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Visually Mediated 'Paratrooper Copulations' in the Mating Behavior of *Agrilus planipennis* (Coleoptera: Buprestidae), a Highly Destructive Invasive Pest of North American Ash Trees

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Abstract The emerald ash borer, *Agrilus planipennis*, is a serious invasive pest of North American ash (*Fraxinus*) trees. In captivity, mating is initiated by beetles at least 10 days old, and appears to be based simply on random contact with a member of the opposite sex. In the field, male *A. planipennis* search the tree during flight, and attempt to copulate with dead beetles of both sexes pinned to leaves, after descending rapidly straight down onto the pinned beetles from a height of from 30 to 100 cm. All evidence suggests that males find potential mates using visual cues. Equal numbers of feral males approach all 'dummy' beetles; however, considerably more time is spent attempting copulation with dead females rather than males, suggesting a contact chemical cue. Sticky traps prepared from dead, pinned EAB capture crawling insects as well as male *A. planipennis*, at a rate similar to that at which small purple sticky traps of similar overall area capture crawling insects and both sexes of feral EAB.

Keyword *Agrilus planipennis* · emerald ash borer · trapping · invasive pest · Buprestidae · *Fraxinus*

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Introduction

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious non-native pest of ash trees (*Fraxinus* sp., Oleaceae). EAB populations are spreading rapidly in the Midwest and Mid-Atlantic states of the USA, as well as in some adjacent areas of Ontario, Canada. The beetle was first identified near Detroit, Michigan and Windsor, Ontario in 2002 (Haack et al. 2002), and has subsequently been detected in Ohio and Maryland in 2003, Indiana in 2004, and Illinois in 2006. A thorough review of the timeline of introduction, host plants, means of spread, and other relevant information is provided by Poland and McCullough (2006). In June 2007, EAB was identified in Pennsylvania as well (Pennsylvania DCNR 2007).

EAB infestations are insidious in that they often go undetected during initial colonization. The characteristic D-shaped exit holes left by emerging adults are only noticeable at ground level by the time the beetle has significantly damaged the tree (Haack et al. 2002). To further complicate matters, EAB larvae can take 2 years to develop in healthy trees, lengthening the time it takes to produce the visible symptoms commonly used for detection (Poland and McCullough 2006; Cappaert et al. 2005; Siegert et al. 2005). Currently, delimiting new infestations involves the use of ‘detection trees’ (also widely referred to as ‘trap trees’) that have been artificially girdled and subsequently sampled for any larvae present. Girdled trees, while effective, come at a huge cost of time and labor and therefore are not optimal for wide-scale detection efforts (Cappaert et al. 2005). A purple panel trap for capturing EAB adults is currently undergoing testing but this trap is not yet being used operationally (Crook et al. 2006; Francese et al. 2005, 2006; Metzger et al. 2006).

Finally, little is known about the mating habits of EAB. There is no evidence to suggest that EAB uses a long range pheromone to locate conspecifics (Otis et al. 2005). Past research has indicated that buprestids appear to first locate their host and then secondarily seek mates by visual, tactile, and other non-pheromonal cues (Carlson and Knight 1969; Matthews and Matthews 1978; Gwynne and Rentz 1983). With the urgent need for early detection of new infestations in mind, we sought to identify any precopulatory behaviors in feral and laboratory-reared EAB that might be significant to either short- or long-range mate attraction. Dead, pinned EAB males and females were used as ‘dummies’ to test for precopulatory behaviors in the field. Subsets of both male and female beetles were washed in dichloromethane to remove chemical cues; we expected that all types of pinned beetles would be approached by feral males if EAB depends heavily on vision for mate finding. If one type of ‘dummy’ were to be favored by males over the other, this could indicate that there are olfactory, visual, or contact chemical differences between the sexes. We also utilized the dead beetles themselves as a trap, and tested the detection effectiveness of these ‘dummy’ beetles compared to small purple traps.

Methods

Insects

All beetles (*A. planipennis*) were kept on a 14:10 light cycle and fed green ash leaves, *Fraxinus pennsylvanica*, collected from outdoors. Beetles were provided by

USDA APHIS PPQ personnel at the experimental station in Brighton, Michigan, 48116. Beetles were reared from infested ash logs collected locally and kept in sealed barrels at ambient room temperature, with a cone at one end leading to an opening. This opening was capped with a removable plastic bottle. Beetles, attracted to the light at the opening, fell into the bottle and were collected every 24 h. Beetles were separated by sex on the day they emerged from the rearing barrels.

Laboratory Behavioral Observations

Individual beetles were removed from sex-specific containers with soft forceps. A male and a female beetle were placed into a small plastic food-storage tub with paper toweling as a substrate as well as ash leaves in a florist's vial. The tub and vial were washed and new toweling and leaves were provided after each trial. Each beetle was randomly chosen from among the age cohorts (1-, 5-, 10-, 15-, and 20-day-old beetles) to form random combinations of beetles in each pairing. Four or five pairs were set up in a similar manner to be observed simultaneously. Beetles were given 15 min to acclimate to their cage and then were observed for 30 min. The following behaviors were noted: contact between individuals, wing fanning, open-wing basking, flight, vibratory interactions, mounting attempts, and copulation. All pairs were observed for 30 min, and the number of instances in which the beetles performed each behavior was noted. Beetles were frozen and discarded after mating trials, thus preventing any introduction of contact compounds into the sex-segregated cages or through the reuse of individual beetles in mating trials.

Field Behavioral Observations

On 7 and 10 June 2006, at night between 9:00 and 11:15 P.M. EDT, 30 feral beetles were counted and observed on each occasion in the USDA/APHIS quarantined area around Brighton, MI. Beetles were located on ash trees by manual inspection while wearing a headlamp equipped with a red LED (Rayovac). The position of each beetle on the tree and its activity and sex were recorded before beetles were released back in to the tree nearby. Similarly, on 12 and 13 June 2006, in the morning between 6:45 and 8:20 A.M. EDT, 30 beetles were counted and observed on each occasion, and their sex, activity and position on the tree were recorded. On 8, 9, 10, 13, and 18 June 2006, from 9:00 A.M. until 5:00 P.M. EDT, ten, random, 1-m sections of branch on green ash trees were assessed for the presence of EAB at 30-min intervals. If only one or two beetles were present on the branch, they were simply counted without being removed from the tree. Beetles in groups of three or more individuals (within the 1-m section of branch examined) were collected and sexed using a hand lens, then returned to a nearby tree. During each day of the mate-finding experiment detailed below, beetles present on the tree trunk up to 2.5 m from the ground were collected and sexed, and then released back onto the trunk.

Mate-Finding

Lab-reared beetles of both sexes were killed in sex-specific vials by freezing, and then, segregated by gender, were immediately pinned through the thorax with a size

2 Monarch insect pin to a Styrofoam board. The beetles were randomly chosen to be pinned in one of the following three postures (Fig. 1): elytra shut (their normal posture on the leaf); elytra pinned at approximately 30° to the body with the hind wings pinned at a 45° relative to the body (this mimics the behavior they perform before flight and while they bask); and with both elytra and wings positioned at an approximately 90° angle to the body (an exaggerated version of the basking posture). Beetles were then allowed to dry for 3 days at standard room temperature, at which point they had assumed these positions permanently.

After 3 days, half of the beetles of each gender and posture were randomly selected to be washed for 10 min in dichloromethane and then returned to dry for 1 day, at which point they were washed again. Washed beetles were not used until at least 24 h after the final washing. All washed or unwashed dried 'dummy' beetles were then taken to ash trees with EAB infestations and pinned onto the terminal leaflet of a leaf facing the sun (Fig. 2). All beetles were pinned to leaflets of approximately the same height (2 m) in a given replicate.

The identity of each 'dummy' beetle was inconspicuously coded by means of a small piece of adhesive tape using one of four colors placed on the underside of the leaflet to which the beetle was pinned. Colors were used as follows: red, unwashed female; orange, washed female; yellow, unwashed male; green, washed male. Insect pins containing no beetles were placed out on sunny terminal leaflets as well. Any beetles arriving on leaves with any of these treatments were sexed and recorded.

To judge overall EAB activity on the tree for comparison, five empty terminal leaf clusters were marked with a wrapping of Teflon tape around the base of the twig. Leaflets with pins only and empty leaflets each had a randomly colored piece of tape placed underneath the leaflet. All six treatments in a replicate were monitored for the presence of EAB for 2 h at a time. Beetles that arrived at any 'dummy' beetle or control leaf cluster were captured by hand or sweep net, sexed and released back into the tree, with the time of attraction and gender of each feral beetle being recorded.

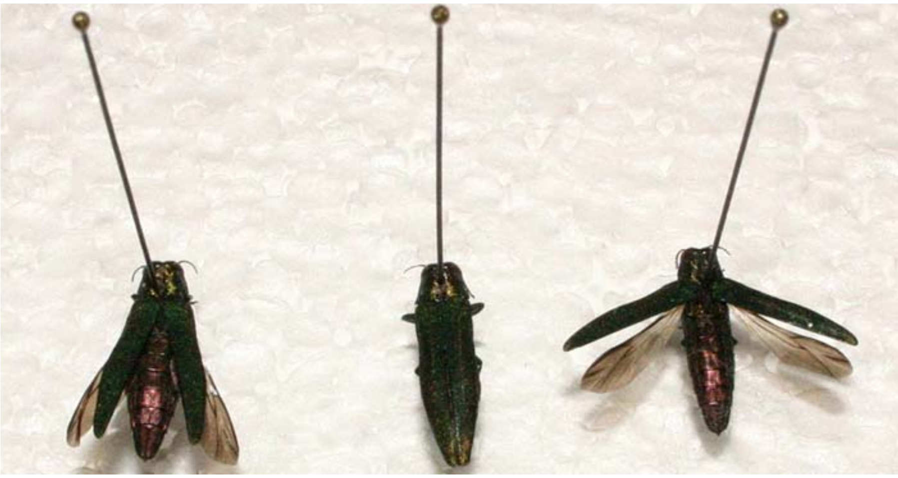

Fig. 1 Three elytra positions tested for dead EAB of each sex in order to determine which was most attractive to feral EAB males. The *model in the center* is pinned with elytra shut, and elicited the most frequent aerial approaches by feral males.

Fig. 2 Close-up view, on the leaf surface, of a pinned EAB used to attract feral males. The *pin* was inserted through the thorax of the beetle, and then used to attach the beetle to the mid-vein of the ash leaflet.

Initially, all three postures of beetles were used. However, responses by feral males to open-winged 'dummy' beetles were very low (Fig. 3), as were responses to those models with a 30° elytral angle. In further observations, therefore, only pinned beetles (of either sex) with elytra closed were used. After 2 h, used 'dummy' beetles were replaced and the locations of each were randomly chosen in the portion of the tree currently facing the sun. In this way, the position of the experiment changed each day depending on the time.

Three infested sites in the area of Brighton, Michigan were used for this experiment, and each was used in a regular rotation on any day in which the weather was suitable. Experimental sites included one isolated tree in a business park; several dead and dying trees surrounding a commercial parking lot; and a combination of dead, dying, and relatively healthy infested trees in a forest edge setting. 'Dummy' beetles were used between 9:00 A.M. and 5:00 P.M. EDT from 20 June 2006 until 29 June 2006. Together, the visual model experiments comprised a total of ca. 40 observation-hours.

EAB Sticky Trapping Using Dummy Beetles

Beetles prepared identically to those used in the visual stimuli assessment were placed on terminal leaflets of ash tree leaves and coated with a layer of Tanglefoot by hand-application while wearing rubber gloves. In addition, other traps were made from: (1) 2.5 cm×2.5 cm squares of purple trap material; (2) 2.5 cm×2.5 cm squares of green metallic paper (HOTP-10371 'Emerald Glimmer', http://www.paperpizazz.com); (3) ovals of green metallic paper the size of an EAB; and (4) ovals of green metallic

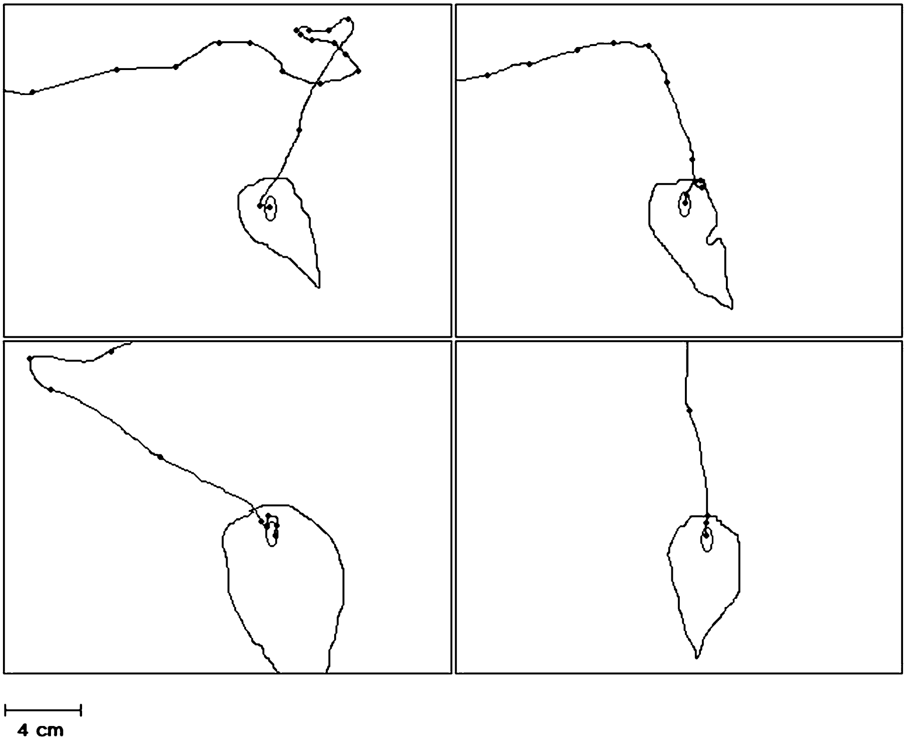

Fig. 3 Various approach flight-tracks, transcribed from video, of feral male EAB descending rapidly onto pinned, closed-elytra models on leaves. The *scale bar at left* indicates 4 cm in each frame. The small oval on each depicted leaflet is the position of the pinned EAB on the leaf. The span of time between each dot is 1/16 s.

paper twice the length and width of an EAB. All such traps were displayed randomly on the terminal leaflets of ash trees in ten replicates of each trap type at each of two field sites. Placement was completed on 30 June 2006. These sticky traps were monitored for the presence of trapped feral EAB on 4 July 2006 and again on 8 July 2006. Any beetles trapped were removed with forceps, rinsed in Histo-Clear (HS-200, National Diagnostics) and sexed under a dissecting scope.

Behavioral Analyses

The approaches of feral male beetles onto leaves containing pinned ‘dummy’ beetles were recorded for illustrative purposes using a Sony HandyCam digital camcorder (Model DCR-TRV350, Sony). Video was transferred via an IEEE 1394 connection to a Dell Inspiron 8600 laptop computer using the Windows XP Home operating system and Windows Movie Maker 2.0 (Microsoft), and the beetle images were captured at 512 kbps at a size of 320 by 240 pixels. Flight tracks (Fig. 4) were prepared by placing transparency sheets over a computer monitor while the video was played frame-by-frame in Movie Maker. At each frame (1/16 s), a marking pen was used to place a dot on the transparency sheet at the center of the feral beetle’s image. The final position of the feral male on the model was recorded as a dot within

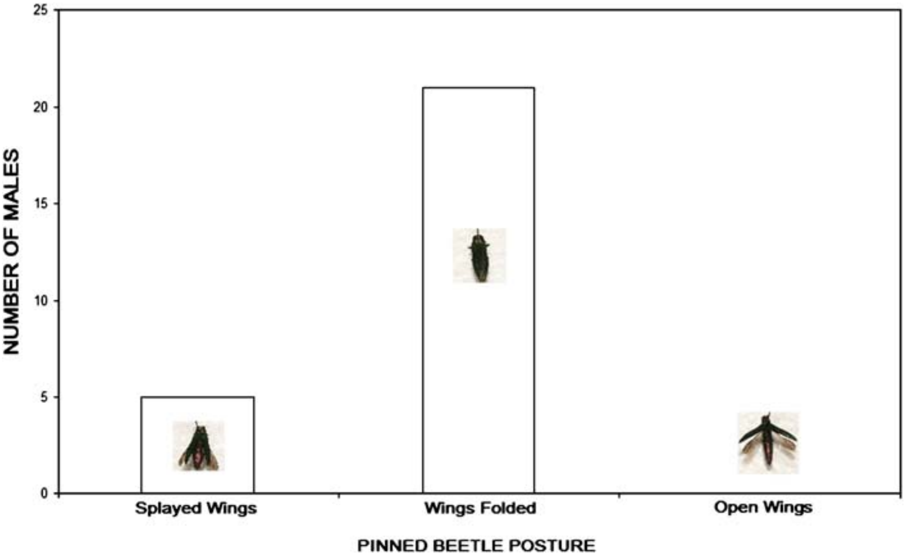

Fig. 4 Effect of model beetle elytra position on the number of paratrooper copulation approaches observed in feral male EAB. Models of either gender with fully open elytra and wings elicited no paratrooper copulation attempts. Models of either gender with fully closed elytra resulted in the highest observed number of paratrooper copulation attempts.

an oval. The dots were connected in sequence and an outline of the leaf was then traced at the position it was in when the feral beetle landed.

Statistical Analyses

Descriptive statistics for lab and field behavioral data were obtained with SPSS version 13 (SPSS 2004) on a Dell Inspiron 8600 laptop computer using the Windows XP (Microsoft) operating system. Analysis of lab behavioral data was performed using PROC PRINCOMP in SAS version 9.1 (SAS Institute 2005). Analysis of feral male approaches by pinned beetle treatment was performed using PROC GLM Type III sum of squares in SAS, including Tukey's Studentized range test. Male approach data was log transformed prior to this analysis. The number of males approaching each pinned beetle treatment and the controls was analyzed using PROC GLM in SAS, again including Tukey's Studentized Range Test for multiple comparisons.

Results

Laboratory Behavioral Observations

During the 260 trials conducted in the laboratory, rates of copulation were extremely low. Principal component analysis revealed no strong correlations between any two behaviors. Among the stronger, although still weak, correlations were male wing fanning to male flight (correlation matrix value=0.5365), female wing fanning to female flight (correlation matrix value=0.5294), and contact to female juddering (a vibration of the body while stilting up on the legs; correlation matrix value=0.4166).

No behavior was strongly correlated with copulation; even male mounting of the female only correlated with a value of 0.5308. The complete correlation matrix is given in Table 1. Contact between the male and female beetles occurred in 85.8% of the trials. Following contact, females clung to males in 2.3% of trials. Males performed wing fanning behavior in 37.7% of the trials, whereas females performed wing fanning in 24.6% of the trials. Basking behavior (open wings) was performed by males in 20.4% of trials and by females in 24.3% of trials. Juddering by males occurred in 38.4% of trials and in 56.2% of trials by females. Flight occurred in 35.4% of the trials for males, and in 22.7% of the trials for females. Males mounted females in 5.3% of trials. For comparison, copulation only occurred in 2.3% of trials. One male mounted a female at 1 day of age (but was kicked off), and another mounted a female at 5 days of age, and was also unsuccessful at achieving a mating. However, most mounting was performed by males of at least 10 days of age (11 out of 14 cases), and copulation was not observed until both the male and female were at least 10 days old.

We terminated all trials at 30 min; therefore it is possible that more matings would have occurred given a longer interval. The lack of any obvious and consistent precopulatory behavior performed by either sex in our trials suggested that some condition necessary for efficiently getting the sexes together was missing in the laboratory. Increasing the density of EAB in a container did increase the number of mating pairs that formed (we used 20 of each sex in one trial and observed four matings in 30 min), but they occurred at the same frequency and in the same way in which single pairs were observed forming: beetles simply contacted one another, and the male quickly attempted to mount the female. To test the possibility that the beetles may use acoustic cues to make contact, we set up a bat detector (Model #T-7407, Acorn Naturalists) approximately 30 cm from the mating arenas, and no ultrasound emissions were detected as having been produced by the beetles during the course of this experiment.

Field Behavioral Observations

In the early morning around sunrise, the 60 beetles examined were found primarily sitting on leaflets (95%) as compared to those found on petioles (5%; Table 2). Of the 95% of EAB on leaflets, 29.8% were found on terminal leaflets, 3.5% on terminal leaflet tips, 21.1% on non-terminal leaflet tips, and 45.6% were found on a leaflet other than the terminal (Table 2). Of those beetles on terminal leaflets or terminal leaflet tips, only 26.3% were male. At this time of day, 58.3% of the beetles observed on any tree location were female. Feeding on leaves was observed in 45% of beetles, while mating pairs comprised 3.3% of the total EAB observed. All other beetles were apparently inactive.

At night, beetles were also found primarily on leaflets (86.7% of 60 EAB observed) (Table 3). Just over 46% (46.7%) of the 60 beetles found on the leaflets at night were male. At night, feeding on leaflets was performed by 8.3% of the beetles, with 91.7% of the beetles being inactive. The five beetles observed to be feeding initiated this behavior after the headlamp was brought to bear on their location. Of

Table 1 PRINCOMP Correlation Matrix of EAB Lab Behavior

	cnt	mwf	fwf	mow	fow	mj	fj	mf	ff	fcm	mm	cop
cnt	1.000	0.0669	−0.0009	0.0421	−0.078	0.2768	0.4166	0.0942	−0.0159	0.0626	0.0972	0.0626
mwf	0.0669	1.000	−0.12.50	−0.0192	0.0417	0.1682	0.0475	0.5365	−0.0045	−0.0667	0.0254	0.0390
fwf	−0.0009	−0.1250	1.000	−0.0633	0.0363	−0.0596	0.0475	0.0508	0.5294	0.0327	0.0639	0.0924
mow	0.0421	−0.0192	−0.0633	1.000	−0.1525	−0.0075	0.0238	0.0448	0.0450	0.0494	0.0908	0.1130
fow	−0.0780	0.0417	0.0363	−0.1525	1.000	−0.0596	−0.0249	0.0508	0.0151	−0.0271	−0.0156	−0.0271
mj	0.2768	0.1682	−0.0596	−0.0075	−0.0596	1.000	0.0772	0.1920	−0.0508	0.0891	0.1266	0.0891
fj	0.4166	0.0475	0.0475	0.0238	−0.0249	0.0772	1.000	0.0541	0.0716	0.0842	0.1421	0.0842
mf	0.0942	0.5365	0.0508	0.0448	0.0508	0.1920	0.0541	1.000	−0.0168	−0.0602	0.0729	0.0470
ff	−0.0159	−0.0045	0.5294	0.0450	0.0151	−0.0508	0.0716	−0.0168	1.000	0.1002	0.1149	0.1002
fcm	0.0626	−0.0667	0.0327	0.0494	−0.0271	0.0891	0.0842	−0.0602	0.1002	1.000	0.1903	0.3176
mm	0.0972	0.0254	0.0639	0.0908	−0.0156	0.1266	0.1421	0.0729	0.1149	0.1903	1.000	0.5308
cop	0.0626	0.0390	0.0924	0.1130	−0.0271	0.0891	0.0842	0.0470	0.1002	0.3176	0.5308	1.000

Variables: *cnt* contact between sexes; *mwf* male wing fanning; *fwf* female wing fanning; *mow* male opens wings; *fow* female opens wings; *mj* male juddering; *fj* female juddering; *mf* male flight; *ff* female flight; *fcm* female clings to male; *mm* male mounts female; and *cop* copulation occurs

Table 2 Position of Feral EAB on Ash Trees at Sunrise

	No. of obs.	Percent of total	Cumul. percent
L	26	43.3	43.3
LT	12	20	63.3
P	3	5	68.3
TL	12	28.3	96.7
TLT	2	3.3	100
Total	60	100	

L Leaflet; *LT* leaflet tip; *TL* terminal leaflet; *TLT* terminal leaflet tip; *P* petiole

the 60 beetles observed, 8.3% were located on petioles, and 5% were located on a branch of the tree rather than a leaflet. We further divided the 86.7% of total beetles observed (52/60) on leaflets into the same categories used above. We observed 15.4% of these to be on terminal leaflets, 5.8% on terminal leaflet tips, 21.2% on leaflet tips, and 57.7% on leaflets other than the terminal. In this case, beetles found on terminal leaflets or their tips, only 33.3% were male (Table 3). This pattern is similar to that noted during the day for leaflet tips.

Many beetles were observed flying near the canopy of the tree as the temperature warmed during the morning observations. We captured 30 flying beetles on the second morning of observation; of the beetles caught in flight, 28 were male. The shift to male bias at night may be a result of those actively flying beetles settling on the tree.

Of the 91 total beetles observed on tree trunks during the day, 70 were female (77% of total), while only 21 (23%) were male. No mating pairs were observed forming on trunks, but nine pairs were observed on trunks already in copula. Highest activity on tree trunks, quantified as number of beetles observed per hour, occurred between 1500 and 1800 P.M. EDT during which time a total of 47 female and 16 male beetles were observed on the trunks. This period appeared to be associated with the highest daytime temperatures (greater than 75°F).

Mate-Finding

Feral male EAB flying rapidly around the foliage suddenly hovered 0.3–1.0 m above beetles pinned to leaves and then rapidly and accurately dove onto the backs of the ‘dummy’ beetles. We term this behavior ‘paratrooper copulation’, because of the

Table 3 Position of Feral EAB on Ash Trees After Sunset

	No. of obs.	Percent of total	Cumul. percent
B	3	5	5
L	30	50	55
LT	11	18.3	73.3
P	5	8.3	81.7
TL	8	13.3	95
TLT	3	5	100
Total	60	100	

B Branch; *L* leaflet; *LT* leaflet tip; *TL* terminal leaflet; *TLT* terminal leaflet tip; *P* petiole rapid, often straight descent by males (Fig. 3). Probing movements with the aedeagus typically followed landing by at most a few seconds, especially when the male landed on an unwashed female. 'Dummy' beetles pinned with the elytra closed elicited frequent paratrooper approaches compared to those pinned with wings splayed. 'Dummy' beetles with open wings were very poor elicitors of feral male behavior (Fig. 4).

Equivalent numbers of feral males were observed on the leaflets containing each of the four types of closed-elytra beetles (ANOVA; $F=0.47$, $p=0.7024$) and these leaflets had significantly more males on them than did either leaflets with an empty pin or completely blank leaves (ANOVA; $F=14.65$, $p<0.0001$).

All four types of closed-elytra beetles evoked equivalent numbers of paratrooper copulation attempts (Fig. 5). No paratrooper approaches were observed in response to blank leaflets either with, or without, a pin (Fig. 5). Once the males had completed their approach, there were significant differences in the amount of time they spent in contact with the dead beetles related to their sex and whether or not they had been washed with dichloromethane. We include mounting, copulation attempts, antennation, and remaining in constant contact with the pinned EAB in the broad category of 'contact' by male EAB. Feral males spent significantly more time (ANOVA, $F=50.87$, $p<0.0001$) in contact with unwashed female EAB than with any other type (Fig. 6,

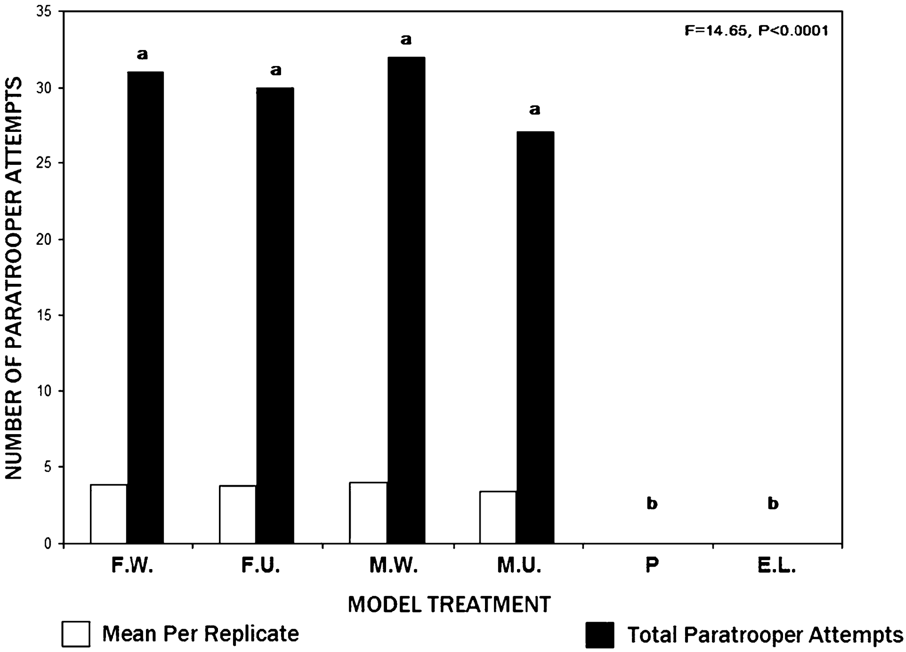

Fig. 5 Mean number of paratrooper copulation attempts observed per replicate (*black bars*) and the total number of paratrooper attempts (*empty bars*) observed in response to the different models during the eight replicates of our experiment. *F.W.* washed female; *F.U.* unwashed female; *M.W.* washed male; *M.U.* unwashed male; *P* leaflet with pin; *E.L.* empty leaflet. Eight replicates of this experiment were conducted, each lasting for 2 h. The number of feral males observed near any of the four types of beetle models is not statistically different (ANOVA, $F=0.47$, $p=0.7024$) but all four of the models had significantly more males than either of the two controls (ANOVA, $F=14.65$, $p<0.0001$).

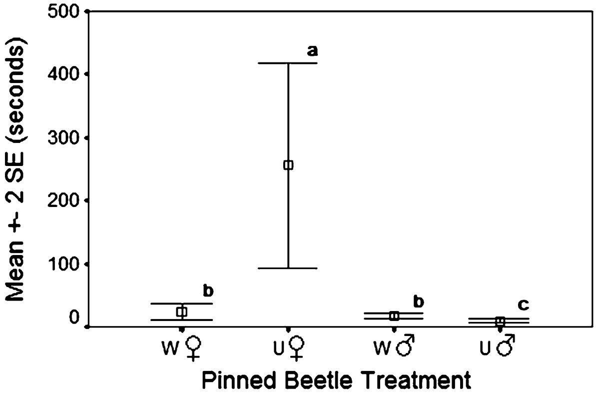

Fig. 6 *Bars* indicate two standard errors about the mean (*squares*). The treatment type of pinned EAB is shown on the *X*-axis, with *W* representing 'washed' beetles and *U* representing 'unwashed' beetles. The time in seconds spent on the pinned beetle is shown on the *Y*-axis. *Bars* having no letters in common are significantly different from one another (ANOVA, $F=50.87$, $p<0.0001$).

Table 4). Also, males spent significantly less time in contact with unwashed male EAB than with any other type. Washed male and washed female beetles evoked equivalent, intermediate durations of contact time by the males (Fig. 6).

EAB Sticky Trapping Using Dummy Beetles

Figure 7 shows the relative captures of feral beetles in response to the five types of traps on leaflets used during summer 2006. During the course of this experiment, only males were captured in response to metallic green ovals or to pinned EAB on leaves. Purple squares and green metallic squares captured both male and female EAB. Some of the trapped beetles managed to crawl short distances through the Tanglefoot layer, and thus we assume that some beetles may have escaped.

Table 4 GLM Analysis of Time Spent on Pinned Beetle Treatments by Male EAB

TRT comparison	Diff. btwn. means	Upp. conf. lim.	Low. conf. lim.	Sig.
FU-FW	1.8515	1.3014	2.4016	***
FU-MW	1.9255	1.3796	2.4714	***
FU-MU	2.7342	2.1644	3.3039	***
FW-FU	−1.8515	−2.4016	−1.3014	***
FW-MW	0.0740	−0.4673	0.6153	
FW-MU	0.8827	0.3172	1.4481	***
MW-FU	−1.9255	−2.4714	−1.3796	***
MW-FW	−0.0740	−0.6153	0.4673	
MW-MU	0.8087	0.2474	1.3700	***
MU-FU	−2.7342	−3.3039	−2.1644	***
MU-FW	−0.8827	−1.4481	−0.3172	***
MU-MW	−0.8087	−1.3700	−0.2474	***

Pinned beetle treatments are as follows: *FW* washed female; *FU* unwashed female; *MW* washed male; *MU* unwashed male. *Asterisks* indicate a significant difference between treatments.

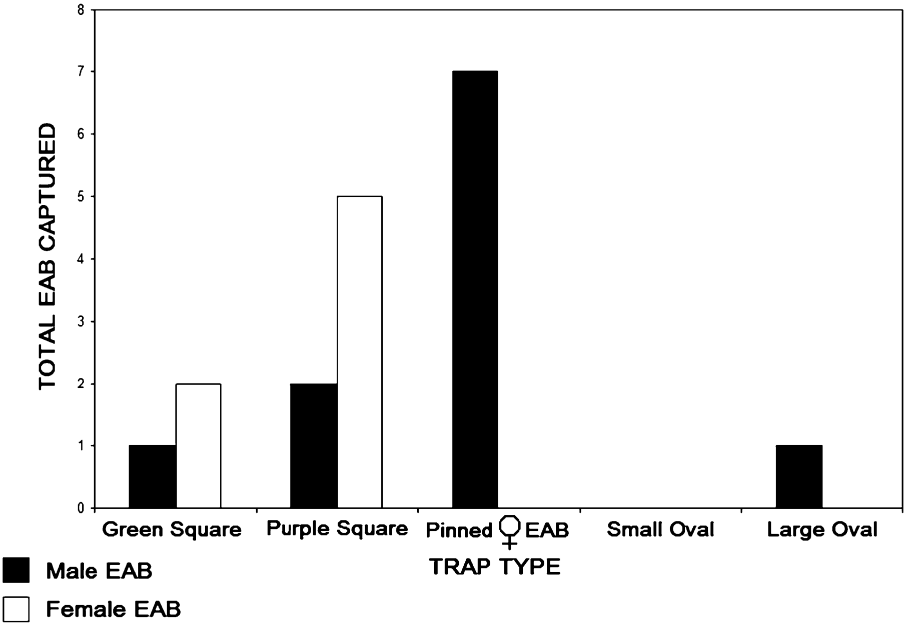

Fig. 7 Number and sex of wild EAB caught on sticky-leaf traps containing different visual cues in June and July of 2006. Trap types used included 2.5 by 2.5 cm metallic green squares, 2.5 by 2.5 cm purple squares, pinned female EAB, small EAB-sized metallic green ovals and large metallic green ovals approximately double the area of an EAB. Note that pinned EAB models attracted only males.

Female Ovipositor Pulsation

Finally, we observed a behavior performed by female EAB in which the ovipositor and its surrounding soft tissue was extended outward at intervals while the female was stationary on a leaf. This soft tissue was extended for between 0.5 and 1 s at a time, interspersed in an apparently random manner by the entire ovipositor being extended as well. Individual females performed this behavior for several to many minutes, with a pulsation of the soft terminal integumental tissue generally continuing throughout this time. Figure 8 depicts a close-up posterior view of a feral female EAB in this posture. No feral EAB adults were observed to approach such females during the greater than 3 h of observation of these EAB females.

Discussion

EAB males appear to seek their mates using visual cues once prospective mates are already present on the host tree. Males initiate approaches equally to all treatments of pinned model beetles having their elytra closed and wings concealed, but the males spend a significantly longer time mounting and attempting to copulate with unwashed female pinned beetles than they do to pinned males, or female washed beetles (Fig. 6). All treatments show significant differences from one another with the exception of washed males as compared to washed females; the lack of

Fig. 8 This image shows a posterior view of the abdominal tip of a feral female EAB performing ovipositor pulsation.

difference here suggests our washing method was effective at removing a contact cue (either antagonist or attractant). It appears that a contact pheromone is involved in copulation and mating after the visual stage of mate-location is finished.

When we washed the dead beetles with dichloromethane, we removed the chemical contact cues that males apparently use to judge whether what they have landed on is a male or a female beetle. The washed models were contacted equivalent amounts of time regardless of their sex. Therefore a contact cue also appears to be involved in male-male repellency, because males that landed on unwashed pinned males spent significantly less time on these beetles than in any of the other treatments (Fig. 6).

Our results indicate that the initial short-range attraction of flying males to sedentary females is visual: males will drop onto and attempt to copulate with male, as well as female, beetles pinned to a leaf. They persist the shortest amount of time when they have contacted an unwashed male beetle and for the longest time when contacting an unwashed female beetle.

In our trapping experiments using visual models pinned to leaves, our preliminary results with a low sample size preclude any statistical analysis. However, if the results hold true at higher sample sizes, the significance for EAB monitoring following this preliminary experiment may be important. Due to the success of sticky traps based on pinned beetles in such a short time during the decline of naturally flying beetles, we suggest that using dead EAB visual lures in this way may be quite effective at trapping male EAB in low-density populations. The specificity of these traps for EAB appears to be higher than the purple traps used currently (i.e. beetle sticky traps caught fewer ants, flies, etc.); we also used purple traps of comparable area to the sticky surface created by leaflets and beetles.

Our observations indicate that males are the more active sex in the canopy, and the observations of others support this (Lance et al. 2006). Therefore, male EAB may be a better target for a trapping program simply because they are highly vagile, fly rapidly around the tree canopies, and are more likely to encounter the 'attractant' visual lure on ash leaflets. It is possible that in this way the EAB mating system could be effectively exploited for monitoring, despite the apparent lack of a long distance sex pheromone. Further, if pinned female EAB were added to the current purple trap this may also enhance this EAB trapping system by visually inducing males to land forcefully on the traps. This concept will be tested during the 2007 EAB flight.

During our observations, an unexpected and striking behavior observed was what we have termed 'ovipositor pulsation'. This behavior was performed only by female EAB, typically while sitting on the epicormic shoots that arise from EAB-damaged ash trees, and was exclusively observed in the afternoon (after 2 P.M. EDT). We note that although this behavior is relatively rarely observed, it is individually persistent. That is, a given female will tend to continue this behavior for many minutes once she has begun to perform it. Despite the superficial resemblance of this behavior to that of a "calling" lepidopteran, we observed no evidence of any attraction of feral male EAB to such females in the many hours of observation that we undertook.

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