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## Behaviors of Adult *Agrilus Planipennis* (Coleoptera: Buprestidae)

### Authors

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**BEHAVIORS OF ADULT *AGRILUS PLANIPENNIS*  
(COLEOPTERA: BUPRESTIDAE)**Cesar R. Rodriguez-Soana<sup>1,2,\*</sup>, James R. Miller<sup>1</sup>, Therese M. Poland<sup>3</sup>,  
Tina M. Kuhn<sup>3</sup>, Gard W. Otis<sup>4</sup>, Tanya Turk<sup>4</sup>, and Daniel L. Ward<sup>5</sup>**ABSTRACT**

A 2-year study was conducted in Canada (2003) and the United States (2005) to better understand searching and mating behaviors of adult *Agrilus planipennis* Fairmaire. In both field and laboratory, adults spent more time resting and walking than feeding or flying. The sex ratio in the field was biased towards males, which tended to hover around trees, likely looking for mates. There was more leaf feeding damage within a tree higher in the canopy than in the lower canopy early in the season, but this difference disappeared over time. In choice experiments, males attempted to mate with individuals of both sexes, but they landed more frequently on females than on males. A series of sexual behaviors was observed in the laboratory, including: exposure of the ovipositor/genitalia, sporadic jumping by males, attempted mating, and mating. Sexual behaviors were absent among 1-3 day-old beetles, but were observed regularly in 10-12 day-old beetles. Females were seen exposing their ovipositor, suggestive of pheromone-calling behavior. No courtship was observed prior to mating. Hovering, searching, and landing behaviors suggest that beetles most likely rely on visual cues during mate finding, although host-plant volatiles and/or pheromones might also be involved.

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*Agrilus* (Coleoptera: Buprestidae) is one of the largest insect genera, with thousands of species (Jendek 2000 and references therein); however, the genus remains rather poorly understood. Reproductive and other behaviors have been studied in only a few economically important species. Carlson and Knight (1969) suggested that *Agrilus* may utilize host trees for mating encounters. Once on trees, beetles could find mates via visual (Gwynne and Rentz 1983), auditory/vibrational (Fenton 1942), tactile, or pheromonal signals, or combinations of these signals. Dunn and Potter (1988) demonstrated that male two-lined chestnut borers, *Agrilus bilineatus* (Weber), landed on cages containing unmated female beetles significantly more often than on cages lacking females; they were not attracted to oak logs or to males. This suggests that female *A. bilineatus* release pheromones or, less likely, produce auditory signals attractive to males. Whether other *Agrilus* species use non-contact signals in mate finding is unknown.

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive species in North America. Native to China, Japan, Korea, Mongolia, and eastern Russia (Yu 1992), this insect borer was first discovered in

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southeastern Michigan, USA, and neighboring Ontario, Canada, in 2002 (Haack et al. 2002, McCullough and Roberts 2002). In eastern North America, *A. planipennis* attacks and can kill all native species of ash (*Fraxinus* spp.). The land area of counties in which *A. planipennis* has been detected in the USA and Canada now exceeds 190,000 km<sup>2</sup>, and it is estimated that more than 20 million ash trees have been killed in the core-infested area (EAB Info 2008).

Little is known about the behavior of *A. planipennis*, mainly due to its recent presence in North America and because this insect is rarely a pest in its native range. In China, *A. planipennis* adults are often active from 0600 to 1700 h, especially when weather is warm and sunny. Mating has been recorded between 0900-1500 h and lasts 20-90 min (Chinese Academy of Science 1986, Yu 1992). Adult beetles often rest in bark cracks or on foliage during rainy or very cloudy weather and usually remain on foliage at night. Adults typically fly in 8-12 m bursts (Yu 1992), but are capable of longer flights. They most often attack ash trees that are growing in the open or along the edge of forest stands (Chinese Academy of Sciences 1986), but entire stands can be killed during outbreaks (Yu 1992).

As in China, *A. planipennis* adults in North America preferentially attack trees in open areas compared to trees within closed-canopy stands (Poland et al. 2005). More beetles were captured on trap trees exposed to full sunlight than on trap trees that were mostly or totally shaded (McCullough et al. 2006). Lelito et al. (2007) found that males search trees during flight and descend rapidly onto pinned beetles of either sex and attempted to copulate, suggesting that visual cues are used in mate finding. Lance et al. (2007) observed that beetles spend most of their time resting on leaves and typically fly from leaf to leaf in the tops of trees.

While the overall behavior of *A. planipennis* in North America appears to be similar to that in China (Poland and McCullough 2006), many details of host and mate-finding and mating behaviors are not clear. Effective management of any insect pest often benefits from a comprehensive understanding of behavior. For instance, knowledge about the distribution of an insect within its host can aid in the development of effective tree surveys (Timms et al. 2006).

Because understanding of host and mate finding and mating behaviors is useful for developing techniques to detect populations, manipulate them, disrupt mating, or reduce attacks on host trees, our main objective was to study these behaviors for adult *A. planipennis* in the field and laboratory. Previous studies showed that adult beetles hover around ash tree trunks (Bauer et al. 2004, Lelito et al. 2007); thus, we postulated that male beetles hover around trees searching for females. Because adult emergence is greater from upper parts of tree trunks (Brown-Rytlewski and Wilson 2005), we postulated that feeding damage within a tree would be greater in the top of canopy at the time of beetle emergence. Specifically, we conducted field studies to determine the searching pattern in *A. planipennis*, the proportion of males and females hovering around trees, the distribution of damaged leaves within a tree, and its mate-searching behaviors. Additional laboratory experiments were conducted to quantify male and female behaviors under controlled conditions. We tested the hypotheses that *A. planipennis* performs gender-specific behaviors and that sexual behaviors appear later in the life of beetles.

## MATERIALS AND METHODS

### Searching behaviors

*Beetle behaviors.* This study was conducted to record adult *A. planipennis* behaviors on and off their host plants. The study was conducted in June and July 2005 at a commercial nursery (Quality Tree Transplanting Nursery, Whitmore Lake, MI), composed of young closely planted ash trees. The nursery was located in a 13-ha field surrounded by mature mixed hardwoods with a significant

component of ash infested by *A. planipennis*. Several tree species were planted in 3-6 ha blocks throughout the nursery. The study was conducted within a 3-ha planting of 6-7 year-old ash trees (~4m tall and ~10 cm diameter). Trees were planted 1.5 m apart in rows 75 m long with 7 m between rows. Varieties included 3 green ash (*Fraxinus pennsylvanica* Marsh.) cultivars (Marshall, Platmore, and Summit) (what is known as green ash in USA is known as red ash in Canada), and 2 white ash (*F. americana* L.) cultivars (Autumn Purple and Autumn Applause). All observations were made on wild beetles of unknown age and mating status, and were conducted between 0900-1400 h under sunny and warm (24-30°C) conditions. This time of day was selected because all beetle behaviors were observed being performed in the morning and midday in laboratory bioassays (see results). Observations were conducted throughout the planting on all ash varieties present.

Wild beetles (n = 33), found on ash leaves, were selected using a "first encountered" approach, and their activities, i.e., resting, walking, eating ash leaves, flying/hovering, and wing-opening (i.e., wings open and exposing the magenta-colored dorsal abdomen) were recorded for a maximum of 5 min or until they flew out of sight. This time period was sufficient to record the full repertoire of beetle activities; for instance, preliminary data showed that doubling this time period did not yield additional behaviors. Time spent performing each of the behaviors was recorded using a hand-held microcassette audio recorder. Observations were conducted on 24 June, 1 July, and 7 July 2005. The time of the first observation coincided with the peak of adult emergence.

Only beetles observed for more than 100 sec were used for data analyses (n = 28). A graphical descriptive summary for the percentage of time a beetle performed each of the behaviors is presented as a box and whiskers plot.

*Beetles flying/hovering around trees.* We examined the entire crown of more than 100 trees and collected all beetles on and off trees to determine the proportion of males and females flying and hovering around the trees. If males search for females, we expect the proportion of individuals caught flying/hovering to be biased towards males. The study was conducted at the Quality Tree Transplanting Nursery under the conditions described above. Hovering/flying beetles were caught using a long-handled sweep net. Collected beetles (n = 195) were placed in vials and their sex determined in the laboratory. Sex was determined under a microscope by sexually-dimorphic external characteristics, including the generally larger size of females (Cappaert et al. 2005), the presence of abundant sternal setae in males, and the narrow tapered abdomen for males compared to the rounded enlarged abdomen for females (Fig. 1). Collections occurred between 1000-1200 h and on four different dates (29 June, 1 July, 7 July, and 8 July 2005).

To compare males and females with regard to the proportion observed hovering, an exact logistic regression model was fit using the LOGISTIC procedure of the SAS System (ver. 9.13, SAS Institute Inc., Cary, NC). The model included gender and date as classification variables as well as their interaction. There was no significant interaction effect (Wald  $\chi^2 = 0.528$ ; df = 3;  $P = 0.9127$ ), so the effect of gender pooled across dates is presented. Proportions hovering and exact (Clopper-Pearson) 95% confidence intervals are presented.

*Distribution of feeding damage.* We quantified the proportion of damaged leaves on trees at three different locations within trees and times in the season. This study was conducted at the Quality Tree Transplanting Nursery under conditions described above. Using clippers, we retrieved branches across the upper, middle, and lower thirds of the tree canopy. A total of 65 different trees was used. For each tree, one branch was collected from each canopy position on 24 June (n = 25), 29 June (n = 20), and 8 July 2005 (n = 20). The number of damaged and undamaged leaves per branch was recorded. Leaf damage was caused almost exclusively by *A. planipennis*, since other herbivores were rarely seen on trees. Damaged leaves had about 10-20% area loss (estimated visually).

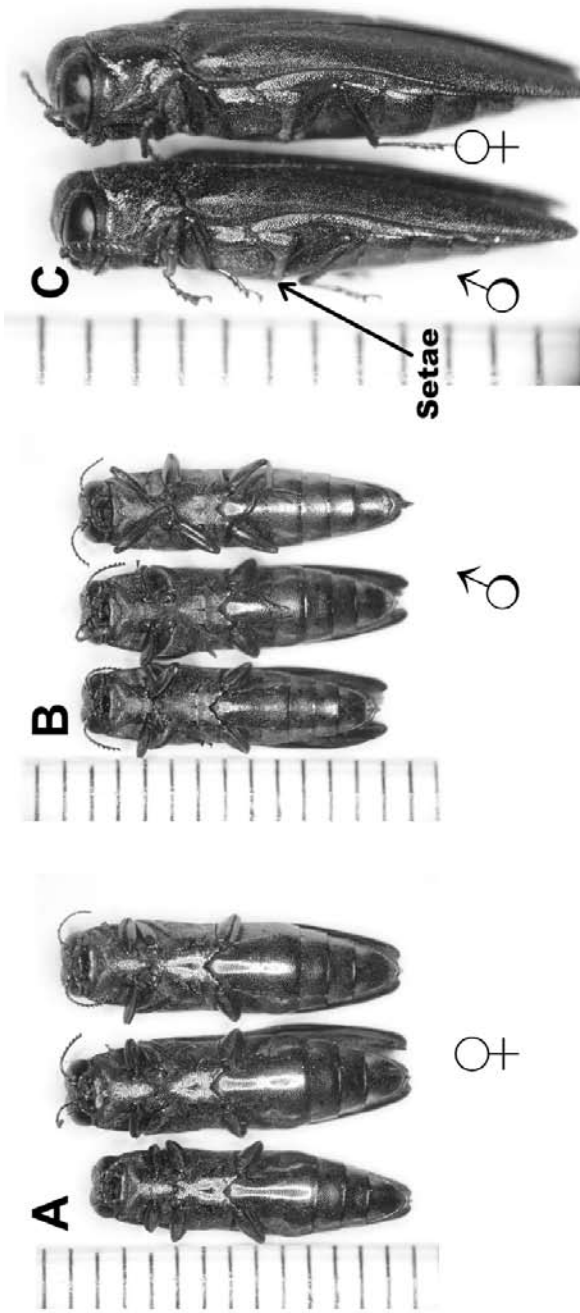


Fig. 1. Photograph of three emerald ash borer females (A), and three emerald ash borer males (B), depicting the overall larger body size for females, the glabrous prosternum of females and rugulose and rough texture of the male pro-sternum, and the enlarged and rounded abdomen of females compared to the narrow and straight-sided abdominal shape for males. Photograph of emerald ash borer male (left) and female (right) depicting the dense setae on the male pro-sternum (C). Scale is in mm. Photographs by Debbie Miller, USDA Forest Service.

We tested for main effects of relative canopy height and date as well as their interaction effect on the proportion of leaves damaged by *A. planipennis* feeding. The experiment was analyzed as a split-plot with date as the whole-plot factor and height as the sub-plot factor. Individual trees were the whole-plots and canopy strata within a tree were the sub-plots. The mixed effects ANOVA was fit using the MIXED procedure of the SAS System. Because the number of leaves sampled varied, the analysis was weighted with the number of leaves per experimental unit. Model adequacy was assessed using plots of standardized residuals. A significant interaction effect was further investigated by pairwise comparisons of means within each date using Tukey's adjustment.

### Mating behaviors

*Male orientation toward mates.* We investigated the role of visual stimuli in male orientation towards potential mates. This study was conducted on 14 and 15 July 2003 at the Ojibway Park Reserve (Windsor, Ontario) that has a mixture of park-like and natural habitats containing many mature green ash trees infested by *A. planipennis*. The study was conducted on ash trees located on the edge of the parking lot adjacent to the visitors' center. We observed beetle behaviors on two trees, with one observer assigned to each tree. All observations were made on feral beetles of unknown age and mating status and were conducted between 1300-1600 h under sunny and warm (24-30°C) conditions. This time of day was selected because sexual behaviors increase at midday-afternoon (see lab results).

Five dead female *A. planipennis* "decoys", mounted on insect pins, were affixed at a height of 1.5 m onto the trunks of two trees on which we observed the greatest beetle activity. We had three decoys on one tree (placed equidistantly on the tree, such that they were 120 angular degrees apart, and a beetle searching the trunk could only see one decoy at a time) and two decoys on the other tree (on opposite sides of the tree). All decoy beetles were dead, oriented vertically (head up), placed at the same height (1.5 m), and their bodies were touching the tree trunk. Inconspicuous black dots (pen-drawn, 0.5 cm in diameter) were marked on the trees 10 cm away from the decoys at the same height. The dots (control treatment) were applied to test whether flying beetles preferentially land on other beetles. The position of the female decoy relative to the black dot was switched every ~15 min, from left to right and back again, to avoid bias of location on tree on male attraction. We recorded the number of males landing on either the decoys or the control spots. Once a male beetle landed on a decoy, we removed and released it so it would not influence other searching males. In a variation of the above test, both male and female decoys were presented simultaneously 10 cm apart at a height of 1.5 m. The position of male decoys and female decoys was switched every ~15 min. The decoys remained in place during interactions with courting males.

Differences in male preference between female decoys and controls and between male and female decoys were compared using the exact binomial test of proportions (FREQ procedure of the SAS System). We tested whether the observed proportions was significantly different from 0.5. The exact 95% confidence intervals are also presented.

*Laboratory bioassay of behavioral ontogeny.* Adult *A. planipennis* beetles (n = 15 males and 15 females, sexed by body size and shape, Fig. 1) were collected from field-infested logs upon emergence on 13 June 2003, separated by sex, and refrigerated overnight. The next morning, these virgin beetles were placed individually in screened containers (made by attaching 2 cups at their open ends; total volume = 568 ml; height = 20 cm) containing green ash leaflets inserted into a water pic. Fresh leaflets were supplied to the beetles every three days. The experiment was conducted in the laboratory, with the temperature in the containers ranging from 23-32°C.

Behavior of each beetle was scanned and recorded in a consistent order, then repeated six times each h. Behavioral scans started at 0600 h and continued until 1800 h. At each scan we scored: resting or inactivity, walking, grooming (consisting of crossing and/or stroking legs, or cleaning antennae with mouthparts), eating ash leaves, raising the abdomen between elytra and/or wing-opening, antennating rapidly, lowering the abdomen, excreting waste or flying, exposing ovipositor (females) or genitalia (males), sporadic jumping behavior of males, attempted mating, and mating. After days 1-3 of recording the behaviors in isolation, males and females were paired and observations were continued on days 4-6 and 10-12. On days 7-9, when observations were not made, the beetles were isolated to prevent mating. When a beetle died, the remaining beetle was paired with another survivor. Sex of all beetles was confirmed by dissection once observations ceased. Data from two pairs had to be discarded because they were same-sex pairings. We also recorded the number and duration of successful matings. A mating was considered successful if the male inserted its aedeagus into the female's genitalia without female rejection. Mating duration was recorded from aedeagus insertion to withdrawal.

To analyze the behaviors, scans were grouped into 3 four-hour time periods: morning (0600-1000 h), midday (1000-1400 h), and afternoon (1400-1800 h) and into age blocks of three days each: young (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> days of adulthood), middle-aged (4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> days of adulthood), and old (10<sup>th</sup>, 11<sup>th</sup>, and 12<sup>th</sup> days of adulthood). The "young" group includes beetles at a pre-mating age, the "middle-aged" group includes beetles at the age of mating (Cappaert et al. 2005), and the "old" group includes beetles at a post-mating age. The counts of observed behaviors were then analyzed using the LOGISTIC procedure of the SAS System to fit a generalized logits model. The generalized logits models multiple nominal discrete responses (the several behaviors) as the probability of their occurring conditioned on the explanatory variables. To reflect the response modeled and simplify interpretation the predicted probabilities were converted to the proportions of time engaged in each behavior. Mating pair was initially included in the model; however, it explained little variation and did not change the effects of the other regressors. Therefore, pairing was omitted from the model in order to include data from the first three dates of observation (before pairing was imposed) in the analysis. The final model included age and gender as classification variables and time of day as a continuous regressor variable.

## RESULTS

### Searching Behaviors

*Beetle behaviors.* Beetles spent more (~70%) time resting and walking on the leaves than in feeding or flying (Fig. 2). Beetles spent as much as 85%, but on average 35%, of their time resting (median = 33%). On average, beetles spent 32% of their time walking (median = 32%), and 70% of beetles were observed performing this behavior. Beetles spent only 15% of their time feeding (median = 0%), and about 60% of all beetles were observed feeding. Similarly, beetles spent 15% of their time flying (median = 12%). For the 64% of beetles observed flying, we recorded a total of 33 flying events; in 88% of these, the beetles landed on the same tree from which they took off, indicating that most beetles were moving within a single tree. Beetles spent the least time (average = 2.5%, median = 0%) opening their wings and exposing their abdomens (Fig. 3). In fact, only 4 of the 28 beetles (14%) were observed to perform this behavior.

*Beetles flying/hovering around trees.* The sex ratio of field-collected beetles was 2.8:1 male:female. More males (62% of all collected male beetles) were found flying/hovering around trees (exact 95% confidence interval (CI): 53% (lower limit), 70% (upper limit)), whereas only 28% of collected female beetles were hovering (exact 95% CI: 17%, 42%) (Wald  $\chi^2 = 5.68$ , df = 1,  $P = 0.0171$ ).



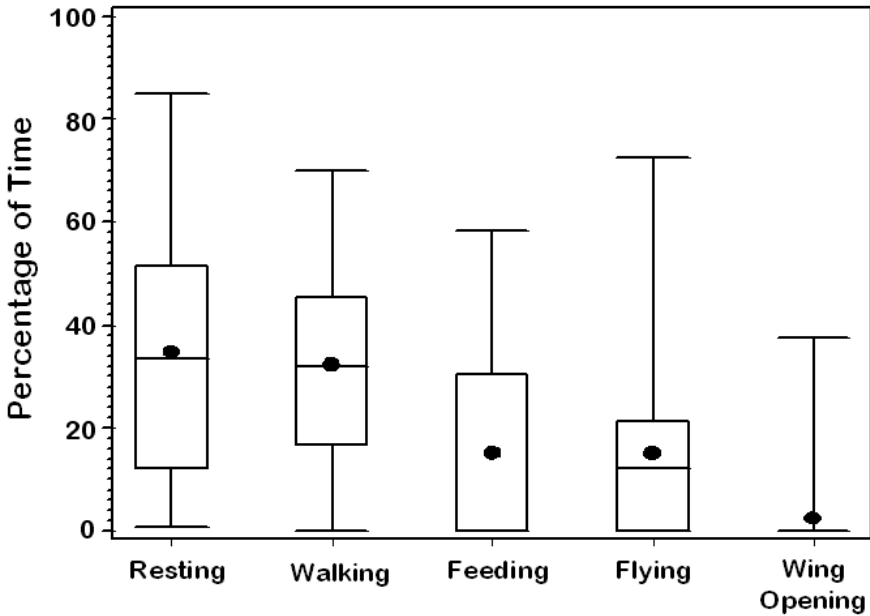


Fig. 2. Percentage of time spent by adult *Agrilus planipennis* performing individual behaviors in the field. Each box represents the inter-quartile range for each of the behaviors and the whiskers represent the range of the data. The line crossing each box indicates the median (the median for feeding and wing opening was zero) and the dot indicates the mean. Observations were conducted between 0900-1400 h in June-July at the Quality Tree Transplanting Nursery in Whitmore Lake, Michigan.  $n = 28$ .

There was also a significant date effect (Wald  $\chi^2 = 13.48$ ;  $df = 3$ ;  $P = 0.0037$ ), indicating that the proportion of beetles hovering varied by date from 30% on 7 Jul to 67% on 8 Jul.

**Distribution of feeding damage.** Leaf damage by *A. planipennis* increased over time ( $F = 9.08$ ;  $df = 2,62$ ;  $P = 0.0003$ ; Fig. 4); the proportion of leaves damaged was below 40% during the first two sampling dates, and increased to 47% by the last sampling date. Leaf damage varied with position within the tree ( $F = 6.41$ ,  $df = 2,124$ ,  $P = 0.0022$ ); initial damage was significantly higher in the middle and upper thirds of the trees than in the lower third ( $P \leq 0.05$ ) (Fig. 4). This difference disappeared over time (significant time-by-position interaction:  $F = 4.16$ ,  $df = 4,124$ ,  $P = 0.0034$ ; Fig. 4).

### Mating behaviors

**Male orientation toward mates.** In beetle decoy experiments, 12 male *A. planipennis* landed on dead female decoys while none landed on or near the controls (black dots), demonstrating a strong preference of males to orient to the dead beetles ( $P = 0.0005$ ; exact 95% CI: 0.74, 1.00). Occasionally, male beetles tried to mate with the dead female decoy (Fig. 5). During the experiment with female decoys, an additional 14 male *A. planipennis* landed on live male and female beetles on the tree with the decoys. When both male and female decoys were presented simultaneously 10 cm apart, 27 out of 35 males landed on the female decoy while 8 landed on the male decoy ( $P = 0.0019$ ; 95% CI: 0.60, 0.90).



Fig. 3. Wing-opening behavior performed by a female *Agrilus planipennis*. The behavior consists of raising the abdomen between elytra and/or opening the wings.

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Thus, males preferentially oriented to and landed on female decoys; however, it is evident that male decoys also presented attractive stimuli to searching males.

*Laboratory bioassay of behavioral ontogeny.* The results of our laboratory observations are summarized in Fig. 6. There was a significant three-way interaction effect (Wald  $\chi^2 = 30.7$ ,  $df = 16$ ,  $P = 0.0145$ ), so the individual predicted probabilities for each of the behaviors at each age by gender by time of day combination were plotted and interpreted.

It was clear from the predicted probabilities that the proportion of time spent resting (R) decreased from morning to afternoon. The decrease in resting was offset by increases in other behaviors, primarily walking (W), grooming (G), and feeding (F), and later in life by sexual behaviors (SB). Grooming (G) was more frequently observed in females than in males and decreased in frequency

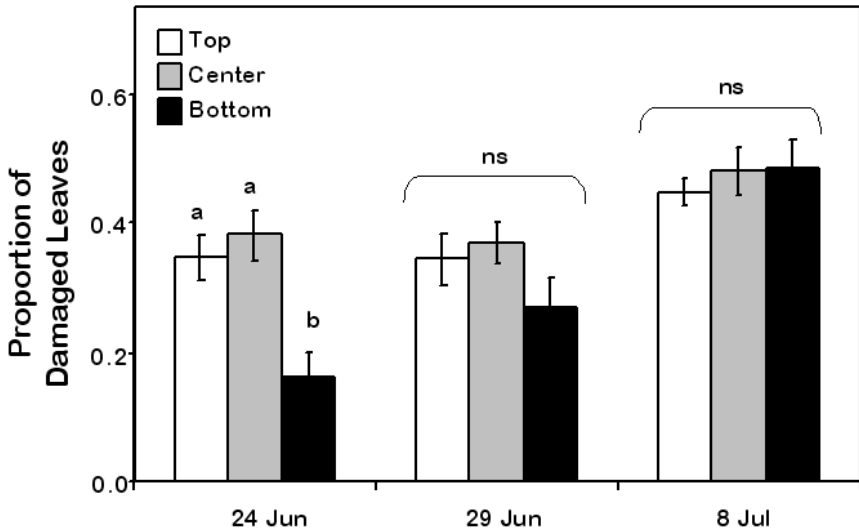


Fig. 4. Proportion ( $\pm$ SE) of damaged leaves per branch taken from the upper (top), middle (center), and lower (bottom) thirds of the ash tree canopy. Samples were conducted on three different and consecutive weeks between June-July at the Quality Tree Transplanting Nursery in Whitmore Lake, Michigan. Columns with different letters are significantly different ( $P \leq 0.05$ ), while ns = not significant ( $P > 0.05$ ) (Tukey tests).  $n = 65$ .

in both sexes with age. Feeding (F) occurred most frequently at midday and increased with age. Raising the abdomen between elytra and/or wing-opening (R/WO) were very infrequent in the morning and declined with age. Rapid antennation (RA) also decreased with age. Lowering the abdomen (LA) and excreting waste or flying (E/F) were the most infrequent behaviors performed under laboratory conditions, and mostly observed at midday and afternoons in both males and females.

Exposing the ovipositor/genitalia, sporadic jumping by males, attempted mating, and mating were completely absent from the behavioral repertoire of beetles 1-3 days old, but were observed regularly in beetles 10-12 days old. We believe that these behaviors are aspects of sexual behaviors (SB), in part because they appeared later in the lives of the insects. However, it is unclear how exposing the ovipositor and male jumping behavior relate to sexual activity. Their frequency was highest at midday.

We observed two successful matings by one male during our laboratory study. No courtship was noticed prior to mating. The successful male mated with the female with which he was paired on Day 10 for 56 min. He attempted to mate with the same female twice on Day 11, but she was unreceptive. That male was then placed with a different female of the same age, and within 1 h he tried to mate but was not successful. On Day 12, he mounted the female twice. On the first attempt, he mounted the female for 36 min; although the female did not actively reject him and the male had his aedeagus fully exposed, no mating occurred. One hour later, he mounted the female again, quickly inserted his aedeagus, and remained in copula for 83 min.



Fig. 5. Male *A. planipennis* mating with a dead female decoy. Larger mean size of female *A. planipennis* is evident in this photo.  
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## DISCUSSION

Altogether, the present study provides evidence for the following conclusions: adult *A. planipennis* spend the majority of time resting, beetles engage in more non-resting behaviors later in the day, early-season feeding is more abundant higher in the tree than towards the bottom, visual cues are important in helping males find females, and sexual behaviors are observed regularly in older beetles (10-12 day-old).

In the field, we observed that *A. planipennis* adults spend about 35% of their time resting, 32% walking, 15% feeding, and 15% flying (Fig. 2). Lance et al. (2007) also reported these four behaviors to be the most common in *A. planipennis* in the field; however, they observed beetles spending more time resting (70%) and less time walking (10%). This discrepancy between studies is likely due to differences in the time of day of the observations and variable environmental field conditions. Our laboratory study shows that beetles can spend between 30 to 75% of their time resting depending on the time of day (Fig. 6), increasing their activity as the day progressed. Field conditions can also influence *A. planipennis* behaviors. In fact, adults are active mostly in warm and sunny conditions (Chinese Academy of Science 1986, Yu 1992). Wing-opening behavior was observed infrequently both in the field and laboratory.

Most beetles observed hovering around trees were males. Although Bauer et al. (2004) also observed adult *A. planipennis* hovering around ash tree trunks, the sex of beetles was not determined. This hovering behavior consisted of a slow upward flight, casting laterally back and forth along the ash tree trunk, suggestive of mate searching. Beetles often landed on the same tree or one close to the one from which they took off, indicating that they tend to fly around a single tree or a few trees in close proximity when searching for mates.

The sex ratio of all field-collected adult *A. planipennis*, found both on and off trees, in our study was biased towards males. This differs from a 1:1 sex ratio for *A. planipennis* adults reared from naturally-infested logs (Lyons et al. 2004). Interestingly, Lyons and Jones (2005) reported more females captured on TangleTrap™-coated sticky traps. For *A. bilineatus*, Cote and Allen (1980) also found a 1:1 adult sex ratio for adults reared from infested logs. However, the sex ratio of beetles drawn to sticky traps placed on trees or on traps containing oak bolts was biased towards females, indicating a greater attraction of females to potential hosts (Cote and Allen 1980). Our male bias may result from females being less conspicuous or less active, making them more difficult to find under field conditions. Alternatively, if female *A. planipennis* become unreceptive after mating and either reduce their activity or disperse to lay eggs, the apparent sex ratio will become male-biased. Taylor et al. (2004) found mated females capable of flying longer, farther, and faster than males or unmated females. Another factor that may contribute to a male-biased sex ratio early in the season is the fact that males emerge slightly earlier than females (Lyons and Jones 2005).

Leaf-feeding damage within trees was greater higher in the canopy than in the lower parts early in the season (Fig. 4). This pattern of leaf damage is consistent with beetle emergence and activity patterns within trees. Brown-Rytlewski and Wilson (2005) found greater adult *A. planipennis* emergence from upper parts of tree trunks. Similarly, Lance et al. (2007) found that flight activity and landing rates were concentrated in the tops of trees. By the end of June the difference in leaf damage within a tree disappeared, indicating an even distribution of leaf damage, and likely adult beetles, within infested trees. Rodriguez-Saona et al. (2006) found that female *A. planipennis* were attracted to leaf volatiles in behavioral assays and that the antennae of both sexes respond to several of these volatiles. Males may use plant volatiles as short-distance cues to locate females or as indicators of the presence of conspecifics. More research is needed to fully understand the role of host-plant volatiles on *A. planipennis* behavior.

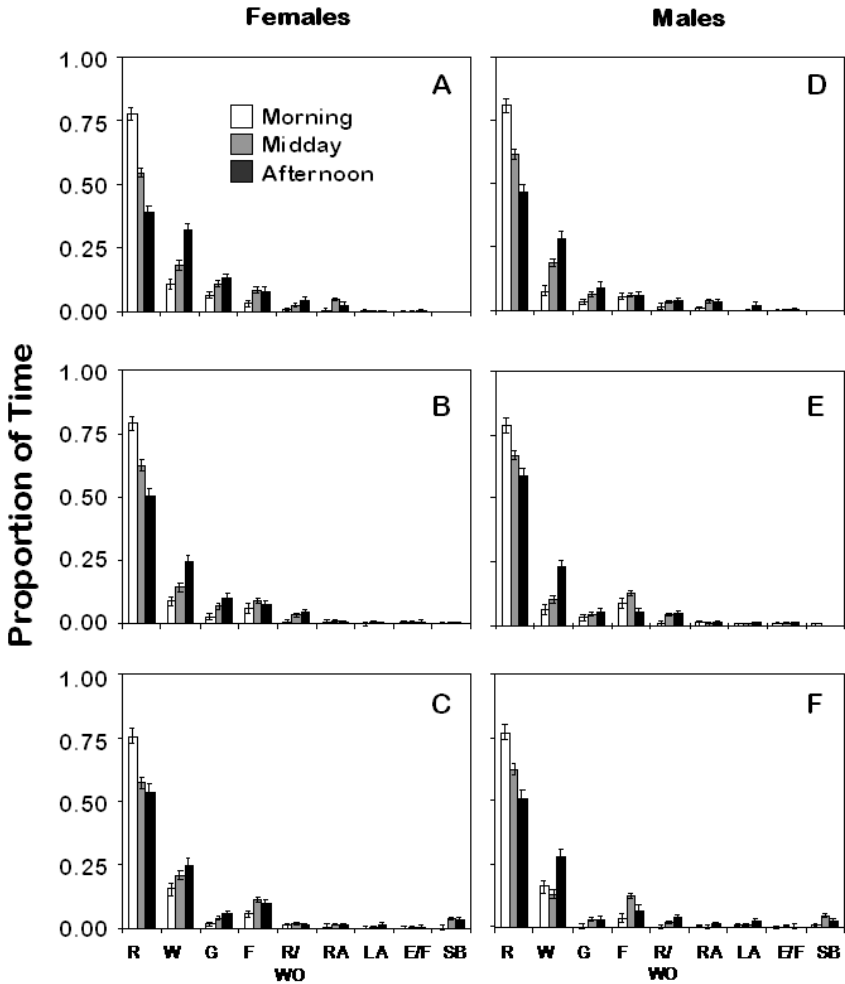


Fig. 6. Proportion of time ( $\pm$  95% confidence intervals) spent by female (A-C) and male (D-F) *Agrilus planipennis* performing individual behaviors in the laboratory. Behaviors include resting (R), walking (W), grooming (G), eating ash leaves (F), raising the abdomen between the elytra and/or wing-opening (wings open and exposing the abdomen) (R/WO), rapid antennation (RA), lowering the abdomen (LA), excreting waste or flying (E/F), and sexual behaviors (SB), which include exposing ovipositor (females) or genitalia (males), sporadic jumping behavior of males, attempting mating, and mating. Recordings were made during the first three days after emergence (A and D), days 4-6 (B and E), and days 10-12 (C and F). Behaviors were grouped into three periods: morning (0600-1000 h), midday (1000-1400 h), and afternoon (1400-1800 h). n = 15 males and 15 females.

Furthermore, the middle and top canopy might emit a higher amount of "attractive" volatiles than the lower canopy at the time of high beetle emergence due to increased leaf damage in those parts of the tree. Our results suggest that traps to monitor early infestations of *A. planipennis* populations should be placed high in trees.

In addition to host-plant derived cues, males might use more reliable cues to find mates, such as those associated with conspecifics. Male beetles oriented identically to both live and dead beetles of either sex, indicating that mate-searching by males appears to be based mainly on visual cues from conspecifics. Males oriented toward beetles they could see and failed to orient toward beetles that were hidden from view by the tree trunk (G.W. Otis, pers. obser.), also suggesting the predominant use of visual stimuli. Upon encountering beetles on tree trunks, searching males landed quickly on them, with no apparent courtship by beetles of either sex (G.W. Otis, pers. obser.). Similarly, Lelito et al. (2007) found that males landed on decoys pinned to leaves of ash trees and quickly attempted mating. The preference of males for female decoys in our study may reflect the greater mean size of females (McCullough and Roberts 2002, Lyons and Jones 2005). Lelito et al. (2007) found no preference of males for female beetles.

*Agrilus planipennis* females begin to mate 5-7 days after emergence (Cappaert et al. 2005). In our laboratory study, shifts in frequency of behavioral activities were likely related to achieving sexual maturity at 4-6 days of age. Older unmated females exhibited sexual behaviors, suggesting that they may switch to more active mate-attraction behaviors if not mated soon after becoming sexually mature. In the laboratory, 10-12 day-old females frequently extruded and slowly rotated their genitalia in a circular motion. Females were also seen exposing their ovipositor, at times in a pulsating motion, suggestive of pheromone-calling behavior exhibited by many other insect species (Tamaki 1985). For example, in the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, calling involves extension and ventral curving of the telescopic terminal abdominal segments; these structures sometimes pulsate rhythmically (Hammack 1995). Female *A. bilineatus* likely emit pheromones; male beetles land on cages containing unmated female beetles in significantly greater numbers than on cages that lacked female beetles (Dunn and Potter 1988). Recently, Bartelt et al. (2007) identified the antennally active macrocyclic lactone, (3Z)-dodecen-12-olide, from *A. planipennis*; this compound was emitted predominantly by females.

A 13-day-old virgin female *A. planipennis* exhibited a behavioral display that greatly enhanced her visibility to human observers (referred to here as wing-opening; Fig. 3). The behavioral sequence appeared to be a fixed action pattern, in which the female first rotated her body, then pumped the head and thorax, and ended with an open wing display in which the magenta-colored abdomen was exposed. The same behavioral components were noted in 10-12 day-old unmated females during the laboratory study. This same behavior was observed in the field. Whether this behavior is a mating display remains unknown.

From our field and laboratory observations we propose a dual mating strategy for *A. planipennis*. Our field observations suggest that the primary mate-finding strategy of *A. planipennis* involves active visual search by males for females on tree trunks. Thus, under high beetle density, it is likely that females will mate soon after reaching sexual maturity. Additionally, our laboratory observations suggest the presence of mating behaviors in *A. planipennis* that develop as the beetles age. We suggest that, if females fail to mate within a few days, they might utilize two alternative strategies to attract mates: active pheromone-calling and a behavioral display sequence that increases their visibility to searching males. These alternative mate-finding strategies are more likely to be used in low-density situations in which females are rarely encountered by males. An analogous dual mating strategy has been documented in the spruce budworm moth (*Choristoneura fumiferana* (Clemens)), with active male

search for females during the daytime at high densities and female pheromone attraction of males at night at low moth densities (Kipp et al. 1995).

The information presented in this study has practical implications for monitoring *A. planipennis* populations. Current efforts focus on the development of traps baited with host-plant volatiles (Poland et al. 2005). This study identified features in the beetle's mating behavior that could help in the improvement of traps. For instance, pheromone-traps or traps using visual stimuli attractive to male beetles could be used alone or in combination to host-plant volatiles in areas of low beetle densities, which are regularly encountered at the leading edge of the expanding distribution of *A. planipennis* in North America. Although Bartelt et al. (2007) identified an antennally active compound from *A. planipennis*, predominantly emitted by females, pheromone traps have yet to be developed for field monitoring of this beetle. Visual cues are important in *A. planipennis* mate-finding (this study; Lelito et al. 2007). One such cue currently tested for monitoring this beetle is color. For example, Otis et al. (2005) and Francese et al. (2005) found purple traps to be more attractive to beetles than black or yellow. Whether this color preference is associated to the wing-opening behavior described here remains unknown. The fact that greater feeding damage was observed higher in the tree canopy, indicates that traps should be placed at that height.

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