

# Novel visual-cue-based sticky traps for monitoring of emerald ash borers, *Agrilus planipennis* (Col., Buprestidae)

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## Abstract

We examined various methods of trapping emerald ash borers (EAB), *Agrilus planipennis* Fairmaire, using solely visual cues based on previous work that has documented the importance of visual cues in EAB mate location. Here, we give the results of two of these methods, coloured sticky cards (yellow or blue), or live ash leaves covered with spray-on adhesive to which dead male EAB visual lures had been pinned. Feral male beetles were captured effectively on the sticky traps made from dead male EAB on ash leaves. These sticky-leaf-traps captured more male EAB when deployed in high-population density areas than low-density areas, but did capture EAB even at lower population densities. More feral males were captured on these traps when they were placed higher in the trees, regardless of the population density of EAB. Very few feral female EAB were captured using the sticky-leaf-traps. This novel method of EAB trapping may allow 'real-time' population detection and monitoring of EAB adults during the active flight period rather than locating larval galleries during the autumn and winter after adult flight and attack. Feral male beetles were also captured using standard yellow- or blue-coloured sticky cards to which male EAB had been affixed with adhesive; however, this type of trap was much less effective overall than using the sticky-leaf-traps. Furthermore, *Agrilus cyanescens*, a species similar in colour to EAB but smaller in size, showed a strong response to blue-coloured sticky traps to which dead male EAB had been affixed with adhesive, suggesting a general use of visual cues in the mating systems of some of the other Buprestidae as well.

## Introduction

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Col., Buprestidae), an invasive pest currently threatening the complete destruction of North America's ash resource, was first detected in the Midwestern United States and adjacent Canada in 2002 (Haack et al. 2002). Effective detection and monitoring of this pest has so far remained elusive (Poland and McCullough 2006). Identifying a reliable species-specific lure has proved to be relatively difficult thus far, despite the elucidation of antennally

active plant volatiles (Crook et al. 2005, 2006, 2007; Poland et al. 2005, 2006; Rodriguez-Saona et al. 2006), as well as a sex-specific macrolide produced by female beetles (Bartelt et al. 2007). The USDA Animal and Plant Health Inspection Service (APHIS) has created purple sticky traps that are still under research and development (Francese et al. 2005a, 2007; McCullough et al. 2006; Poland et al. 2006; Poland and McCullough 2007), but these are generally effective at detecting EAB adults only at high population densities. Girdling ash trees and stripping the bark to reveal larvae during the following

autumn and winter remains the most reliable method of EAB detection, especially at low population densities (Cappaert et al. 2005; Poland et al. 2005; Fraser and Mastro 2007; Poland and McCullough 2007). One of the key shortcomings of relying on the girdled-tree method is that EAB detection is delayed until eggs have hatched and larvae have developed until the point that their galleries are evident. Detection is also delayed by the necessarily time-consuming cutting and stripping of the logs cut from girdled trees. Detecting EAB infestations by the girdled-tree method therefore results in reliable, but delayed and costly, detection (Cappaert et al. 2005). Current efforts are also underway to determine the effects of ash tree densities and EAB population densities on the efficacy of trap types across the entire area of the United States that is under APHIS-mandated quarantine (Metzger et al. 2007).

Identification of the expanding 'edge' of a given infestation is critical to the subsequent removal of infested trees in an effort to prevent further spread of EAB. Early studies of outlier sites suggested that EAB could infest trees at least 600 m from a point-source (McCullough et al. 2005). Sawyer (2007) described an incident in which a tree containing EAB in Maryland was left standing just outside the estimated edge of an infestation (in this case, trees had been removed out to 800 m from a point-source focus). It was originally declared that EAB had been eliminated from the area, but the infested tree and several others nearby were subsequently discovered, and this omission subsequently resulted in re-quarantine of the area. Based on population simulations, careful monitoring for EAB must continue for several years to ensure eradication (Sawyer 2007).

We have previously shown that male EAB locate conspecifics predominantly using visual cues once the beetles are near or on a host tree (Lelito et al. 2007). The role of visual cues has also been investigated via changes in trap colour (Otis et al. 2005) and silhouette (Francese et al. 2007; Poland and McCullough 2007). Our previous work had also described preliminary tests of the use of these visual mate-finding cues in order to create a selective and sensitive trap for EAB in the wild (Lelito et al. 2007). The goal of the present study was to explore the utilization of visual mate-location behaviour of male EAB in capturing beetles at both high and low population densities. With potentially improved visual-cue-based methods of trapping, we sought to monitor EAB infestations in 'real-time', i.e. detecting invading active EAB adults rather than their progeny, or evidence

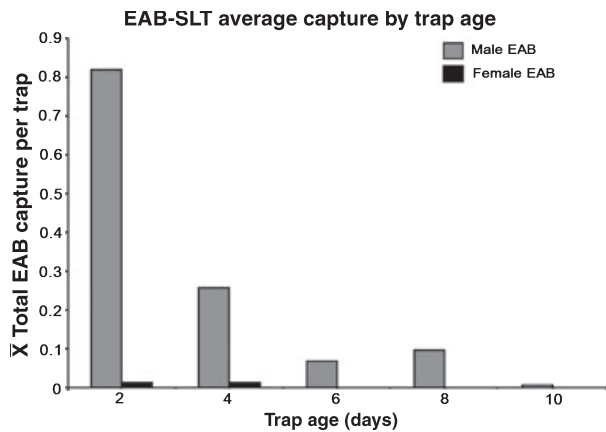
thereof in the form of galleries, the following autumn and winter.

## Methods

### EAB sticky-leaf trapping

Our low density EAB population density sites were to the west and south-west of the town of South Lyon, in Oakland County, Michigan. No survey traps were present in this area during the course of the study; however, this area is characterized by nearly complete ash mortality, with mature trees having died at least two seasons previously and thus unlikely to support a high density of EAB. The only live ash remaining consisted of the epicormic shoots arising from dead stumps. We also used two high-population density EAB populations: one to the south and another to the north-west of the town of Howell, both in Livingston County, Michigan. The high density sites were characterized by live ash trees in a state of moderate to severe dieback from borer damage. Unbaited purple survey traps were present at both high density sites, and captured a mean total ( $\pm$ SE) of  $243.67 \pm 108.43$  EAB north-west of Howell, and a mean total of  $118.00 \pm 60.19$  south of Howell. All four of our sites were dominated by green ash, *Fraxinus pennsylvanica*, but white ash (*Fraxinus americana*) was found at both high density sites as well. A small number of the trees at the South Lyon sites were blue ash (*Fraxinus quadrangulata*).

Dead EAB males were pinned to the terminal leaflets of ash leaves (one EAB per leaflet) in the field, and then covered with Spray-On Tangle-Trap (The Tanglefoot Company, Grand Rapids, MI). We hereafter will refer to this trap type as an EAB-Sticky-Leaf-Trap (EAB-SLT). We also placed control traps, which consisted of a terminal ash leaflet sprayed with Tangle-Trap, without a pinned EAB. Our experimental configuration was as follows: two sites at each EAB population density, each of which contained six ash trees, at least 10 m apart and of at least 10 cm diameter at breast height, onto which EAB-LSTs were deployed. On each tree, we placed three EAB-LSTs and three control traps at each of two heights in the tree (2 and 4 m from the ground), for a total of 72 EAB-LSTs at each population density. All of the EAB-SLT deployments were carried out over the course of a single day (16 June 2007). Traps were subsequently monitored every 2 days after placement. If EAB males were found stuck to traps they were counted and then removed with forceps, bagged by trap type, and then returned to the



**Fig. 1** Mean per-trap capture of feral emerald ash borers (EAB) on EAB Sticky-Leaf-Traps (LSTs) pooled across all trap treatments at our four field sites in south-east Michigan during June and July 2007.

laboratory where they were frozen. These captured EAB were then washed in Histo-Clear (HS-200; National Diagnostics, Atlanta, GA), and their sex was determined under a binocular microscope. Other buprestids were also found on the traps, and these were also collected and returned to the laboratory where they were identified and tallied, but not sexed. By the eighth day after placement, many traps showed serious deterioration because of phytotoxicity from the propellants in the Tanglefoot application and/or the blockage of leaf respiration. Trap catch declined rapidly as traps aged (fig. 1); therefore, on the 12th day after deployment, traps were examined for beetles, none were found, and all the remaining traps were removed.

#### Coloured-card sticky trapping

Blue- plus yellow-coloured sticky cards (10 by 25 cm) were purchased from Hummert International (Earth City, MN Cat. Nos 0136051 and 0136001). Two experimental treatments and one set of controls (Blue-Control, 'B-C' and Yellow-Control, 'Y-C' traps) were prepared from these base sticky cards. Experimental traps consisted of sticky cards with one vertical column of four dead adult male EAB [Blue 1-Column ('B-1') and Yellow 1-Column ('Y-1') traps] evenly spaced down the centreline of the card or two vertical columns of four dead adult male EAB [Blue 2-Column ('B-2') and Yellow 2-Column ('Y-2') traps] evenly spaced down the length of the card. Dead male EAB were attached to the trap by gentle pressure against the adhesive. A total of 24 cards of each treatment (colour  $\times$  number of beetles affixed)

were prepared. These sticky cards were hung from outer branches of ash trees at 4 m height in the tree, at a site of high EAB population density south of the town of Howell, Michigan. They were allowed to rotate freely, such that the side of the trap with beetles on it was not bound in a certain position. Traps were placed on 31 May 2007, and monitored every 4 days through July 2 2007. Any EAB and other buprestids found stuck to the traps were removed, returned to the laboratory and frozen. As above, EAB were then sexed, while non-EAB buprestids were not. In a manner similar to EAB-LSTs, trap catch declined with trap age, and in this case it was most likely due to the accumulation of dust, debris and dead insects on the cards.

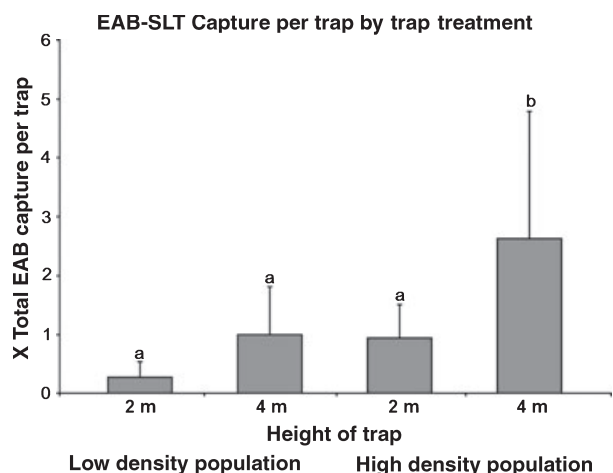
#### Statistical analyses

Mean capture, per 48-h period, of feral EAB and other buprestids caught on EAB-LSTs was log-transformed to achieve normality. The transformed data were then compared by height and EAB population density treatments, as well as by an interaction effect between these two factors, using PROC GLM, SAS 9.1.3 (SAS Institute 2006), and including Tukey's Studentized Range Test for multiple comparisons. We used a CONTRAST statement within PROC GLM to perform an orthogonal contrast of the number of EAB males captured on those coloured cards without EAB affixed to those with EAB affixed. Trap capture data for each 48-h period, of each species of buprestid sampled on coloured-card traps, were log-transformed to achieve normality and compared by trap colour and beetle-lure treatments, including Tukey's Studentized Range Test, using PROC GLM, SAS 9.1.3 (SAS Institute 2006).

## Results

#### EAB sticky-leaf trapping

The EAB-SLTs placed high in the tree at high density sites outperformed the other three experimental treatments overall (ANOVA,  $F = 10.18$ ,  $P < 0.0001$ ). Control traps caught no beetles at low EAB population density, and a total of only three female and one male EAB at high EAB population density. Both height in the tree (ANOVA,  $F = 14.56$ ,  $P = 0.0005$ ) and EAB population density (ANOVA,  $F = 13.31$ ,  $P = 0.0008$ ) had significant effects on experimental trap success (fig. 2). At high EAB population densities, 2.64 beetles were captured on average per EAB-SLT when the trap was placed at 4 m height, and

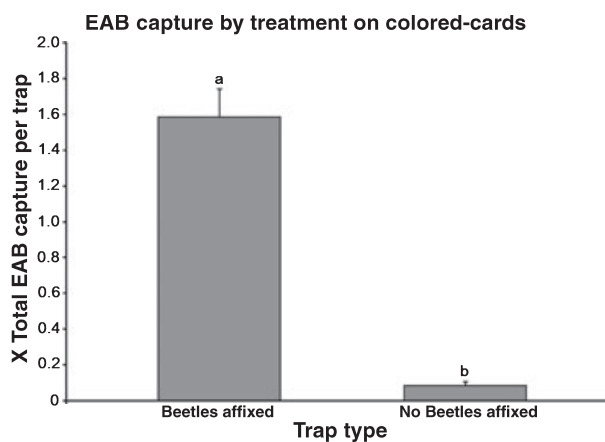


**Fig. 2** Mean total capture per trap of feral male emerald ash borers (EAB), per EAB Sticky-Leaf-Trap (SLT), by treatment. Significantly more feral male EAB were captured on 4 m high traps at high EAB population density sites (ANOVA,  $F = 10.18$ ,  $P < 0.0001$ ). Means having no letters in common are significantly different according to Fisher's LSD ( $P < 0.05$ ). Bars represent one standard error about the mean.

0.94 beetles were captured on average per trap when the trap was placed at 2 m height. At low EAB population densities, an average of one beetle was captured per trap at 4 m height in the ash tree, and an average of 0.28 beetles were captured per trap when the trap was placed at 2 m height. Individuals of three non-target buprestid species were captured on these traps as well, almost always in close proximity to the dead EAB pinned to the leaf, as was also typical of the male EAB stuck to the EAB-SLT. Seventy-two individuals of *Agrilus cyanescens* Ratzeburg were captured in this manner on EAB-SLTs. The height of the trap in the tree significantly influenced trap catch of *A. cyanescens*, with more individuals being caught at 4 m height than at 2 m height (ANOVA,  $F = 15.80$ ,  $P = 0.0003$ ). EAB population density had no effect on the capture rate of *A. cyanescens* (ANOVA,  $F = 1.76$ ,  $P = 0.1935$ ). Forty-eight individuals of *Agrilus subcinctus* Gory were found on EAB-SLTs in total and overall there were significant differences in *A. subcinctus* capture between treatments (ANOVA,  $F = 9.85$ ,  $P < 0.0001$ ). Significantly more *A. subcinctus* were captured on the higher traps in the tree (ANOVA,  $F = 21.76$ ,  $P < 0.0001$ ), and at the higher EAB population density (ANOVA,  $F = 6.72$ ,  $P = 0.0137$ ).

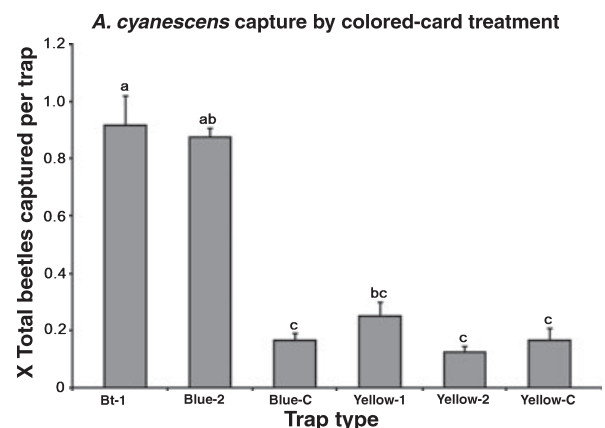
**Coloured-card sticky trapping**

In total, 40 feral EAB males were captured on coloured card traps (fig. 3). Male EAB did not show a preference for trap colour (ANOVA,  $F = 0.00$ ,



**Fig. 3** Mean total capture, per coloured sticky trap, of feral male emerald ash borers (EAB) by trap treatment. More feral male EAB were captured on coloured sticky traps to which male EAB had been affixed than on traps without affixed EAB according to an orthogonal contrast (d.f. = 2,  $F = 4.80$ ,  $P = 0.0198$ ). Bars represent one standard error about the mean.

$P = 1.00$ ), but seemed to be influenced within a given colour treatment by the presence of affixed conspecifics (d.f. = 2,  $F = 4.80$ ,  $P = 0.0198$ ). An average of 0.38 male EAB were captured on each B-1 trap. The average capture of male EAB on other trap treatments was 0.42 per B-2 trap, 0.04 per B-C trap, 0.33 per Y-1 trap, 0.46 per Y-2 trap and 0.04 per Y-C trap. Eleven female EAB were also captured in this experiment, but these did not show a preference



**Fig. 4** Mean number of *Agrilus cyanescens* captured, in total per trap, on coloured sticky traps by treatment. All traps were hung at 4 m height. Significantly more *A. cyanescens* were captured on B-1 traps than all other treatments. B-2 and Y-1 traps captured significantly more *A. cyanescens* than the remaining treatments (ANOVA,  $F = 3.13$ ,  $P = 0.0330$ ). Means having no letters in common are significantly different according to Fisher's LSD test ( $P < 0.05$ ). Bars represent one standard error about the mean.

for the colour of the sticky-card or the presence of dead male EAB affixed to the trap (adjusted  $\chi^2$ , d.f. = 1,  $P = 0.55$ ).

Greater numbers of both *A. subcinctus* and *A. cyaneescens* were captured on the coloured card traps than were EAB. Two-hundred and fifty-one *A. subcinctus* were captured in total, but neither the trap colour nor the presence of affixed EAB on the trap influenced the trap catch (ANOVA,  $F = 1.53$ ,  $P = 0.2298$ ). Sixty *A. cyaneescens* in total were collected from coloured card traps, with significantly more *A. cyaneescens* captured on B-1 traps than on all other treatments (fig. 4; ANOVA,  $F = 3.13$ ,  $P = 0.0330$ ). B-2 and Y-1 traps caught more *A. cyaneescens* than Y-2, B-C and Y-C traps (fig. 4; ANOVA,  $F = 3.13$ ,  $P = 0.0330$ ).

## Discussion

The attraction of feral male EAB to conspecifics on adhesive surfaces for trapping purposes had previously been demonstrated on a limited basis (Lelito et al. 2007) and we now confirm these results on a broader scale. If we could produce a longer period of trapping effectiveness (i.e. extending the period before leaf decay or dust accumulation), it is likely that more EAB could be captured and trap efficacy at low EAB population densities could be increased. We are currently exploring alternative adhesives that are designed to reduce dust accumulation and still retain trapping efficacy for EAB. Testing these improved traps will take place during the 2008 EAB flight.

In terms of surface area, coloured cards were a much less efficient trapping method than EAB-SLTs, considering that 183 EAB were captured on approximately 1.11 m<sup>2</sup> (1728 in.<sup>2</sup>) of sticky surface for EAB-SLTs, and 51 EAB were captured on 7.43 m<sup>2</sup> (11 520 in.<sup>2</sup>) of coloured card traps, given here by generously estimating each terminal ash leaflet as 0.1016 m × 0.0762 m (4 in. × 3 in.). This amounts to one EAB captured for every 0.006 m<sup>2</sup> (9.44 in.<sup>2</sup>) of EAB-SLT surface area, and one EAB captured for every 0.146 m<sup>2</sup> (225.8 in.<sup>2</sup>) of coloured card surface area, even when females are included in the total coloured-card trap capture but not included in the total EAB-SLT trap capture. EAB-SLTs also outperform coloured cards on a per-trap basis even at high EAB population densities at 4 m height. EAB-SLTs captured an average of 2.64 EAB per trap, more than a fivefold increase over Y-2 traps, the most effective coloured card treatment (with an average capture of 0.46 EAB per trap).

Species specificity of trap capture was also greater for EAB-SLTs than for coloured traps: EAB

represented 44.4% of the total number of buprestids captured on EAB-SLTs vs. 13.7% of buprestids captured on coloured card traps, when counting both male and female EAB captured on each type of trap. This may be critical, as the capture of similarly coloured non-EAB buprestids such as *A. cyaneescens* on traps may lead to false-positive EAB detection. However, our results suggest that due to the attractive nature of objects resembling conspecifics to the buprestids studied here, some cross-attraction is likely to result from any trap design incorporating a visual lure. In addition to buprestids, a variety of other insects were captured on our traps. The most numerous of these were Diptera, followed by Coleoptera, Hemiptera and Hymenoptera. We did not pursue the identification of these insects beyond the level of order.

Sex specificity of trap capture was also greater for EAB-SLTs: 97.8% of EAB captured on EAB-SLTs were male compared with 78.4% of EAB on coloured card traps. Although a more male-specific trap may not be useful for slowing the spread of this species, this approach does represent an increase in trap specificity over standard coloured card traps. Also, the greater apparent vagility of males (Lelito et al. 2007) makes them a better target for detection of spreading populations, and the EAB-SLTs are well suited for this effort.

Other researchers have used coloured sticky traps (usually purple) when testing potential volatile lures for EAB (Francesca et al. 2005b, 2007; Crook et al. 2006; Poland et al. 2006) and our data suggest such coloured card approaches may predispose these efforts to low rates of EAB capture. In fact, purple panel traps had been originally conjectured to be inadequately attractive to EAB (Otis et al. 2005). Our experiments suggest that finding the most efficient method of trapping EAB may first hinge on designing a trap that can catch EAB at low population densities even without volatile lures. Based on our current results, EAB-SLTs appear to be a useful initial design around which to improve this type of synthetic trap.

Our data provide support for the possibility that EAB might be able to be successfully monitored using a hand-deployable, low-cost, and mass-produced 'visual-lure' trap. The success of these traps may possibly be further enhanced by the emission of ash volatiles, specifically those found in ash bark or foliage already shown to increase the capture of EAB on purple traps (Crook et al. 2006; Poland et al. 2006; Poland and McCullough 2007). However, work by others suggests that even traps baited with

plant compounds in this manner cannot outperform girdled trees as detection agents for EAB (Anulewicz et al. 2007).

Our current results also agree with those of others who have noted increased EAB activity (Lelito et al. 2007) and greater trap catch (Fraser et al. 2006; Lance et al. 2007) higher in ash trees. Despite this, it remains a possibility that we can capture EAB adults with EAB-SLTs for monitoring purposes, even at low heights on ash trees.

The capture of *A. cyanescens* on coloured card traps with EAB affixed suggests that objects similar in colour and size to a conspecific can act as a functional visual lure for buprestids, including EAB. This also agrees with previous work regarding male buprestid beetles and their attraction to objects by visual means (Gwynne and Rentz 1983). The implications of these findings are clear: a species- or sex-specific trap for EAB will likely require further research and outside-the-box thinking, beyond merely adding plant volatiles to an arbitrarily coloured sticky card. Our future experiments will aim at examining the effects of visual lures on EAB, and perhaps other buprestids as well, in the context of providing a useful detection tool for monitoring expanding populations of EAB.

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