

Detecting emerald ash borers (*Agrilus planipennis*) using branch traps baited with 3D-printed beetle decoys

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Abstract Small visual-decoy-baited traps for the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), have been researched as an alternative to current technologies, but have relied on real beetles rather than synthetic materials. We hypothesized that visual decoys created by three-dimensional (3D) printing can provide such a substitute. Branch traps displaying decoys consisting of real EAB females or 3D-printed decoys were compared to controls without decoys. Traps of the three varieties were placed on neighboring branches along with one (*Z*)-3-hexen-1-ol lure per tree and checked daily. Both real and 3D-printed decoys similarly increased EAB trap captures compared to controls. The numbers of both sexes were higher on the decoy-baited traps, but the increase in male captures was more pronounced. Males were also ensnared closer to the decoys than females. Daily trap–capture patterns showed sparse activity of EAB adults before June 18, 2013 followed by a peak in captures of both

males and females until June 28, 2013. Beginning at approximately July 1, 2013, there was a second peak of EAB captures, which consisted almost entirely of males caught on the decoy-baited traps. The native ash borer *Agrilus subcinctus* was found earlier in the season and was also significantly attracted to both the real EABs and the 3D-printed decoys compared with control traps. Four purple prism traps were also deployed concurrently and captures tallied on three different days within the season. The results demonstrate efficacy of a small, inexpensive, and fully synthetic decoy-based branch trap system for EAB.

Keywords 3D printing · Visual decoy · Forest pest · Invasive species · Visual attraction

Introduction

The recent proliferation of three-dimensional (3D) printing presents a tool that has already been used to mimic a vast array of biological structures. The potential for synthetic structures to affect the behavior of living organisms has been demonstrated for bacteria that are sensitive to the microstructured profiles of their environments (Connell et al. 2013). Furthermore, an even greater body of work has been dedicated to using fabricated 3D structures to influence intra-organismal cellular environments, with widespread relevance for biomedical applications involving tissue and organ growth and regeneration, as reviewed by Gagg et al. (2013). At the same time, applications for the manufacture of biomimetic structures for influencing the behavior of multicellular organisms such as insects have rarely been explored, with most examples involving macro-scale constructs such as artificial harborage for nesting insects (Lye et al. 2011; Gazal et al. 2014; Enríquez et al.

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2013) or replicas of host-fruit shapes and colors (Prokopy and Owens 1983). For insects, semiochemical-influenced behavior is usually the most powerfully exploitable sensory modality. Thus, for several decades the emphasis for applied research has been the identification of pheromones and host attractants that can be used to direct insect behaviors toward and into trapping devices. In many cases, pheromones have been synthesized that can lead to the trapping of hundreds to thousands of insects when unbaited control traps catch few to none of the same species (Cardé et al. 1997, Moser and Browne 1978; Roelofs et al. 1977).

Ecological and evolutionary circumstances have occasionally led to the emergence of invasive insect pests of grave concern that do not have such pronounced exploitable sex-pheromone communication or host volatile-based attraction systems. One such example of a species whose semiochemical interactions are difficult to exploit is the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). The EAB is an exotic buprestid beetle from Asia that has been causing severe mortality of North American ash (*Fraxinus*) trees during the last decade (Haack et al. 2002). The biology of the insect is typical of tree-feeding buprestids and is described in detail elsewhere (Yu 1992; Cappaert et al. 2005). EABs, like many tree-feeding buprestids, emerge and disperse as adults early in the summer, with peak flight observed in late June to early July in most of the temperate areas of North America where it has been introduced. Unlike many other bark-feeding insects such as scolytid beetles, which burrow into the bark as adults, buprestids spend their entire adult lives outside their hosts. During the adult dispersive stage in early summer, adults can be seen feeding and mating on ash leaves, while mated females begin laying their eggs under the loose bark or bark crevices of susceptible host trees. The larvae feed on the inner bark until the fall season, and they will overwinter there as prepupae in places where there is a 1 year life cycle. Buprestids are also known for their intense metallic coloration, which has at times been associated with mating-related attraction. For example, it was once noted that males of one species of Australian buprestid, *Julodimorpha saundersii*, were attracted to and would actively try to copulate with the glass surfaces of highly reflective beer bottles of similar color (Gwynn and Rentz 1983).

A more thorough examination of potential visual and chemical attraction mechanisms for EAB began in the years following this beetle's recent American outbreak, with many tangible benefits for trapping outcomes. Color-based attraction has most commonly been employed in the form of large green- or purple-colored "prism traps" forming a three-sided triangular structure (Francese et al. 2005, 2008). More recently, green-colored funnel traps have been shown to also be effective at capturing EAB

(Francese et al. 2011). The capture rates with such traps have been successfully augmented by the use of host or host-related semiochemicals. Bark extracts such as Manuka oil and Phoebe oil, whose many volatiles contain some components that are the same as those found in ash bark, increase trap capture of EAB adults (Crook et al. 2008). The general green-leaf volatile (*Z*)-3-hexen-1-ol that is omnipresent in green plant volatile emissions is equally as good at increasing EAB trap capture (deGroot et al. 2008; Grant et al. 2010, 2011). One beetle-produced compound, a lactone, (*Z*)-3-dodecen-12-olide (Bartelt et al. 2007), has been shown to increase the attraction toward green prism traps when co-emitted with (*Z*)-3-hexen-1-ol (Silk et al. 2011; Ryall et al. 2012), but is not likely to be a long-range attractant. Thus, the ability to trap and detect EAB at low population densities has almost certainly been hampered by the lack of a long-range pheromone that actively attracts beetles to the traps.

In addition to the progress made to maximize local attraction to traps based on semiochemical and non-specific visual cues, there have also been attempts to exploit the short-range visual mate-attraction behavior that tree-dwelling buprestids typically employ. Male EAB has been observed flying directly onto females resting on the foliage from up to 1 m away (Lelito et al. 2007). Similar behavior has been observed on a variety of hosts for other *Agrilus* species, including *Agrilus cyanescens*, *A. subcinctus*, *A. bugutatus*, *A. sulcicollis*, and *A. angustulus* (Lelito et al. 2011; Domingue et al. 2011). Although this behavior occurs at a relatively short distance, it has provided an impetus over the past several years for developing and optimizing a visual-EAB-decoy-based trap that is small and easy to deploy in large numbers (Lelito et al. 2008; Domingue et al. 2013a, b).

Initial attempts at decoy-based trapping involved a simple "sticky-leaf trap" consisting of a dead EAB pinned to an ash leaflet, both of which were then coated with Tanglefoot (Lelito et al. 2008). In such a trap, decoy beetles significantly increased the capture of male EAB on the sticky-leaf surfaces in comparison to blank sticky leaves. Furthermore, when dispensers containing either Manuka oil or Phoebe oil were hung in the trees containing these traps, the proportion of small sticky-leaf visual-decoy traps detecting the presence of at least one EAB was not significantly different from that of the much larger prism traps (Domingue et al. 2013a). Despite the detection efficacy of such traps, the use of natural-leaf surfaces is not realistic for widespread and sustained trapping efforts. The Tanglefoot glue causes leaf necrosis, and also darkens the color of the dead EAB decoys. The EAB decoys commonly need to be replaced throughout the season as the body parts can gradually separate from each other and sometimes even fall from the trap. Furthermore, the use of real EAB decoys

requires the ability to obtain sufficient numbers of specimens for trap deployment.

In previous studies there have been efforts to substitute green plastic cards for real leaves, while still utilizing real beetle decoys in traps targeting either EAB (Domingue et al. 2013a) or European oak buprestids (Domingue et al. 2013b). In both instances the visual decoy significantly increased the attraction and detection of targeted buprestid species. For EAB trapping, decoy-baited sticky plastic cards, though effective, did not perform as well as sticky-leaf traps. However, for the European buprestids, the sticky cards performed significantly better. These experiments were not directly comparable because the study targeting oak buprestids was performed a few years later and employed several improvements to the trap design including a way to easily fasten the traps to head-level sunlit branches, which has led to the use of the term “branch trap”.

For the current study, we developed and tested the efficacy of a small branch trap made of fully synthetic materials that could be mass-produced at a low cost and deployed in large numbers wherever the presence of EAB might be a concern. We manufactured plastic beetle replicas using a rapid-prototyping system that allows 3D printing of polymer objects. These decoys were affixed to small green branch traps and compared to traps baited with dead EAB decoys as well as to blank control traps. The traps were deployed within a heavily infested 35-year-old plantation in central Pennsylvania. To further our understanding of the behavioral attributes of EAB exploited by these traps, they were checked daily to assess the activities of both males and females. Purple prism traps were also deployed at the site to compare the within-season variation in captures using the two trap designs, as well as any difference in sex-specific attraction. By demonstrating that synthetic decoys can increase trap captures on small economical branch traps, we hope to immediately provide a new detection tool that is worthy of being further investigated to determine its efficacy in comparison to other traps. Apart from any potential applications of the branch-trapping system, the creation of biologically active visual decoys may also provide the impetus for the development of other novel buprestid-detection technologies.

Materials and methods

Field site

Traps were deployed on May 28, 2013 at a site on the Pennsylvania State University campus in University Park, PA, (40°48'40"N, 77°50'41"W, 318 m altitude). The site is occupied nearly entirely by green ash (*Fraxinus*

pennsylvanica), uniformly planted in 1978 as a replicated test of population progenies representing nearly the entire natural distribution of the species (Steiner et al. 1988). As such, the plantation is assumed to contain a representative sample of the entire genome of the species. The trees are arranged in rows and columns at a spacing of 3.7 m. There were originally more than 2,000 trees on this site, with mortality at 10–15 % at the time of the experiment. The trees are arranged such that there are usually 20 rows planted on an uphill gradient with a 15 m elevation rise, moving from north to south along most of the site. There are two sections of this plantation separated by a 20 m wide grassy field, with approximately 60 % of the trees to the west of this dividing point. During the 2013 trapping experiments the eastern end of this site was exhibiting very strong symptoms of tree decline due to EAB infestation, with nearly all trees exhibiting crown dieback, epicormic sprouting, or mortality. Buprestid exit holes were commonly observed. These symptoms were less frequent in the western versus the eastern end of the plantation. Surveys of exit holes visible between 1.25 and 1.75 m on the trunks of the trees were performed in July 2012 and June 2013, showing greater EAB activity on the eastern side of the plantation (Table 1). The entire plantation is primarily surrounded by land used for residential and agricultural purposes, as well as a power plant and stormwater catchment basin.

Decoys

All dead EABs used as decoys originated from a colony at the USDA-APHIS EAB rearing facility in Brighton, MI, USA, which is maintained in *F. pennsylvanica* logs for larval feeding and leaves for continual adult beetle feeding. Females were collected from the colony at approximately 30 days before they were to be deployed in the field and were killed by freezing for 48 h. They were then shipped to University Park, PA, USA, where they were pinned through the prothorax in preparation for deployment on traps.

To fabricate synthetic decoys, first the profile of a sitting EAB female with its elytra folded naturally was drawn and measured as it appears when viewed from the side (sagittal profile). The dorsal view of the manufactured beetle is provided in Fig. 1a, while the outline of the sagittal profile used to construct the blueprint for 3D printing is shown in Fig. 1b. Features that were captured in the profile were the overall length, shape of the elytral region, and a slight dip between the female's head and the elytral region. Next, the sagittal profile was then rotated through 180° to produce a 3D model which roughly matched the shape and size of the EAB female. The 3D model was uploaded into the control software for a Dimension 1200es-SST (Stratasys, Edina,

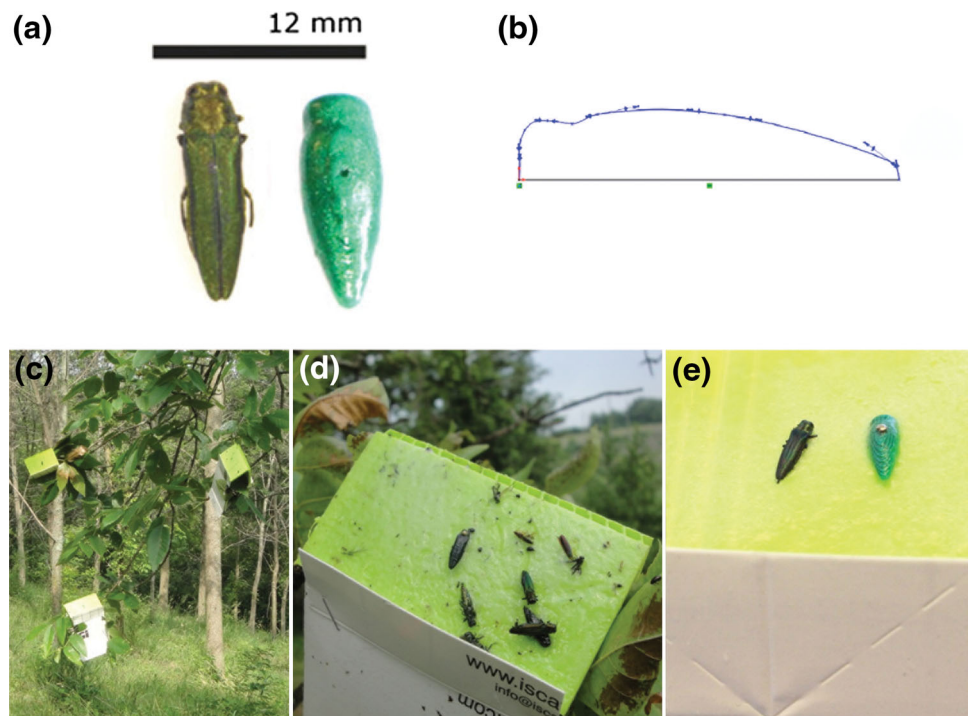
Table 1 Comparison of western and eastern sections of the ash plantation with respect to exit holes at 1.25–1.75 m and EAB captures on branch traps

Section*	Exit holes				EAB/trap/day ^a	
	All trees		Trees with branch traps		Sex	
	2012	2013	2012	2013	Male	Female
East	0.55 ± 0.08	4.60 ± 0.24	0.19 ± 0.14	4.64 ± 0.23	0.091 ± 0.085	0.023 ± 0.005
West	0.069 ± 0.017	0.75 ± 0.08	0	0.20 ± 0.20	0.074 ± 0.014	0.011 ± 0.005

^a Statistical analyses were performed using a cumulative logit model and considering decoy type and plantation section as factors. Plantation section was not a significant factor at $\alpha = 0.05$ for either sex. Decoy type was significant at this level, but details are not shown here (see Table 2)

* The exit hole sample sizes were 1,088 trees in the eastern section and 695 in the western section in 2012, and respectively 1,116 and 714 trees in those sections in 2013. There were 17 trees with branch traps in the eastern plot and 5 in the western one

Fig. 1 Branch traps for emerald ash borers: (a) a dead-female-EAB decoy (left) and a 3D-printed decoy (right); (b) sagittal profile of EAB used to construct 3D-printed decoy; (c) a typical block-replicate of branch traps comprising a three-treatment triad of a blank trap (upper right), a dead-female-EAB decoy trap (upper left), and a 3D-printed decoy trap (below-middle) with a Z3-6:OH packet at far right; (d) closer view of a dead-female-EAB decoy (red arrow) on a branch trap surface with several ensnared EABs; (e) closer view of a 3D-printed decoy trap with the synthetic decoy and a real male next to it. (Color figure online)



MN, USA) 3D printer for additive manufacturing. In this process, the model was arrayed to allow the printing of 300 decoys of white acrylonitrile butadiene styrene (ABS). Each decoy was printed as 11 discrete layers, which averaged 0.254 mm in thickness. For one-half of the decoys produced by the additive manufacturing equipment, the resulting visibly stepped structure was smoothed prior to being painted by hand-rubbing each decoy with acetone, which is a solvent for ABS. All synthetic decoys were then painted with Testor's Mystic Emerald spray paint (Testor Corp., Rockford, IL, USA). The paint selected has a reflection spectrum with a peak near 520 nm, just like a real emerald ash borer, but does not reflect light in the near-infrared region of the spectrum (700–800 nm), as ash leaves do (Fig. 2). While it is not known if the beetles

detect and are behaviorally affected by light at these higher wavelengths, the most realistic representation possible of the color of EAB was desired. An awl was used to pierce a hole through the decoy at approximately the corresponding prothoracic position used for pinning the real beetles. This hole was then used for pinning the fabricated decoys to the traps (Fig. 1a).

Branch traps

The branch traps were built from halved, inverted white delta traps (ISCA Technologies; Fig. 1c–e). The traps had two $5 \times 9 \text{ cm}^2$ surfaces that were covered by green corrugated plastic cards of the same size. A highly reflective green plastic material with a peak reflectance at

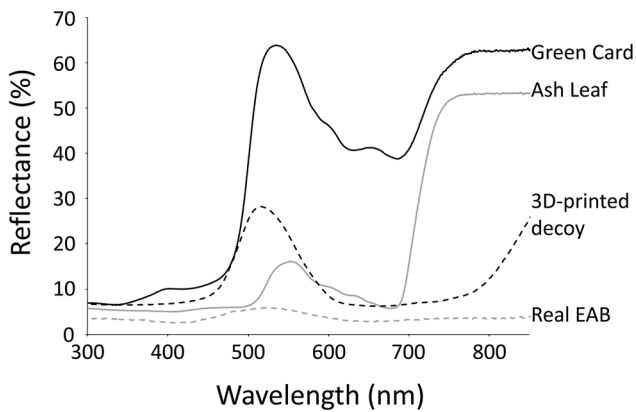


Fig. 2 Reflectance spectra of real EABs and the ash leaves they normally feed and mate upon compared to synthetic decoys and plastic surfaces for branch traps. The spectrophotometer used was a Perkin–Elmer Lambda 950 with a 150-mm integrating sphere equipped with a micro-focus lens and mechanical iris with a minimum beam size of 2 mm, meaning the elytra of the EAB at 1 mm was smaller than the beam, while the other samples were fully illuminated

535–540 nm was used (Crook et al. 2009; Fig. 2). The material was obtained from ChemTica Internacional (Heredia, Costa Rica). The traps were placed around leaf clusters and fastened with clips using the leaves and/or their twigs such that the green surfaces were oriented toward the sun at approximately 45°. Descending from each surface and wrapping around the branch were two $9 \times 13 \text{ cm}^2$ white cardboard pieces. Traps were always placed on the south-facing tree branches that were adjacent to openings that allowed the direct rays of sunlight to strike the top surfaces of the traps. Traps were placed within the reach of the personnel deploying them and thus tended to be 2.0–2.5 m from the ground. A lightweight portable stool was sometimes used to facilitate trap checking.

After fastening traps onto the tree branches, decoys were added. Either a dead female *A. planipennis* or a 3D-printed decoy was pinned to the center of each of the two green trap surfaces as visual lures (Fig. 1c–e). Before applying Tanglefoot glue, the traps were also fitted with $2 \times 9 \text{ cm}^2$ glossy cardboard strips that were stapled at the lower margins of the traps. These strips formed “gutters” to prevent the beetles from falling off the sloped trap surface onto the ground. Next, Tanglefoot glue was applied over the entire horizontal surface of the branch traps. A very thin layer of the Tanglefoot glue was applied to the decoy itself as was shown to be effective in EAB trapping (Lelito et al. 2008; Domingue et al. 2013a, b).

Distribution of branch traps

All the green branch traps were placed in clusters of three traps per tree with the following treatments: (1) a blank sticky trap, (2) a trap with dead female EAB decoys, and

(3) a trap with 3D-printed decoys. The three treatments were always placed on neighboring south-facing branches such that they were all exposed to direct sunlight for most of the day. Approximately 1 m equally distant from the traps and toward the interior of the tree canopy, a high-emission-rate (*Z*)-3-hexen-1-ol (*Z*3-6:OH) (“green-leaf volatile”) dispenser was added per tree. Each dispenser consisted of pre-made plastic packets (ChemTica Internacional), which had been determined to release 25 mg per day for 45 days by measuring weight loss at room temperature (22 °C). The three trapping treatments were initially placed on 13 different trees on May 28, 2013. Replicates were added on June 20 at nine additional trees so that there were 22 replicates for most of the season. One-half of the traps containing the 3D-printed decoys consisted of the step-layered (unsmoothed) version of these decoys and the other set of traps used the smoothed, slightly textured version. Most of the traps (17) were placed on the higher density, eastern half of the plantation. This discrepancy was intentional because we wanted to maximize captures to ensure strong statistical power for comparing the different decoys to the blank traps. However, because healthier trees with live branches were usually selected for hanging traps, nearly all of the individual trees used had no exit hole recorded during the 2012 survey, with just three existing on two trees (Table 1).

Traps were checked daily between 10:00 a.m. and noon beginning May 29, the day after their deployment, until July 17, by which time very few if any beetles were still being caught on a daily basis. After that, the traps were checked weekly until August 7th, when no beetles were found on any of the branch traps. Following each trap-check, all insects or plant materials ensnared on the sticky material were removed. Specimens of Buprestidae and Elateridae were retained in plastic bags for later identification. These were the most common beetle families caught in the traps, and when such specimens were covered with Tanglefoot glue, it was often difficult to discriminate between them without magnification.

For the branch-trap experiments, observations of beetle positions on the traps were made nightly from June 20 to July 3 at approximately 7:00 p.m. Because we often saw beetles moving on the traps as they struggled to free themselves from Tanglefoot glue, this observation time allowed for a better assessment of the positions of newly arriving beetles relative to the decoys, as opposed to waiting until the morning collection when their positions could have shifted substantially.

Purple prism traps

On May 28, four purple prism traps (Synergy Semiochemicals Corp., Burnaby, BC, USA) were placed within

the infested area, on their own distinct trees located at least 10–20 m from those containing the branch traps. Prism traps were hung over high branches and pulled up with ropes such that the bottoms were approximately 3 m from the ground. The traps were each baited with one Manuka oil and one (*Z*)-3-hexen-1-ol lure, which were replaced once during mid-season. They were all hung next to trees with no visible exit holes in the 2012 survey.

These traps were lowered so that all the EAB could be collected from them on June 18, July 10, and August 7. No other specimens were saved from these traps other than the EAB, but we informally observed several *A. subcinctus*, *A. cyanescens*, and Elateridae.

Handling of specimens

All specimens were placed in plastic bags during each day's collection and labeled specifically to each trap and collection date. They were frozen for a period of 2 to 4 months before the Tanglefoot glue was removed to facilitate identification. To remove the Tanglefoot glue, the specimens from each trap capture event were placed in a vial with Histoclear (National Diagnostics, Atlanta, GA, USA) for 24 h. At the end of this period, the vials and the specimens were separated and successively rinsed with hexane, acetone, and ethanol before being recombined with 2 ml of 80 % ethanol for preservation. Nearly all buprestids were either EAB or *A. subcinctus*. In addition, 11 individuals of *A. cyanescens* were found on the branch traps plus an undetermined number on prism traps. These specimens were not included in any analyses. Honeysuckle plants (*Lonicera* spp.) were interspersed at a low density among the ash trees at this site, and these were the likely host of this species. Individual EABs were also dissected to examine the genitalia so that sexual identity could be assigned to each specimen. None of the elaterid samples were identified past the family level.

Statistical analysis

For the branch-trap experiment, a cumulative logit model (McCullagh and Nelder 1989) was used to explore the effects of the factors including the date of collection, individual tree, high or low exit hole area, and the decoy type. Individual tree was included as a random effect to account for local variations in beetle density or penetration of sunlight that might affect trap captures. This model provides likelihood ratios to test the significance of each factor. Comparisons of individual parameters within the decoy type effect (such as real EAB vs 3D-printed decoys) were performed using Wald's χ^2 . Proc GENMOD in SAS version 9.2[©]2008 (SAS institute, Cary, NC, USA) was used for all calculations. The cumulative logit model was used

to separately explore these effects on captures of male EAB, female EAB, *A. subcinctus*, and elaterid specimens. For all analyses, dates were deleted where there were no captures such that the model would reach convergence criteria. For the same reason, we deleted observations from trees that had no captures for all of the analyses except those concerning male EAB captures. We were not as strongly interested in distinguishing effects of date of capture or tree location as we were in the efficacy of decoys, but we did wish to partition these sources of variation in the models. Interaction effects between these factors were never found to be significant, and thus were not included in the final models.

Trap costs

All costs are expressed in US dollars (\$). The cardboard base of each branch trap was \$1.00. The 90 cm² green plastic surface for each trap was \$0.50. The insect pins were \$0.05 each (two per trap). Approximately \$10 of brush and spray-on Tanglefoot was used for the 66 traps deployed, adding \$0.15. The combined cost of the two binder clips per trap to fasten to the foliage was \$0.13. While the cost of 3D-printing techniques is projected to decline in coming years as quality increases (Wohlers 2014), the 300 3D-printed decoys created for this project cost only \$43 at a Penn State workshop. Five dollars were spent for the green spray paint. Thus, the decoy cost was \$0.16 per unit and \$0.32 per trap. With these parameters, the manufacturing cost of a branch trap to us, without considering labor, ranged from \$1.78 to \$2.20, depending on whether decoys were used. The reusable binder clips, pins, and decoys accounted for \$0.55. While estimating the material cost of producing such traps at an industrial scale is difficult, it can be safely assumed that it would be low enough to offset necessary labor costs, because many of the materials used were purchased at retail rather than wholesale prices. The purple prism cost \$7.40 for each trap and \$1.44 for each reusable hanger unit. Odor lures were \$5.62 per packet regardless of which trap was used.

Results

EAB captures in branch traps

For the entire season, eight males and eight females were captured on the blank traps, 89 males and 25 females on the EAB-baited traps, and 84 males and 23 females on the 3D-printed decoy-baited traps. EAB were caught on branch traps on 21 of the 22 trees on which they were deployed, the exception being one ash tree that was located at the far western end of the plot away from the infested area and

Fig. 3 Mean (\pm SE) number of male (a–c) and female (d–f) EABs captured each day on branch traps for the three trap treatments (a, d = blank controls; b, e = dead-female-EAB decoys; c, f = 3D-printed decoys). There were 13 traps of each type until June 20, after which that number was increased to 22. Trap captures were collected daily until July 17, and so the last two data points (July 24 and July 31) represent weekly captures on those dates. Vertical dashed lines are provided to allow better visualization of the dates marked on the x axis

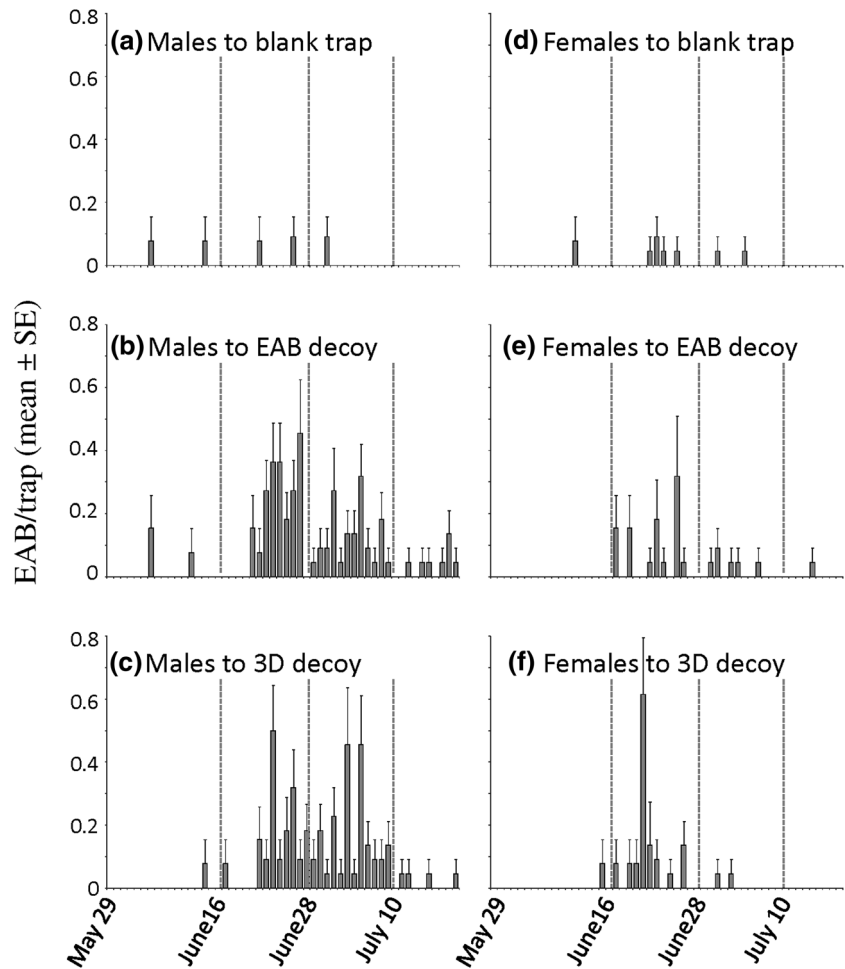


Table 2 Summary of statistical analysis of the effects of trap date, tree, and decoy on capture of male and female EAB

Factor	Male			Female		
	DF	χ^2	<i>p</i>	DF	χ^2	<i>p</i>
Date	31	105	<0.0001	20	46	0.00076
Tree	21	96	<0.0001	10	32	0.00047
Decoy	2	100	<0.0001	2	9.1	0.010
Blank vs EAB	1	48	<0.0001	1	6.8	0.0092
Blank vs 3DP	1	43	<0.0001	1	6.1	0.013
EAB vs 3DP	1	0.45	0.502	1	0.025	0.88

A cumulative logit model was used with comparisons of individual decoy types performed using Wald’s χ^2 . To ensure that the models used achieved convergence criteria, all dates exhibiting no captures were not considered for males, and all dates and trees with no captures were not considered for females

near non-host *Quercus* and *Juglans* trees. The traps at this location were also shaded for much of the day by other trees, with only 1 or 2 h of direct sunlight reaching them.

Statistically, for both male and female EAB, there were significant effects of date of collection, individual tree, and decoy status of trap (Fig. 3; Table 2). For both sexes, captures were significantly greater on either real EAB-baited traps or 3D-printed decoy-baited traps versus the blank control traps. However, catches on the two types of decoy-baited traps were not significantly different. No difference in capture performance was observed with respect to stepped versus smoothed decoys, the details of which are not presented.

Examination of the patterns of daily captures (Fig. 3) also revealed seasonal variation in activity of the sexes. Early in the season before June 16, there were only rare sporadic captures of either sex. Subsequently, there was a peak in captures of both sexes on the decoy-baited traps over the next 10 days. After June 26, the decoy-baited traps experienced another peak in attraction with a much heavier male bias. This peak reached a maximum by July 4 and lasted until July 10, after which there was a diminished frequency of captures for the rest of the season, consisting almost entirely of males.

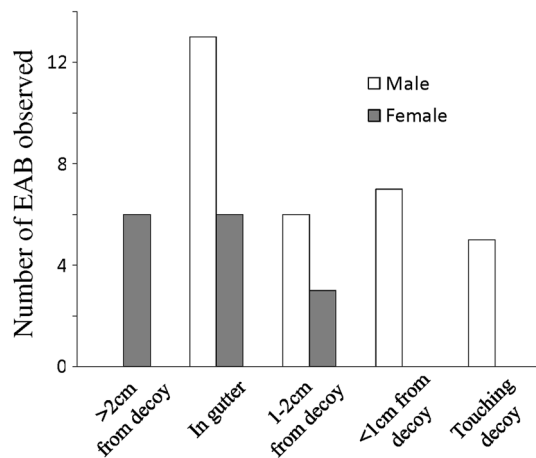


Fig. 4 Frequency at which captured male and female EAB were observed to be ensnared at different locations relative to the decoys during evening observations of freshly captured beetles. Data are combined for both dead-female-EAB decoys and 3D-printed ones

Spatial distribution on decoy traps

There were 46 observations made of EABs on the decoy-baited branch traps in the evenings just a few hours after their daytime ensnarement. Males were more likely to be found on, or very close to, the decoys than females, which were never observed in such positions (Fig. 4). Both sexes were often found in the gutter of the trap, which is where the green plastic surface meets the cardboard gutter, protecting them from falling off the side of the trap. There was a significant difference in frequencies at which males and females were found at the various categories of positions indicated in Fig. 4 ($\chi^2 = 18.1$, $d.f. = 9$, $p = 0.03$). We combined the observation data from the EAB-decoy-baited traps with those from the 3D-printed decoy traps here because the frequency distributions in these spatial categories were not significant for males ($\chi^2 = 2.26$, $d.f. = 7$, $p = 0.94$) or females ($\chi^2 = 3.7$, $d.f. = 5$, $p = 0.60$). Furthermore, for the male-capture positions observed, two of the five instances of a beetle found touching the decoy, and three of the seven instances of a beetle found less than 1 cm away, involved a 3D-printed decoy.

Exit hole distribution and branch trap captures

Both the western and eastern sides of the plantation experienced increases in exit hole numbers in 2013 roughly tenfold over 2012, with the numbers consistently much higher on the eastern side (Table 1). The specific trees the branch traps were hung upon showed a similar increase in exit hole counts from close to zero in 2012, to values that mirrored those of all the trees in 2013 (Table 1). There were more males and females found on the 17 traps in the high density eastern side of the plantation versus the five on

Table 3 Summary of statistical analysis of the effects of trap date, tree, and decoy on capture of *A. subcinctus* and all Elateridae

Factor	<i>A. subcinctus</i>			Elateridae		
	DF	χ^2	p	DF	χ^2	p
Date	13	31	0.0039	21	51	0.0003
Tree	17	23	0.16	21	75	<0.0001
Decoy	2	11	0.0045	2	0.90	0.63
Blank vs EAB	1	4.1	0.042	1	0.62	0.43
Blank vs 3DP	1	9.2	0.0025	1	0.0030	0.96
EAB vs 3DP	1	1.3	0.25	1	0.72	0.40

A cumulative logit model was used, with comparisons of individual decoy types performed using Wald's χ^2 . To ensure that the models used achieved convergence criteria, all dates and trees with no captures were not considered

the lower density western plot. This effect seemed more pronounced for females, but the differences in captures between the two sides of the plantation were not significant for either sex at $\alpha = 0.05$ (Table 1). An attempt to correlate exit hole counts and trap captures on each tree was strongly affected by an outlier. One tree had 20 exit holes recorded on the trunk in 2013, while all others had six or fewer. This same tree also had 22 males and 15 females caught per season, while the average was only 6.90 males and 1.14 females for all the other traps. When the traps from that particular tree with a large number of exit holes were included, there were positive correlations between both male ($r = 0.419$) and female ($r = 0.752$) branch-trap captures and the number of exit holes. However, if data from that outlier were excluded, the correlations became negative for both males ($r = -0.226$) and females ($r = -0.257$).

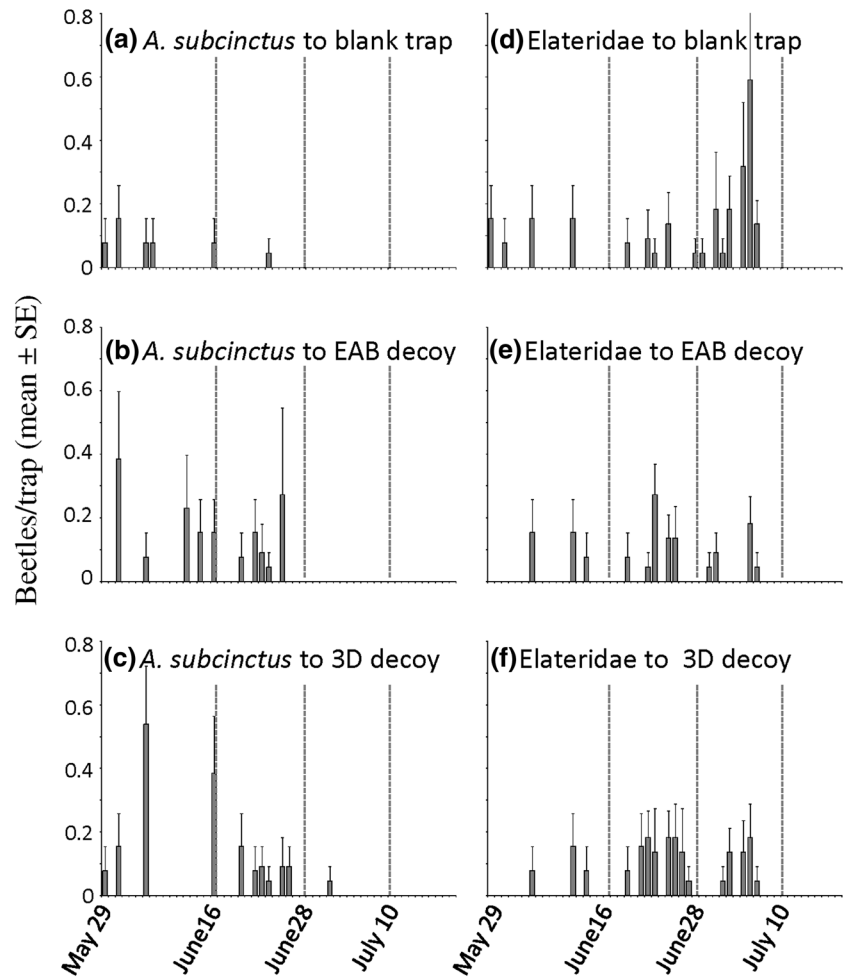
Other species on branch traps

Although the *A. subcinctus* captures were not separated by sex, in many respects the data exhibited similar patterns to that for EAB. There was a significant effect of decoys on captures (Table 3). Similar to EAB, there were significantly more *A. subcinctus* caught in decoy-baited traps of either variety (Fig. 5; Table 3) and there was no significant difference in the attraction to real EAB decoys versus the 3D-printed ones. However, *A. subcinctus* differed from EAB in that they were caught earlier in the season than both male and female EAB (Fig. 5). The elaterid specimens collected did not show any preference for any of the three branch traps (Fig. 5; Table 3).

Purple prism traps

There were 750 EAB caught on the four purple prism traps throughout the 2013 season (Fig. 6). When comparing the

Fig. 5 Mean (\pm SE) numbers of *A. subcinctus* (a–c) and Elateridae beetles (d–f) collected each day on branch traps of the three treatments. For each group, blank control traps (a, d); EAB decoy-baited traps (b, e); and 3D printed decoy-baited (c, f) were deployed. There were 13 traps of each type until June 20, after which that number was increased to 22. Trap captures were collected daily until July 17, and so the last two data points (July 24 and July 31) represent weekly captures on those dates. Vertical dashed lines are provided to allow better visualization of the dates marked on the x axis



prism traps to the branch traps for activity at different periods (Fig. 6), the purple prism traps, unbaited branch traps, and decoy-baited branch traps all caught some beetles in the earliest time period, from May 28 to June 19, and there was no indication of sexual bias in captures on any of the traps. There was a large increase in capture numbers between June 18 and July 9 for the purple prism and decoy-baited branch traps, but not the unbaited branch traps. This increase was female-biased in the prism traps, and heavily male-biased in the decoy traps. Only the purple prism and decoy-baited traps caught beetles in the latest period after July 10th, with a male bias and a much reduced rate of capture in both cases. For the prism traps, only the second collection period, where large numbers of insects were trapped, showed a significant difference from the expectation of equal numbers of the sexes, with a female bias ($\chi^2 = 39.7$, *d.f.* = 7, $p < 0.0001$).

Finally, approximately three times more beetles were collected on the prism traps in the eastern, higher-density end of the plantation than in the western end. The two traps on the western end had 60 ± 27 males and 43.5 ± 17.5 females, while the two on the eastern end had 143 ± 4 males and 128.5 ± 47.5 females (mean \pm SE). No

statistical analyses were attempted with such a small sample size. All four traps had been hung on trees without exit holes in 2012, but one of the trees in the eastern end had four exit holes recorded in 2013.

Discussion

On the branch traps, the 3D-printed decoys exhibit almost identical attraction ability versus real EAB decoys, while both decoys caused greater captures than blank traps. The frequency and timing of beetle captures, along with the relative proportions of males and females, were all quite similar for traps baited with visual decoys of either type. This finding demonstrates that small, fully synthetic decoy-based traps could potentially be cheaply manufactured and successfully deployed for the purpose of monitoring EAB populations. Furthermore, it is conceivable that such decoys may have applications beyond the specific traps deployed here, which can now more confidently be explored knowing that effective, inexpensive synthetic decoys are available.

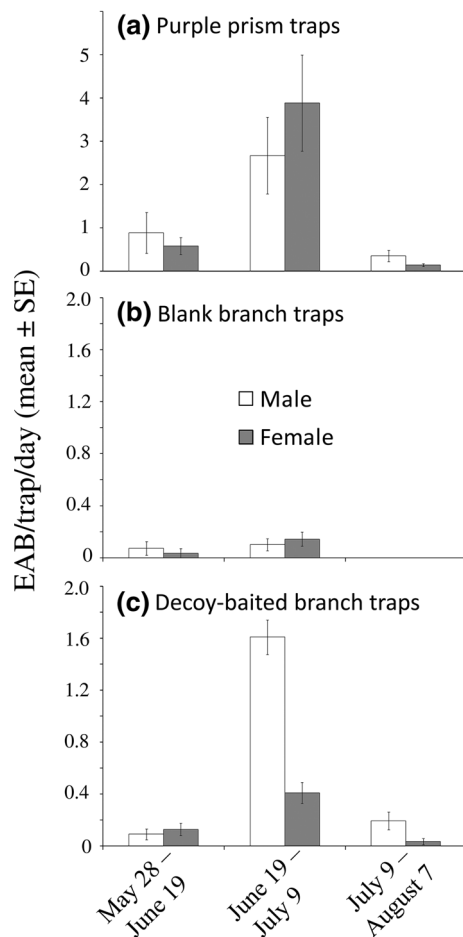


Fig. 6 Daily capture rates of each trap design during early-, mid- and late-season periods. Because of nearly identical capture profiles (Fig. 3), dead-female-EAB and 3D-printed decoys' captures were combined for part C. There were four purple prism traps (a), 13–22 blank green branch traps (b), and 26–44 decoy-baited branch traps (c). Each of the three branch trap treatments increased from 13 replicates to 22 replicates on June 20

However, concerning the specific branch-trap design used here, it would be worthwhile to perform further research to clarify how they might be useful as a management tool. The site used for this study, and those used for previous demonstrations of EAB visual decoy effectiveness (Lelito et al. 2007; Domingue et al. 2013a), very clearly had quite high population densities. All of these sites were selected intentionally to ensure that there would be enough captures to evaluate whether decoy-baited traps perform better than controls. Thus, efficacy at low populations has not been verified. Indeed attempts to compare trap functions at high and low density EAB sites have been rare, but have been performed quite recently to compare different colors and variations of prism and funnel traps (Marshall et al. 2010; Francese et al. 2013; Poland and McCullough 2014). We are hopeful that the branch traps will also function well in low-density populations, because

they function to attract males, who are expected to continue to forage through the canopy for mates for as long as they are alive. The observation in this study that male branch trap captures were less affected by local variations in beetle density than female branch-trap captures or prism-trap captures (Table 1) suggests that such male dispersibility may have a real effect on branch trap outcomes. However, it should be cautioned that none of the population exit hole density-dependent trends were significant in the current experiment and a more thorough examination of the phenomenon is warranted.

One potential challenge for implementing such decoys at low population densities is that often feeding and mating activity among EAB begins high in the canopy, progressing downward as the tree declines (Cappaert et al. 2005). Thus, further innovations that retain elements of the branch-trap design, but would allow deployment of decoys at the tops of trees to maximize low-density detection may be necessary. A highly desirable mechanism would attach the traps to a releasable pole that would allow one to hook the trap onto the higher branches from the ground.

Most of the EAB detection methods devised to this point involved labor-intensive aspects at various stages of their deployment. For example, branch-sampling has been proven to be an effective monitoring tool for locating EAB in asymptomatic trees (Ryall et al. 2011), but requires a large amount of labor, and has thus been proposed as a tool primarily for urban management. Likewise, the monitoring of buprestid beetles found in the nests of predatory *Cerceris* wasps has been implemented as a detection tool, leading to an initial detection of EAB in Connecticut (Rutledge et al. 2013). However, this technique also requires a fair amount of labor in the field during the season, relying on an educated volunteer work force to monitor wasp colonies. Traps such as the branch traps and prism traps deployed in the current study also require some time for deployment, servicing, collection, and processing of results. While we have not quantified all of these factors, from our experience it seems like the cost of using branch traps should be low in this respect. Because they did not require the use of ropes, and had much less sticky material, they were much easier for a single person to deploy quickly. Each trap required less than a minute to service per person. Although trap-checking times at each visit would likely grow if longer intervals between checks were to be employed in more realistic trapping programs, it would still not likely approach the scale of our experience of approximately 45 min required for three persons to clear the purple prism traps. The more recently developed green funnel traps (Francese et al. 2011) are also easier to service than prism traps, but are large and require the use of ropes to be deployed, and appear to be somewhat less able to

detect EAB than prism traps at low densities (Francese et al. 2013).

Another aspect of the potential deployment of the branch traps to consider is that all experiments with decoys-based trapping in this and other studies have involved frequent checking of traps at intervals ranging from 1 to 3 days (Lelito et al. 2008; Domingue et al. 2013a). In the current study, daily collections were performed for the purpose of tracking the phenology of adult activity at a fine scale. It is unknown if trap efficacy will improve or decrease if checked at weekly, monthly, or seasonal intervals, which would be necessary in trapping programs. It is conceivable that trapped buprestids that are not removed will serve as additional attractive decoys and thus facilitate greater capture numbers. At the same time, the accumulation of non-target insects on the trap surface could produce a negative effect. Previous work also indicated that host and host-like volatiles such as (*Z*)-3-hexen-1-ol, manuka oil, and phoebe oil increase attraction to decoy-baited traps (Domingue et al. 2013a), but further work could be done to optimize the blend and release rates of such volatiles as has been done for prism traps (Crook et al. 2012). Moreover, the EAB-produced lactone compound, which has been shown to synergize attraction of males to (*Z*)-3-hexen-1-ol (Silk et al. 2011), would be an interesting compound to test with decoy-baited traps which are designed to evoke the male visual mating response.

One noticeable difference from previous studies is that the female-capture rate on the decoy-baited branch traps increased compared to controls. For EAB, there have never been reports of such a decoy effect for females on sticky-leaf traps (Lelito et al. 2008; Domingue et al. 2013a). However, this observation is not completely unprecedented because the attraction of females of *Agrilus biguttatus* to decoy-baited branch traps occurred when these traps were deployed in a Hungarian oak forest (Domingue et al. 2013b). The significantly larger capture surface of the branch traps compared to the surface of a single ash leaflet likely contributes to our ability to observe this phenomenon. For EAB females in the present study in University Park, PA, USA, it was clear that the females were found ensnared further away from the decoys than were males (Fig. 4), and at distances that would not be possible on a sticky-leaf trap. The behavioral significance of female attraction to decoys at this point is unclear. Perhaps such females intend to initiate mating contact directly with males that are maturation feeding alone on the foliage. Alternatively, the presence of another beetle might indicate a favorable position for a female to feed and signal its availability for mating, prompting her to land nearby without the immediate intention of mating with the resting beetle she had initially observed.

The daily collection of beetles from branch traps throughout the season allows us to make some inferences concerning the seasonal phenologies of male and female EAB throughout the adult dispersive stage (Fig. 3). Early June was marked by sporadic captures of either sex on all the traps. Between June 16 and June 28, there was an increase in the captures of both sexes on the decoy-baited traps. The prevalence of both sexes on the traps declined throughout the season, but this decline was much more rapid for females, while males experienced a second peak of captures in decoy-baited traps between June 28 and July 11. This is likely explained by a female shift in time allocation from maturation feeding and mating to oviposition-related behaviors. However, males continued to patrol the foliage searching for mates, as evidenced by the continuing attraction to the decoy-baited traps. It should also be noted, however, that four of the authors of this paper all independently observed males and females copulating on the trunks of ash trees at 1–2 m heights. Thus, it should not necessarily be assumed that all male foraging for mates occurs in the canopy. Perhaps, decoys could also be applied to further document and understand this behavior.

The decoy-enhanced captures of *A. subcinctus* early in the season in this experiment, coupled with similar previous captures of a large array of European oak *Agrilus* species on branch traps baited with real EAB (Domingue et al. 2013b), suggests that decoy-baited traps might be useful for a large array of buprestid species. If the traps were not cleared often, it is uncertain whether the accumulation of *A. subcinctus* early in the season could have later affected EAB captures positively or negatively. In order to devise optimal branch-trap deployment strategies, such non-target species effects should be a consideration for future research, and if necessary a date established for an early season clearing of traps to remove the effects of such non-targets. Additionally, the effect of the accumulation of common non-buprestids such as the Elateridae often found on the traps in this experiment merits future consideration.

Because decoys work by attracting buprestids to a more fine-scaled spatial area than that possible with other buprestid traps, non-sticky trapping techniques might be more easily explored. Electrocutation and collection of beetles approaching decoys in a trap with an upper surface similar to these branch traps has been accomplished (Domingue et al. 2014), using nanofabricated decoys rather than the 3D-printed decoys described in this study. Other means of detecting male beetles approaching decoys for mating can be envisioned by means of sensing vibrational, electrical, or light changes associated with these events. Such technologies could also conceivably be fashioned into remote reporting devices such that real-time reporting of events is

possible. Finally, because there is substantial evidence of cross attraction among buprestid species (Lelito et al. 2011; Domingue et al. 2011), work to improve and vary the visual properties of the decoys may allow further study of such phenomena in more detail, with the aim of perhaps providing more general versus specific signals for trapping systems as applications might require.

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