanycolus cappaerti</i>	Adult, Male	5	Pinned
Braconidae	<i>Atanycolus cappaerti</i>	Adult, Female	5	Pinned
Braconidae	<i>Atanycolus cappaerti</i>	Adult, Male	5	Ethanol
Braconidae	<i>Atanycolus cappaerti</i>	Adult, Female	5	Ethanol

Appendix B

Table B.1. List of study sites by Michigan county and geographic coordinates.

Study Sites	County In Michgian	Latitude	Longitude
Core Sites			
Dansville State Game Area	Ingham County	42.53°N	84.34°W
Highland State Recreational Area	Oakland County	42.63°N	83.55°W
Hines Drive, Hawthorn Ridge	Wayne County	42.34°N	83.36°W
Indian Springs Metro park	Macomb County	42.71°N	83.48°W
Maybury State Park	Wayne County	42.43°N	83.52°W
Michigan State University Campus, South Lot	Ingham County	42.67°N	84.47°W
Proud Lake Recreation Area	Oakland County	42.57°N	83.55°W
University of Michigan-Dearborn Environmental Interpretive Center	Wayne County	42.32°N	83.24°W
Crest Sites			
Central Park	Ingham County	42.73°N	84.42°W
Ionia State Recreational Area	Ionia County	42.95°N	85.12°W
Lincoln Brick Park	Eaton County	42.77°N	84.76°W
Meridian Township Natural Area	Ingham County	42.74°N	84.41°W
Riverfront Park, East Gate	Ingham County	42.71°N	84.37°W
Rose Lake Wildlife Research Area	Clinton County	42.82°N	84.36°W
Sleepy Hollow State Park, Site 1	Clinton County	42.92°N	84.41°W
Sleepy Hollow State Park, Horse Trail	Clinton County	42.95°N	84.40°W
Cusp Sites			
Augusta Creek Fish & Wildlife Area	Kalamazoo County	42.41°N	85.36°W
Barry State Game Area	Barry County	42.58°N	85.41°W
Crockery Creek	Ottawa County	43.05°N	86.07°W
East Bur Oak Landing	Ottawa County	43.01°N	86.01°W
Kellogg Experimental Forest	Kalamazoo County	42.37°N	85.36°W
Maple River State Game Area	Clinton County	43.13°N	84.65°W
Middleville State Game Area, Site 1	Barry County	42.72°N	85.39°W
Middleville State Game Area, Site 2	Barry County	42.75°N	85.38°W

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