Entomologia Experimentalis et Applicata

DOI: 10.1111/eea.12083

Trapping of European buprestid beetles in oak forests using visual and olfactory cues

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Accepted: 2 April 2013

Key words: invasive species, *Agrilus*, forest pest, Coleoptera, Buprestidae, *Quercus*, Fagaceae, Elateridae, decoy

Abstract

Trapping approaches developed for the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), were adapted for trapping several European oak buprestid species. These approaches included the use of natural leaf surfaces as well as green and purple plastic in sticky trap designs. Plastic surfaces were incorporated into novel ‘branch-trap’ designs that each presented two $5 \times 9\text{-cm}^2$ rectangular surfaces on a cardboard structure wrapped around the leaves of a branch. We used visual adult *Agrilus* decoys in an attempt to evoke male mating approaches toward the traps. Our first experiment compared the attractiveness of visual characteristics of the surfaces of branch-traps. The second looked at the effect on trap captures of adding semiochemical lures, including manuka oil, (*Z*)-3-hexen-1-ol, and (*Z*)-9-tricosene. In total, 1 962 buprestid specimens including 14 species from the genus *Agrilus* were caught on 178 traps in a 22-day time-span. Overall, the green plastic-covered branch-traps significantly out-performed the other trap designs. We further found that the presence of an EAB visual decoy placed on the trap surface often increased captures on these green traps, but this effect was stronger for certain *Agrilus* species than for others. The visual decoy was particularly important for the most serious pest detected, *Agrilus biguttatus* Fabricius, which was captured 13 times on traps with decoys, but only once without a decoy. There were some small but significant effects of odor treatment on the capture of buprestids of two common species, *Agrilus angustulus* Illiger and *Agrilus sulcicollis* Lacordaire. There were also 141 Elateridae specimens on these traps, which were not influenced by trap type or decoys. The results suggest that small branch-traps of this nature can provide a useful new tool for monitoring of buprestids, which have the potential to be further optimized with respect to visual and olfactory cues.

Introduction

Until the recent emergence of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), as an invasive tree-killing pest in North America from Asia, there had been relatively little behavioral research aimed at the development of trapping methods for forest buprestids. Nevertheless, there are other forest buprestid species capable of causing tree mortality and other economic concerns. These include the oak (*Quercus* spec.) feeding species, *Agrilus bilineatus* Weber in North America (Haack & Benjamin, 1982) and *Agrilus biguttatus* Fabricius in Europe (Moraal & Hilszczanski, 2000; Vansteenkiste et al., 2005), which are both linked to oak mortality in their native ranges when forests become stressed by drought and/or defoliation (Csóka & Kovács, 1999; Muzika et al., 2000; Csóka & Hirka, 2006; McManus & Csóka, 2007). The potential for non-native buprestids to threaten oak forests has been underscored by the recent finding of the less aggressive European oak species *Agrilus*

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sulcicollis Lacordaire in North America (Haack et al., 2009). In addition, the recent range expansion of the North American gold spotted oak borer, *Agrilus auroguttatus* Schaeffer into California from Arizona (both USA) has been implicated in widespread tree mortality of coast live oak, *Quercus agrifolia* Née (Coleman & Seybold, 2008; Coleman et al., 2011). Thus, effective tools for detecting and monitoring buprestids in oak forests are needed to recognize and manage such emerging threats.

After nearly a decade of intense research into different trap designs for detecting EAB, it is not yet fully understood how well these technologies will apply to other species. The most widespread trapping technique for EAB involves the use of green and purple sticky plastic 'prism' traps. There has also been research documenting evidence that pinned dead adult EAB decoys on sticky leaves can capture males with a high detection rate (Lelito et al., 2008; Domingue et al., 2013). Such traps were developed after field observations showed that male EAB are visually attracted to other adult conspecifics of either sex, whether alive or dead, resting on the foliage in bright sunlight. They rapidly descend to land on such beetles from ca. 1 m above, before attempting to copulate (Lelito et al., 2007).

Furthermore, some semiochemicals are known to be attractive to EAB adults and are useful for trapping when presented in particular contexts. Commercially available bark extracts such as manuka oil and phoebe oil, which share some of the components of ash bark, have been shown to increase trap catch of EAB adults in the field (Crook et al., 2008). The green-leaf volatile (*Z*)-3-hexen-1-ol also increases trap catch (DeGroot et al., 2008; Grant et al., 2010, 2011), especially when green prism traps are used (Silk et al., 2011). A lactone, (*Z*)-3-dodecen-12-olide, was extracted from female EAB feeding on ash foliage (Bartelt et al., 2007), and this lactone has been shown to synergistically increase the attraction toward green prism traps when co-emitted with (*Z*)-3-hexen-1-ol (Silk et al., 2011). Finally, after males perform their visually mediated flight toward females, contact sex pheromones are known to promote copulatory behavior (Lelito et al., 2009). The cuticular hydrocarbons 3-methyl-tricosane (Lelito et al., 2009) and 9-methyl-pentacosane (Silk et al., 2009), have been shown to be active components of this signal. As of yet, there have been no attempts to incorporate such contact pheromones into trap designs.

Other species of buprestids have been caught while attempting to catch EAB on traps directed toward EAB in North America (Lelito et al., 2008; Haack et al., 2009). Specifically when using EAB decoy-baited traps, Lelito et al. (2008) were able to collect large numbers of the non-target species *Agrilus subcinctus* Gory and *Agrilus cyanescens* Ratzeburg. Recent behavioral studies have also confirmed that several other species of *Agrilus* use visually mediated mating approaches similar to EAB (Domingue et al., 2011; Lelito et al., 2011). Males of the European oak-infesting species *A. biguttatus*, *A. sulcicollis*, and *Agrilus angustulus* Illiger will often perform such mating attempts to heterospecific decoys, even when given a choice of multiple decoys that include a conspecific decoy (Domingue et al., 2011). *Agrilus planipennis* decoys were particularly attractive in this context, eliciting approaches by all three of these oak *Agrilus* species as often as the conspecific decoys. Underscoring the degree of attraction, *A. biguttatus* males also vigorously attempted to copulate with EAB females, spending on average a significantly longer time on these copulation attempts vs. those on conspecific decoys.

In this context, we developed trap designs for European forest buprestids, which were deployed in an oak forest in Hungary in 2011. Traps included design modifications of sticky cards previously used for EAB (Lelito et al., 2008; Domingue et al., 2013). Traps with or without EAB decoys, and having green plastic, purple plastic, white cardboard, or natural leaf surfaces were tested for buprestid attraction. The base of such traps was a folded cardboard sheet designed to fit around leaf clusters for deployment at a height of 2.5 m. The novel traps were also compared to the use of undisturbed sticky leaves with or without decoys.

In another experiment, we tested for the possibility that semiochemicals that are attractive to EAB might increase oak buprestid captures. The three semiochemicals used were manuka oil, (*Z*)-3-hexen-1-ol, and (*Z*)-9-tricosene. The latter compound has a somewhat similar chemical structure to one of the cuticular hydrocarbon contact sex pheromone components of EAB females and was tried because it was commercially available. It was not known whether the contact sex pheromone would be sufficiently volatile to be able to attract beetles to the trap vicinity. Furthermore, *A. biguttatus* is the only oak-infesting *Agrilus* species known to have compatibility with EAB contact pheromones; males exhibited strong copulatory activity toward the dead EAB female decoys that they landed on (Domingue et al., 2011). Despite how speculative the potential attractiveness (*Z*)-9-tricosene to oak buprestids is, this possibility was easily testable due to the availability of the compound.

Materials and methods

Field sites

Trapping was performed in June 2011 at two sites in an oak forest near Mátrafüred, Hungary (47°50′17″N, 19°59′50″E, 367 m altitude) where active logging occurs annually. This oak forest consists of ca. 85% sessile oak, *Quercus petraea* (Mattuschka) Liebl., with a sporadic mix of Turkey oak, *Quercus cerris* L., downy oak, *Quercus pubescens* Willd. (Fagaceae), hornbeam, *Carpinus betulus* L. (Betulaceae), and goat willows, *Salix caprea* L. (Salicaceae), on the forest margins. The trapping sites were situated ca. 2 km within the southern border of a 40 000-ha continuously forest region. One site had several piles of large recently cut sessile oak logs stored adjacent to trees along the roadside (hundreds of 20-cm-diameter × 3-m-long pieces). The other site had just a few log piles consisting of smaller sessile oak trees and branches (dozens of 10-cm-diameter × 1-m-long pieces). The logs had been cut and stacked within a month of the experiment. In the previous year, three *Agrilus* species (*A. biguttatus*, *A. sulcicollis*, and *A. angustulus*) were observed in the foliage above similarly stacked logs (Domingue et al., 2011). The proximity of traps to cut wood piles was intended to utilize the possibility that host odors might be attracting more buprestids to the area. It was also thought possible that the oak logs might include some emerging buprestids if infested, although characteristic D-shaped emergence holes were never found on the logs.

Branch- and leaf traps

In an effort to design an effective and efficient method of sampling buprestid presence in the foliage, novel sticky traps were designed and compared to our well-researched 'sticky-leaf' EAB trap that involves applying sticky material directly to visual decoys and the leaves to which they are pinned (Lelito et al., 2008; Figure 1A). The novel traps were built from halved, inverted white delta traps (ISCA technologies; Figure 1B), which were placed around a leaf cluster and fastened with clips using the leaves and/or their twigs. The traps had two $5 \times 9\ \text{cm}^2$ surfaces that were exposed to the sun at ca. 45°. Descending from each surface and wrapping around the branch were two $9 \times 13\text{-cm}^2$ white cardboard pieces. The traps were modified by stapling various materials to the sun-exposed surfaces. These included either green or purple plastic rectangles (Figure 1C and D), or freshly picked oak leaves (Figure 1E). 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. The green and purple plastic had peak reflectance at 540 and 430 nm, respectively, and were obtained from ChemTica International (Heredia, Costa Rica).

One dead female *A. planipennis* originating from the USDA-APHIS EAB rearing facility in Brighton, MI, USA, was pinned to the center of each of the top two trap surfaces as visual decoys for each type of novel branch-trap deployed (Figure 1D and E). Beetles were killed by freezing to be pinned and stored at room temperature for ca. 1 month before deployment. For the sticky-leaf traps, one such beetle was placed in the center of each trap and covered with Tanglefoot™ (Grand Rapids, MI, USA) glue along with the leaf (Figure 1A). Similar to the sticky-leaf traps, Tanglefoot was applied over the entire horizontal surface of the branch-traps. A very thin layer of Tanglefoot was applied to the decoy itself as shown to be effective in EAB trapping (Lelito et al., 2008; Domingue et al., 2013). After 2 days of deployment, sticky material was added to

Figure 1 Trapping designs that were employed both with and without visual decoys, including (A) sticky leaves, (B) a white branch-trap, (C) purple plastic branch-traps with a (*Z*)-3-hexen-1-ol packet suspended between them, (D) a green plastic branch-trap with a (*Z*)-9-tricosene packet attached to the underside, and (E) a leaf-covered branch-trap.

the vertically descending white sides of all branch-traps to further maximize the capture rate of any beetles that would potentially slide off the roof of the trap. Leaf surfaces of the branch-traps or sticky-leaf traps were replaced using new leaves if necrosis of the leaf tissue became apparent.

Visual-attraction experiment in lower branches

Our first experiment involved comparison of all the five visual trap designs, each duplicated with and without an EAB decoy on the same tree. A replication of these 10 traps took place on two trees at each of the two sites described above. Within each site, the trees selected were separated by ca. 100 m and had particularly large numbers of southern facing branches accessible for hanging traps. Each of the following traps, as described in more detail above and depicted in Figure 1, was deployed both with and without EAB lures: (1) sticky-leaf; (2) white cardboard branch-trap; (3) purple plastic branch-trap; (4) green plastic branch-trap; or (5) leaf-covered branch-trap. The 10 traps were placed on neighboring branches, each separated by ca. 0.5 m. They were also deployed such that identical trap types with and without lures were never adjacent. Table 1 lists the frequency with which each trap type was deployed in this experiment.

Trap positions were re-randomized three times. The same branches were used for the branch-traps (types 2–5), but a trap with a different surface color was employed at each spot on each rotation. Also, the presence or absence of a pinned EAB decoy was alternated on each branch at each rotation. At each rotation, new leaves were selected for sticky-leaf traps (Figure 1A), both with and without visual decoys. We also refreshed the leaves on top of each leaf-covered branch-trap (Figure 1E).

Traps were checked daily, with the exception of excessively rainy or cloudy periods, when traps were checked after 2 days. Because buprestids are only active during sunny periods, none were found in traps when it was consistently rainy or overcast. After each trap check, all other insects or plant materials ensnared on the sticky material were removed. Buprestidae and Elateridae specimens were retained in plastic bags for later identification. These were the most consistently prevalent beetle families present across all traps. The two families can be difficult to discriminate quickly when covered by Tanglefoot. Furthermore, it is also possible that members of the two families may have divergent response characteristics that reveal generic characteristics of each family.

Odor-baiting experiment in lower branches

In the same locations used for the previous experiment, traps were deployed to evaluate the effectiveness of three potential semiochemical attractants. These attractants included the bark extract manuka oil, the green-leaf volatile component (*Z*)-3-hexen-1-ol, and the housefly pheromone component lure consisting of (*Z*)-9-tricosene (Carlson et al., 1971). Manuka oil and (*Z*)-3-hexen-1-ol dispensers were provided as pre-made plastic packets (ChemTica Internacional). These dispensers had been measured to release 25 mg per day for 45 days by measuring weight loss at room temperature (22 °C). The Z9-tricosene pads were a 5 × 7 cm thick non-woven polymer with 50 mg of Z9-tricosene applied using hexane. Traps were deployed in trees that were either baited with one of the three odors, or else left as semiochemically unbaited control trees. In each tree with a distinct odor treatment, four traps were deployed. Two were sticky-leaf traps with

Table 1 Total number of each trap and decoy combination that were deployed in four replicated blocks of the visual-attraction and odor-added experiments

			Odor-added experiment			
Trap type	Decoy	Visual experiment	Blank	(*Z*)-3-hexen-1-ol	Manuka oil	(*Z*)-9-tricosene
Leaf	+	4	8	8	8	8
	–	4	8	8	8	8
Branch						
Green	+	4	4	4	4	4
	–	4	4	4	4	4
Purple	+	4	4	4	4	4
	–	4	4	4	4	4
White	+	4				
	–	4				
Leaf-covered	+	4				
	–	4				

or without a pinned EAB decoy and the other two were branch-traps of the same color (either green or purple), each either having or lacking a pinned EAB visual decoy. Table 1 provides the total number of traps deployed with each trap type and odor combination.

In the case of the manuka oil or (*Z*)-3-hexen-1-ol-lure-baited trees, a single odor packet was dispensed on a tree branch with all four traps clustered ca. 0.5 m from the odor lure (Figure 1C). Previous research with *A. planipennis* had shown that within-tree odor placement of these lures was all that was necessary to increase trap captures in both sticky-leaf traps and prism traps (Domingue et al., 2013). A different approach to odor placement was used for (*Z*)-9-tricosene. Because the (*Z*)-9-tricosene bait was intended to potentially mimic cuticular hydrocarbon components emitted from the EAB itself, we stapled the odor pads of this treatment to the cardboard directly below the plastic surface of the branch-traps (Figure 1D). Thus, two odor pads were deployed in two traps within each tree containing each of the branch-traps, with or without EAB decoys. Sticky-leaf traps were also deployed on these trees, but without such a point-source odor lure.

The odor-added experiment was arranged across a total of four blocks adjacent to each of the four trees that were used for the visual decoy attraction experiment described above. Within each block, two roughly parallel trap lines were established that ran east to west away from the visual-trapping experiment on the south-facing branches of trees, which were at least 5 m away from other trees in the block. The experiment began with each of the two trap lines in the block having a complete series of green or purple branch-traps with one of the four odor treatments as described above. Thus, overall a total of 32 trees were selected and provided with one of the branch-trap color (green or purple) and odor lure [control, manuka oil, (*Z*)-3-hexen-1-ol, or (*Z*)-9-tricosene] combinations.

Similar to the visual-attraction experiment, traps were checked daily or every second day after rain or cloudiness, when buprestid beetles are not actively flying. Again, the traps were cleaned at each check and all Buprestidae and Elateridae specimens were kept in small plastic bags for later identification. The traps were rearranged three times after their initial deployment such that within each block, a new odor lure and trap color combination was assigned to each tree at each rotation. The four rotations were planned, so that each tree received each odor treatment just once and green vs. purple plastic traps exactly twice. At each rotation event lures were moved with each set of traps, and new sticky leaves were prepared with and without EAB decoys.

Traps in all the visual and odor experiments at the more lightly logged sites were deployed on 5 June 2011, whereas those at the more heavily logged sites were deployed on 7 June. The last day for collection was 22 June 2011. Rotations were performed after 2–4 trap checks, the timings of which were dependent on the weather conditions.

High canopy green sticky traps

Trapping in the canopy was conducted at Julianna Major (47°32′56″N, 18°55′37″E, 344 m altitude) from 29 May to 14 July 2011. This forest area is ca. 1 600 ha on the outskirts of Budapest. This experimental site also consists mostly of sessile oak, with a scattering of black locust [*Robinia pseudoacacia* L. (Fabaceae)], common hawthorn [*Crataegus monogyna* Jacq. (Rosaceae)], cherry dogwood [*Cornus mas* L. (Cornaceae)], and hazel [*Corylus avellana* L. (Betulaceae)]. Traps were set up in a randomized complete block design, with four blocks per site. Traps were spaced 10–15 m apart, and were hung from the canopy of trees at a height of 5–6 m. Traps were inspected once weekly, when captured insects were collected. Lures were replaced every 2nd or 3rd week.

Field tests were carried out with VARb3 modified funnel traps from the CSALOMON® trap family produced by the Plant Protection Institute (Hungarian Academy of Sciences, Budapest, Hungary; www.julia-nki.hu/traps) (Imrei et al., 2001; Schmera et al., 2004). This trap design, with a fluorescent yellow (non-sticky) upper funnel and a vertical plastic sheet mounted in the funnel for interception of flying insects, has been shown to be effective in catching other beetle species (Tóth et al., 2005; Toshova et al., 2010). In the present experiments, four 10 × 16-cm fluorescent yellow sticky sheets covered with Tanglefoot glue were fixed on the transparent upper funnel, two on both sides. The plant volatile dispensers were suspended from the vertical plastic sheet, so that they hung in the middle of the funnel opening.

The bait dispensers consisted of a 1-cm piece of dental roll (Celluron®; Paul Hartmann, Heidenheim, Germany), which was placed into a tight 1.5 × 1.5-cm polythene bag made of 0.02 mm linear polyethylene foil attached to a 8 × 1-cm plastic strip. For making up the baits, 300 mg of Manuka oil or 100 mg of (*Z*)-3-hexen-1-ol alone or 100 mg of each green-leaf volatile compound (*E*)-2-hexenal, hexan-1-ol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol were administered onto the dental roll and the opening of the polythene bag was heat-sealed. Earlier experience showed that the bait did not lose its activity during several weeks of field exposure; hence we decided that it was safe to renew the lures at 2- to 3-week intervals. The Manuka oil bait was always combined with the (*Z*)-3-hexen-1-ol bait on the fluorescent yellow traps, whereas the green-leaf volatile bait was applied on these traps alone.

Handling of specimens

All specimens were placed in plastic bags specific to each trap and collection date. They were frozen for several months before the Tanglefoot was removed to aid in identification. To remove the Tanglefoot, 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. All buprestids were identified to species using the characters listed in Muskovits & Hegyessy (2002), by the lead author of that publication. Individuals of only the largest species, *A. biguttatus*, were also dissected to examine the genitalia so that sexual identity could be assigned to each specimen.

Statistical analysis

For each analysis, a cumulative logit model was used to explore the effects of factors such as trap type, decoy presence, site location, rotation period, and daily variation. This model provides likelihood ratios to test the significance of each factor. Comparisons of individual parameters within each effect (such as green vs. purple branch-traps) were performed using Wald's χ^2. Proc GENMOD in SAS version 9.2 (SAS institute, Cary, NC, USA) was used for all calculations.

Results

Species distribution of trap captures

In total, 1 962 buprestid and 141 elaterid specimens were trapped in both the visual decoy and semiochemical odor experiments involving the 168 branch-traps and leaf traps deployed in Matrafured. Of these captures, 1 475 were on the 16 green branch-traps deployed. The total buprestids included nine *Agrilus*, three *Anthaxia*, one *Chrysobothris*, and one *Coraebus* species (Table 2). All of the species are listed as being found on oak in Hungary by Muskovits & Hegyessy (2002). However, the rarely caught *Anthaxia* species are more commonly associated with other trees and flowering shrubs that are also found in this oak forest (Rosacea, Ranunculaceae, *Salix* spp.). The 14 species of buprestids, which we caught, are among the currently known 12 *Agrilus* and 18 other buprestid species (belonging to the three other genera listed above) found on oak in Hungary (Muskovits & Hegyessy, 2002). Slightly more than half of these beetles collected in our traps were *A. angustulus* (Table 2). This and the next four most common species, *A. sulcicollis*, *Agrilus obscuricollis* Kiesenwetter, *Agrilus laticornis* Illiger, and *Agrilus graminus* Laporte & Gory accounted for 97% of the buprestids trapped.

Table 2 Total numbers of Buprestidae caught in the branch- and leaf traps deployed in June 2011 in Matrafured, Hungary. All buprestids were identified to species. There was no significant difference in the distribution for the seven most common *Agrilus* species in the visual-attraction-only vs. the odor-added experiments (χ^2 = 8.30, d.f. = 13, P = 0.82)

Taxon	Visual-only experiment	Odor-added experiment	Total no.	% total
All Buprestidae	489	1473	1962	100
Agrilus angustulus	281	823	1104	56.3
Agrilus sulcicollis	86	237	323	16.5
Agrilus obscuricollis	66	172	238	12.1
Agrilus laticornis	30	136	166	8.5
Agrilus graminus	14	62	76	3.9
Agrilus olivicolor	3	15	18	0.9
Agrilus biguttatus	3	11	14	0.7
Agrilus hastulifer	1	3	4	0.2
Agrilus convexicollis	0	2	2	0.1
Anthaxia nitidula signaticollis	2	5	7	0.4
Anthaxia salicis	2	3	5	0.3
Anthaxia fulgurans	0	2	2	0.1
Chrysobothris affinis	0	2	2	0.1
Coraebus florentinus	1	0	1	0.1

Table 3 Total numbers of Buprestidae caught in the high canopy traps deployed in June 2011 near Budapest, Hungary. All buprestids were identified to species. There was no significant difference in the distribution for these species with respect to the odor baits (χ^2 = 5.90, d.f. = 11, P = 0.88)

Taxon	Manuka + (*Z*)-3-hexen-1-ol	GLV mixture	Total no.	% total
All Buprestidae	17	8	25	100
Agrilus angustulus	1	1	2	8
Agrilus sulcicollis	1	3	4	16
Agrilus obscuricollis	1	0	1	4
Agrilus laticornis	10	4	14	56
Agrilus olivicolor	3	0	3	12
Anthaxia nitidula signaticollis	1	0	1	4

Comparatively many fewer buprestids were caught in the canopy traps in Budapest, with only 25 captures in eight traps over the entire season (Table 3). The distribution of species was significantly different from that observed for the captures in the lower branches in Matrafured (χ^2 = 104, d.f. = 11, P<0.001), with an obviously greater proportion of *A. laticornis* and smaller proportion of *A. angustulus* caught. Because of the scarcity of beetles collected in this experiment, no further analyses were performed using these data, whereas a more detailed examination of the trap capture patterns for the two experiments involving the lower branch-traps is provided below.

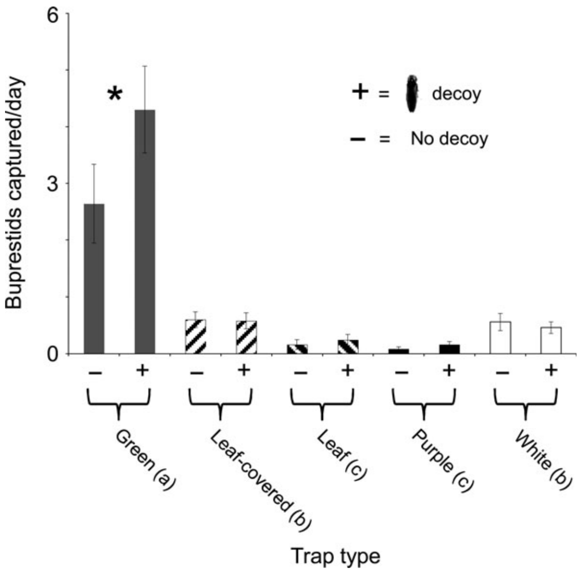

Figure 2 Mean (± SE) number of buprestids per day on each trap in the visual-attraction experiment. Data are organized by trap design and presence of visual decoy. There were n = 48 total observations for each decoy and trap type combination. Similar letters following the trap type listed on the x-axis indicates that there are no significant differences ($P>0.05$) in the buprestid attraction (pooling decoy and non-decoy traps of each type). The asterisk indicates a significant difference between trap captures on green plastic traps with vs. without beetles ($\chi^2 = 7.49$, d.f. = 1, $P = 0.006$).

Visual attraction in lower branches

The green plastic branch-traps captured significantly more buprestids than any of the other designs (Figure 2). The cumulative logit model indicated significant effects of trap type ($\chi^2 = 185$, d.f. = 4, $P<0.0001$), experimental block ($\chi^2 = 10.7$, d.f. = 3, $P = 0.014$), day ($\chi^2 = 34.28$, d.f. = 12, $P = 0.0006$), decoy ($\chi^2 = 3.85$, d.f. = 1, $P = 0.050$), and trap rotation period ($\chi^2 = 8.72$, d.f. = 3, $P = 0.033$). The different experimental blocks involved variation in the size of the log piles stacked nearby. In this experiment, more buprestids were consistently caught on the two trees near which the larger oak logs were being piled (Figure 3A).

Further individual comparisons were made with respect to the buprestids caught on different trap types (Figure 2). Green plastic branch-traps caught significantly more buprestids than any of the others. The leaf-covered and white-uncovered traps caught fewer buprestids, at levels that were of similar statistical significance. The purple plastic branch-traps and the sticky-leaf traps caught a similar number of beetles, both numbers being lower than those of the other trap designs. Because of the strong obvious functional superiority of the green plastic traps over the other designs for catching buprestids, for most further statistical analyses we included only the data from green branch-traps. When the cumulative logit model is applied to the total number of buprestids caught in only the green traps, the effect of the visual decoy in attracting beetles (the '+' in Figure 2) becomes strongly significant ($\chi^2 = 7.49$, d.f. = 1, $P = 0.0062$).

For each of the five most common species, it was also readily apparent that the green plastic branch-traps were much more effective than other designs (Figure 4). For all

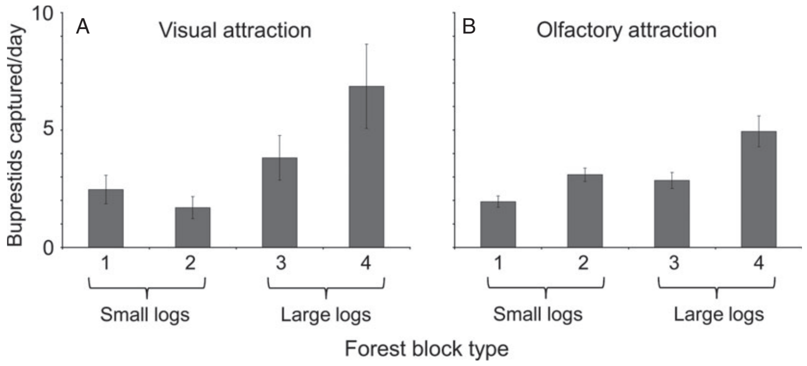

Figure 3 Mean (± SE) number of buprestids per day on green branch-traps in the (A) visual-attraction and (B) odor-lure experiments with respect to the forest type blocks where larger or smaller log piles were stacked near the traps as described in the text. For the visual-attraction experiment, n = 22 and 26 at the two small and two large log pile sites, respectively, which significantly differ using the cumulative logit model ($\chi^2 = 4.82$, d.f. = 1, $P = 0.028$). For the olfactory attraction experiment, n = 88 and 104 at the small and large log pile sites, respectively, which are not significantly different using the cumulative logit model ($\chi^2 = 0.29$, d.f. = 1, $P = 0.49$). If the data for the two experiments are combined, there is no significant difference between the sites ($\chi^2 = 1.93$, d.f. = 1, $P = 0.16$).

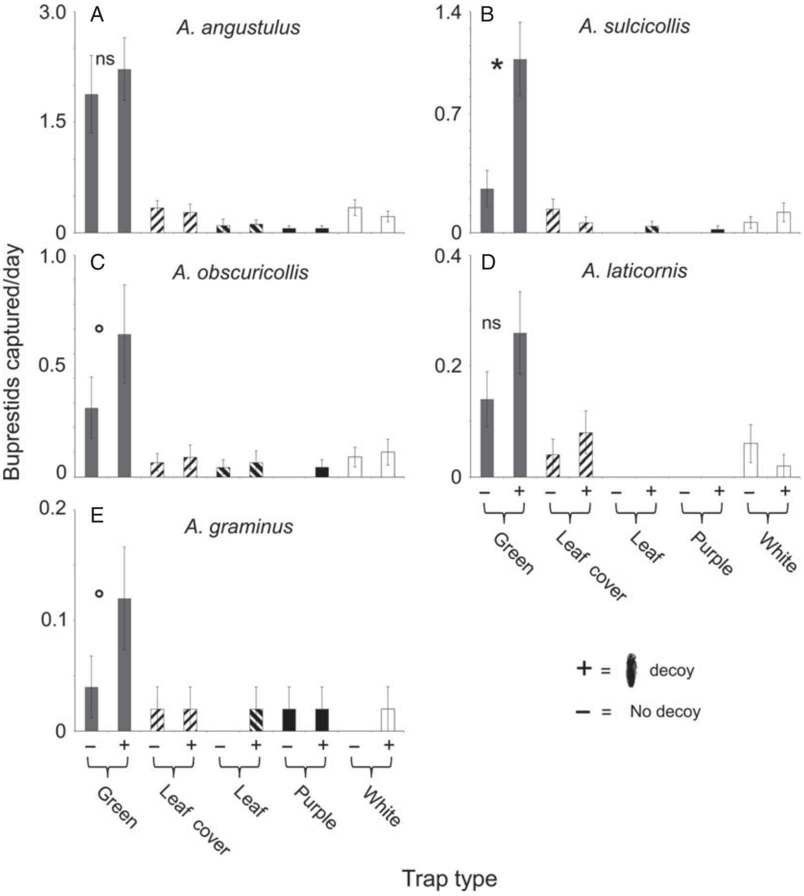

Figure 4 Mean (± SE) number of common buprestid species caught per day on each trap in the visual-attraction experiment. Species include (A) *Agrilus angustulus*, (B) *A. sulcicollis*, (C) *A. obscuricollis*, (D) *A. laticornis*, and (E) *A. graminus*. There were n = 48 total observation for each decoy and trap type combination. + and – signs indicate the presence or absence of the visual decoy. Following the cumulative logit model described in the text, the effect of the visual decoy on the green branch-traps was evaluated with significance levels: ns, $P>0.05$; *$P<0.001$; °$0.05<P<0.06$.

of these species, more beetles were caught on traps having EAB visual decoys on them. However, this effect was only strongly significant for *A. sulcicollis* (Figure 4B). The captures of both *A. obscuricollis* and *A. graminus* were marginally significantly increased when a visual decoy was added, whereas captures of *A. angustulus* and *A. laticornis* were not influenced by the presence of the decoy to a statistically significant degree.

It was not possible to estimate several of the factors in the cumulative logit model for the less common buprestid species. For these more rare species, a more limited analysis is performed below in combination with the data from the odor experiment. However, it was possible to do such a cumulative logit model analysis for the Elateridae collected, which saw an overall daily trap collection rate of 0.92 beetles per trap. For elaterids, there were effects attributable to the day of trapping ($\chi^2 = 28.98$, d.f. = 12, $P = 0.004$), rotation cycle ($\chi^2 = 8.69$, d.f. = 3, $P = 0.034$), and block location ($\chi^2 = 28.90$, d.f. = 3, $P<0.0001$), whereas there were no significant effects of trap type ($\chi^2 = 8.44$, d.f. = 4, $P = 0.077$) or visual decoy ($\chi^2 = 0.65$, d.f. = 1, $P = 0.42$).

Odor baiting in lower branches

It was again obvious that green plastic branch-traps were far superior to purple branch-traps or sticky-leaf traps in the odor-baiting experiments (Figure 5). For this reason, all statistical analyses concerning the odor-baiting experiment were performed only using the data for these green traps. There were strongly significant effects of day of trap collection ($\chi^2 = 110$, d.f. = 12, $P<0.0001$), trap rotation ($\chi^2 = 17.3$, d.f. = 3, $P = 0.0006$), and block location ($\chi^2 = 17.9$, d.f. = 3, $P = 0.0005$). There was also a significant effect of odor ($\chi^2 = 8.91$, d.f. = 3, $P = 0.031$). All of the odor-baited treatments caught more buprestids than the controls, but such a comparison was only significant when comparing (*Z*)-9-tricosene to the controls (Figure 5). There was only a marginally significant effect of visual decoy for total trap captures in this experiment ($\chi^2 = 3.39$, d.f. = 1, $P = 0.065$). The block location effect, although significant overall, did not include a significant difference with respect to the size of the log piles stacked nearby (Figure 3B).

For individual species, we again consider only the green plastic branch-traps (Figure 6). The captures of

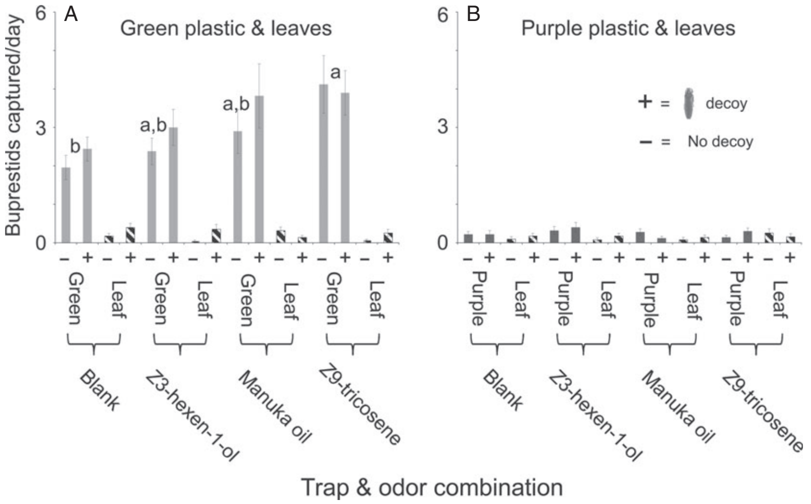

Figure 5 Mean (± SE) number of total buprestids per day on each trap in the odor-lure experiment. Data are organized by odor type, trap design, and presence of visual decoy. In some cases, leaf traps were co-localized (A) on trees with green plastic traps, and in others (B) with purple plastic branch-traps. There were n = 96 trap collections for each odor and trap combination. A cumulative logit model as described in the text was applied to only the green branch-traps. Similar letters associated with the odor type for these traps indicate no significant difference in the buprestid attraction at $\alpha = 0.05$.

A. angustulus (Figure 6A) closely mirror those of total buprestids (Figure 5). When the cumulative logit model is applied, there is a significant effect of odor ($\chi^2 = 14.8$, d.f. = 3, P = 0.002), but not of visual decoy ($\chi^2 = 2.11$, d.f. = 1, P = 0.15). When individual comparisons were made, captures on (*Z*)-9-tricosene traps were greater than those on unscented traps of the other odor treatments (Figure 6A). Trap capture of *A. sulcicollis* in this experiment was also significantly increased by the presence of odor ($\chi^2 = 22.3$, d.f. = 3, P<0.0001), with all the odor lures significantly increasing captures vs. the control (Figure 6B). Among all the species trapped, *A. sulcicollis* was the only one to exhibit even a marginally significant effect of the presence of a visual decoy lure ($\chi^2 = 3.54$, d.f. = 3, P = 0.060). When performing individual comparisons regarding visual decoy effects within odor treatments, only the manuka oil-baited traps showed a significant effect (Figure 6B). None of the remaining common species were significantly affected by the presence of odor lures or the visual decoy (Figure 6C–E, details of statistical analyses not shown).

The cumulative logit model was also applied to the Elateridae collections in the odor-baiting experiment. There were significant effects of day of trapping ($\chi^2 = 53.7$, d.f. = 12, P<0.0001), rotation cycle ($\chi^2 = 13.3$, d.f. = 3, P = 0.004), block location ($\chi^2 = 26.3$, d.f. = 3, P<0.0001), and with respect to the use of the larger branch-traps vs. the smaller sticky-leaf traps ($\chi^2 = 33.1$, d.f. = 1, P<0.0001). There was an average of 0.18 click beetles per leaf trap and 1.7 per branch-trap. However, there were no significant differences between green vs. purple branch-traps ($\chi^2 = 2.31$, d.f. = 1, P = 0.13), among the odor treatments ($\chi^2 = 3.62$, d.f. = 3, P = 0.31) or between decoy-baited vs. visually unbaited traps ($\chi^2 = 0.11$, d.f. = 1, P = 0.74).

Trap distribution of rare species in the branch- and leaf traps

For *A. biguttatus*, *A. olivicolor*, and *Anthaxia* spp., the total numbers trapped were too small to analyze with the complete cumulative logit model. However, there were sufficiently high captures to reveal certain patterns which were of particular importance for the aggressive pest *A. biguttatus.* Of 14 captures of *A. biguttatus*, 13 were on traps with EAB visual decoys (Table 4). Because there were equal numbers of traps possessing vs. lacking visual decoys, we could perform a simple χ^2 test vs. the expectation that visually unbaited vs. visually baited traps would have equal numbers of captures. This test shows a highly significant effect of the decoy. Ten of the captures were on green plastic branch-traps, whereas three were on simple sticky-leaf traps and one was on a white branch-trap. In a χ^2 test comparing captures on green vs. purple plastic branch-traps, which were equally dispensed throughout both experiments, there was a strongly significant preference of *A. biguttatus* for the green branch-traps. However, it is also interesting to note that even with the strong

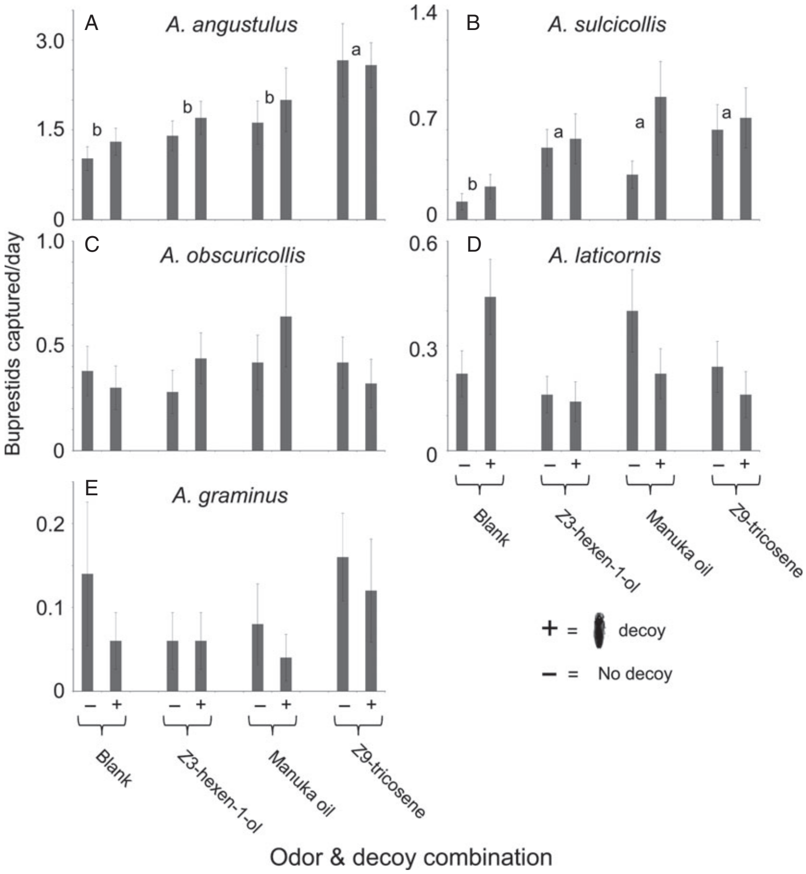

Figure 6 Mean (± SE) number of buprestid from common species caught per day on each green plastic branch-trap in the odor-lure experiment. Species include (A) *Agrilus angustulus*, (B) *A. sulcicollis*, (C) *A. obscuricollis*, (D) *A. laticornis*, and (E) *A. graminus*. There were n = 96 trap collections for each odor and trap combination. For *A. angustulus* and *A. sulcicollis*, similar letters within the subfigures indicate no statistically significant differences among the odor baits ($\alpha = 0.05$); there are no significant odor effects for the remaining species.

tendency of attraction toward green (green plastic branch or simple sticky-leaf) traps with decoys, there were equal numbers of females and males caught in this experiment. Despite the small sample size, it is notable that only males were caught on sticky-leaf traps and on green branch-traps baited with (*Z*)-3-hexen-1-ol. Alternatively, the few captures on green branch-traps baited with manuka oil or (*Z*)-9-tricosene were all females.

We could find no preference for visual decoy-baited vs. unbaited traps for *A. olivicolor* (Table 5). Also, this species was caught six times on purple plastic traps, vs. 10 times on green plastic traps, which does not indicate a significant difference. This species was caught just once on a sticky-leaf trap, and also once on one of the simple white branch-traps that were only deployed in the visual-attraction experiment.

Finally, we combined the 14 total captures among the three *Anthaxia* species to perform similar analyses (Table 6). Members of this genus were found more often on traps having visual decoys than without, and on green vs. purple branch-traps. All of the remaining species were caught only four times or less (Table 2), making the statistical power too low for any statistical analysis with respect to trap type distribution.

Discussion

The two largest *Agrilus* species in our trap collection, *A. biguttatus* and *A. sulcicollis*, exhibited the clearest attraction to EAB visual decoys in the green plastic branch-traps. These also happen to be the species of greatest economic concern, owing to the known tree killing outbreaks of *A. biguttatus* in Europe (Moraal & Hilszczanski, 2000; Vansteenkiste et al., 2005), and the potential impacts of the recent introduction of *A. sulcicollis* in North America (Haack et al., 2009). Although there were only 14 overall captures of *A. biguttatus*, it is quite noteworthy that on traps lacking visual decoys, this species was only captured once and thus nearly escaped detection. There was no tree mortality associated with *A. biguttatus* reported in this region in 2011, suggesting that the population was likely relatively low, yet 10 green plastic branch-traps caught individuals of this species. Thus, distributing a wide array of these small economical

Table 4 Description of the 14 captures of *Agrilus biguttatus* during both the odor- and visual-lure experiments, including the sex and the trap characteristic information. Comparisons were evaluated against the assumption of equal likelihood of landing on green vs. purple traps (ratio = 10:0; $\chi^2 = 10.0$, d.f. = 1, $P = 0.002$) and decoy-baited vs. visually unbaited traps (ratio = 13:1; $\chi^2 = 10.3$, d.f. = 1, $P = 0.001$)

Frequency	Sex	Trap type	Surface	Decoy	Odor
2	Male	Leaf	Leaf	+	–
1	Male	Leaf	Leaf	+	(*Z*)-3-hexen-1-ol
1	Male	Branch	Green	+	–
3	Male	Branch	Green	+	(*Z*)-3-hexen-1-ol
3	Female	Branch	Green	+	–
1	Female	Branch	Green	+	(*Z*)-9-tricosene
1	Female	Branch	Green	–	Manuka oil
1	Female	Branch	Green	+	Manuka oil
1	Female	Branch	White	+	–

Table 5 Description of the 18 captures of *Agrilus olivicolor* during both the odor- and visual-lure experiments with respect to trap characteristic information. Comparisons were evaluated against the assumption of equal likelihood of landing on green vs. purple traps (ratio = 10:6; $\chi^2 = 1.00$, d.f. = 1, $P = 0.32$) and decoy-baited vs. visually unbaited traps (ratio = 9:9; $\chi^2 = 0$, d.f. = 1, $P = 1.0$)

Frequency	Trap type	Surface	Decoy	Odor
1	Leaf	Leaf	–	–
3	Branch	Green	–	–
2	Branch	Green	–	Manuka oil
1	Branch	Green	–	(*Z*)-9-tricosene
2	Branch	Green	+	–
1	Branch	Green	+	(*Z*)-3-hexen-1-ol
1	Branch	Green	+	Manuka oil
1	Branch	Purple	–	Manuka oil
1	Branch	Purple	+	–
2	Branch	Purple	+	(*Z*)-3-hexen-1-ol
1	Branch	Purple	+	Manuka oil
1	Branch	Purple	+	(*Z*)-9-tricosene
1	Branch	White	–	–

traps may prove to be an effective detection tool for *A. biguttatus*. Future field comparisons of these traps vs. the established, large prism, and funnel traps for *A. planipennis* detection will be necessary to evaluate the feasibility and cost-effectiveness of using these two trapping approaches.

It is also interesting that the increased captures in green plastic branch-traps containing visual decoys was much more pronounced in the visual-attraction-only experiment, in which there was no deployment of odor packets (Figures 2 and 4), compared to the odor-added experiment (Figures 5 and 6). This difference cannot be attributed to the odor lures because captures in the non-odor control traps in the odor-baiting experiment also were not strongly increased by the presence of the visual decoy. It is possible that the characteristics of the trees selected for the two experiments explain this phenomenon. Large trees in the very open areas with numerous sun-exposed branches were selected for the visual-attraction-only experiment, whereas trees in more densely forested areas having fewer branches exposed to sunlight were used for the odor-added experiment. Similar to *A. planipennis* (Lelito et al., 2007), both *A. biguttatus* and *A. sulcicollis* have been directly observed to only mate on the surfaces of brightly sunlit foliage (Domingue et al., 2011), and these were the two species' captures that were most strongly increased by the presence of visual decoys.

Previous trapping of EAB using visual decoys indicated a strong male bias (Lelito et al., 2008; Domingue et al., 2013). However, in this study both males and females of *A. biguttatus* were attracted. This result was somewhat unexpected given that the traps were designed based on our previous field observations of *A. biguttatus* males' visually mediated approaches to, and prolonged copulation attempts with, decoys on leaves (Domingue et al., 2011). Our observations mirrored for *A. biguttatus* what had previously been described for EAB males' in their responses to visual decoys (Lelito et al., 2007). A key difference, though, is that our present study involved captures occurring primarily on green cards having a different size, shape, and color emission from leaves; the few cases of *A. biguttatus* being captured on sticky leaves with decoys were of males only. It may be that female *A. biguttatus* are being attracted to other beetles for some yet-to-be-determined purpose, such as for locating preferential feeding and mate-attracting sites. Better replicated observations of female *A. biguttatus* behavior toward traps and visual decoys will be needed to understand this phenomenon.

The diversity of *Agrilus* species captured in these studies suggests that green plastic branch-traps may be useful for a range of species, especially those known to be of concern in North America: *A. planipennis* (Haack et al., 2002), *A. bilineatus* (Cote & Allen, 1980), *Agrilus anxius* Gory (Ball & Simmons, 1986), *A. auroguttatus*, and *A. coxalis* Waterhouse (Hespenheide & Bellamy, 2009), or in Europe: *Agrilus populneus* Schaefer (Csóka & Kovács, 1999). At the same time, there may be a limit to the generality of such a trap for non-*Agrilus* spp. buprestids. Only one specimen of *Coraebus florentinus*

Table 6 Description of the 14 captures of *Anthaxia* species during both the odor- and visual-lure experiments with respect to trap characteristic information. Comparisons were evaluated against the assumption of equal likelihood of landing on green vs. purple traps (ratio = 11:1; $\chi^2 = 8.33$, d.f. = 1, P = 0.004) and decoy-baited vs. visually unbaited traps (ratio = 11:3; $\chi^2 = 4.57$, d.f. = 1, P = 0.033)

Frequency	Species	Trap type	Surface	Decoy	Odor
1	*A. nitidula*	Leaf	Leaf	+	–
1	*A. nitidula*	Branch	Green	–	–
1	*A. nitidula*	Branch	Green	–	(*Z*)-9-tricosene
1	*A. salicis*	Branch	Green	–	–
1	*A. fulgurans*	Branch	Green	+	–
1	*A. fulgurans*	Branch	Green	+	Manuka oil
1	*A. nitidula*	Branch	Green	+	(*Z*)-3-hexen-1-ol
1	*A. nitidula*	Branch	Green	+	(*Z*)-9-tricosene
2	*A. nitidula*	Branch	Green	+	–
1	*A. salicis*	Branch	Green	+	(*Z*)-3-hexen-1-ol
1	*A. salicis*	Branch	Green	+	–
1	*A. salicis*	Branch	Purple	+	(*Z*)-3-hexen-1-ol
1	*A. salicis*	Branch	White	+	–

Herbst was collected in this experiment, despite the expectation that a relatively robust population should be present at this location. This species has received recent attention as a pest associated with crown dieback in European oak forests (Jurc et al., 2009). Although it is a protected species recently in Hungary, time to time this *Coraebus* species causes rather widespread and locally significant branch mortality (Koltay & Leskó, 1991). Further research into the behavioral attributes of this species would be needed to determine whether green branch-traps with visual decoys could be adapted toward its detection.

The odors used to bait the traps in the odor-added experiments did tend to increase captures, but not to a very large extent, and not consistently for all of the species (Figure 6). *Agrilus angustulus* and *A. sulcicollis* were the only species showing a clear pattern of trap-catch increases when odors were added. Furthermore, *A. sulcicollis* was the only species whose captures in the odor-added experiment were additionally increased by the presence of a visual decoy, but there was a far greater effect of the decoys in the visual-only experiment (Figure 4 compared to Figure 6). This difference may again be due to the more sunlit trees used for the visual-trapping experiment, underscoring the importance of trap placement.

It was somewhat surprising that (*Z*)-9-tricosene was the odorant whose emission increased *Agrilus* trap captures to the greatest degree. We chose this odorant based on its inexpensiveness and its chemical similarity to the more volatile of the two known *A. planipennis* contact sex pheromone components, 3-methyltricosane (Lelito et al., 2009) and 9-methylpentacosane (Silk et al., 2009). No research has directly been focused on determining whether contact pheromones are used by any of the European oak buprestid species trapped in this study. Further research into the possible contact pheromones of these species would be necessary to explain this attraction and perhaps improve such compounds as potential long-range attractants. In the meantime, it appears that (*Z*)-9-tricosene should be examined to see whether it might be a useful addition to *A. planipennis* monitoring traps for improving their detection capabilities.

In a previous study, green plastic cards were not as effective as sticky leaves for catching *A. planipennis* (Domingue et al., 2013). However, in that trapping study a ‘dry’ adhesive-type was used that lost its tackiness after less than 2 days in the sunlight. For this study, we used Tanglefoot™ for all traps, which is a standard wet and highly tacky adhesive. Because of this and other design changes, the past result is not comparable to the result of this study. However, it is interesting that across all species the green plastic branch-traps performed better than the simple sticky-leaf traps. They also performed much better than the leaf-covered branch-traps, which controlled for other trap characteristics.

It is also noteworthy how poor the purple plastic branch-traps were at capturing significant numbers of buprestids. For *A. planipennis* purple plastic has sometimes been more effective than green plastic in the prism trap design, in which trapping surfaces are oriented perpendicularly to the ground (Francese et al., 2005). Because our branch-traps are oriented skyward to catch rays of direct sunlight in foliage where *Agrilus* spp. have been seen to be active, it seems likely that the visual image of the green plastic surface evokes attraction to leaves, whereas the purple does not.

Acknowledgements

Funding was provided by the USDA-APHIS program supporting the Development of Detection Tools for Exotic Buprestid Beetles. Miklós Tóth of the Hungarian Academy of Science also helped to facilitate travel arrangements and contributed to discussions about the research. The Hungarian Forest Research Institute aided in the location of field sites (grant TAMOP-42.2A-11/1/KONV). Bettina Pásztor of Corvinus University and Levente Szőcs of the Hungarian Forestry Research Institute provided assistance with trap collection.

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