

ORIGINAL CONTRIBUTION

Visual and chemical cues affecting the detection rate of the emerald ash borer in sticky trapsM. J. Domingue¹, J. P. Lelito¹, I. Fraser², V. C. Mastro³, J. H. Tumlinson¹ & T. C. Baker¹¹ Department of Entomology, Center for Chemical Ecology, The Pennsylvania State University, University Park, PA, USA² USDA APHIS PPQ, Brighton, MI, USA³ USDA APHIS PPQ CPHST, Otis Laboratory, Otis ANGB, MA, USA**Keywords***Agrilus planipennis*, Buprestidae, manuka oil, mate-finding, phoebe oil**Correspondence**Michael J. Domingue (corresponding author),
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Abstract

Using sticky traps, we compared the efficacy of chemical and visual lures, both alone and in combination, for improving the detection of populations of the emerald ash borer (EAB), *Agrilus planipennis*. Ash leaflets to which EAB visual decoys were pinned and coated with sticky material were able to trap EAB with as high a rate of detection as large sticky visually unbaited 'prism traps' currently used in wide-scale EAB surveillance programs in North America, in a high-density area. Both the sticky leaf traps and prism traps captured more EAB when a point source of plant odours, either manuka or phoebe oil, was deployed with the trap. For the sticky leaf traps, the shape of the EAB visual decoy lure was found to be important in optimizing the detection rate. Either an entire dead beetle or else two elytra placed side by side to mimic a resting beetle resulted in optimal trap performance. When two elytra were placed end to end or else other body parts were deployed, the traps lost their efficacy. Small green plastic surfaces to which EAB visual decoys were pinned were found to be fairly good substitutes for live ash leaflets, but the rate of beetle detection was reduced significantly from that of the ash leaflet plus EAB decoy. Throughout all experiments, a clear male bias occurred in sticky leaf traps when EAB visual decoys were placed on the traps. The implications of these findings for developing new trapping designs for EAB and other forest buprestids are discussed.

Introduction

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is an invasive beetle species that is an increasingly serious threat to North America's native ash trees (Haack et al. 2002). EAB attacks all North American ash tree species in the genus *Fraxinus* and is causing high ash mortality wherever it has spread (Poland and McCullough 2006). Efforts to develop an easily deployable, inexpensive and effective means of detecting EAB have been limited by the inability to identify a sex pheromone or another strong, behaviour-modifying volatile attractant (Cap-

paert et al. 2005; Poland and McCullough 2006). Furthermore, the currently used 3-sided large sticky traps ('prism traps') must be placed high in trees to increase effectiveness (Francese et al. 2008).

One early and continuing focus of research into improving the effectiveness of sticky traps for EAB has been the colour and structural characteristics of prism traps (Francese et al. 2005, 2011). At the same time, other studies have sought to develop host odour-related attractants after it was noted that EAB are attracted to stressed ash trees (Poland and McCullough 2006; Crook et al. 2008; McCullough et al. 2009). Several components found in ash tree

volatile collections also exist in essential oils, such as manuka and phoebe oil, which increase EAB captures on prism traps (Crook et al. 2008). 'Green leaf' volatiles have also been identified that evoke electroantennogram responses (Rodriguez-Saona et al. 2006; de Groot et al. 2008). One of these common plant volatiles, (*Z*)-3-hexenol, was found to increase EAB trap capture in field experiments (de Groot et al. 2008; Grant et al. 2010).

Several seasons of field observations showed that mate location is dominated by males responding visually to females sitting in bright sunlight on ash leaflets (Lelito et al. 2007, 2008). Males patrolling the tree canopy rapidly descend to land directly onto females from a height of ca. 0.3–1 m (Lelito et al. 2007, 2008). The descending flights of males can be evoked readily by dead EAB males or females that have been pinned to leaves (Lelito et al. 2007). Removal of any possible semiochemicals from the dead 'visual decoy' models by solvent washing does not reduce the attraction of males. Recent studies involving other species of tree-feeding buprestids have all shown similar visually mediated flight towards beetle models, even towards heterospecific models, indicating a common exploitable behavioural template (Domingue et al. 2011; Lelito et al. 2011). When an EAB visual decoy is coated with a spray-on sticky material, this behaviour can be exploited to produce a highly effective trap (Lelito et al. 2008).

Overall, our goal was to gain insight into creating a more durable and effective EAB trap by building upon the trapping knowledge that had accumulated from these many earlier studies. First we wanted to elucidate the effect of the shape of the visual decoy in attracting EAB males to the sticky leaf trap surfaces. We also wished to evaluate the importance of the spatial precision of essential oil additions to different traps, including sticky leaves with or without EAB visual decoys and sticky prism traps.

Materials and Methods

Visual cue test

First we compared the use of different portions of the bodies of dead EAB specimens as visual decoy lures. Whole beetles or their parts were pinned to the terminal leaflet of an ash leaf. Unbaited leaves were used as blank controls. The experiment consisted of the following treatments, all on an ash leaflet, and all comprised of female EAB body parts. The treatments were the following: (i) a blank ash leaf-

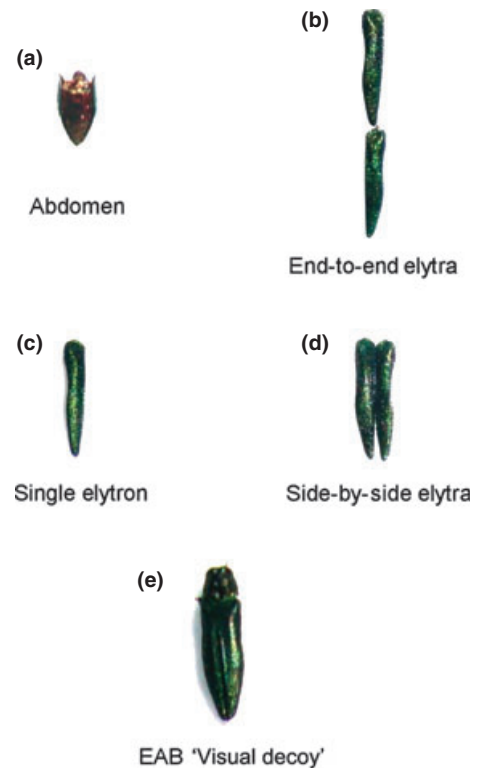


Fig. 1 Visual stimuli used in the visual cue test: (a) Emerald ash borer (EAB) abdomen, (b) two elytra arranged end to end, (c) a single elytron, (d) two elytra placed side by side, and (e) a whole dead EAB 'visual decoy'.

let, (ii) an EAB abdomen (fig. 1a), (iii) two EAB elytra pinned longitudinally, end to end (fig. 1b), (iv) a single EAB elytron (fig. 1c), (v) two EAB elytra pinned side by side (fig. 1d) and (vi) a whole, dead, pinned EAB (fig. 1e).

On 11 June 2008, a complete set of each of these treatments of leaf-based traps was pinned to the terminal leaflets of ash leaves on the southern side of an ash tree at 4 m height, all of which were sprayed with Tangle-Trap[®]. The treatments were removed and replaced onto the next nearest fresh leaflets on 20 June 2008 and 28 June 2008 to avoid decay (Lelito et al. 2008). We replicated this experiment on four ash trees that were between 20 and 25 m apart. Each tree had visible EAB emergence holes in the trunks, but also had remaining viable foliage on at least half the tree. Here, and in other experiments, we used known infestation sites with high densities of EAB to ensure that all trap designs had some chance for success at detecting EAB. As an additional control in each tree, one unbaited purple prism trap and one unbaited green prism trap were hung from a rope at 4 m height. All leaf-based and prism traps

were separated by at least 2 m from one another. The prism traps were scraped to remove the Tangle-Trap and any captured insects. The prism traps were then re-glued at the same time as the leaf-based traps were replaced.

We checked all the traps for the presence of adult EAB and other insects twice weekly until 8 July 2008. Adult EAB found on the traps were removed, sexed, counted and discarded. Other buprestids and insects similar in size to EAB were collected from these traps, but they were rare and their abundance was not further analysed. This experiment was performed at two EAB-infested sites near the town of Williamston in Ingham County, Michigan.

Green plastic sticky cards

In the next experiment, we attempted to find an alternative to using living ash leaflets as the surface for decoys. We used green 10 × 10 cm plastic cards having peak reflectance at 540 nm similar to ash foliage, which we obtained from ChemTica International S.A., Costa Rica. We prepared a total of 64 such cards, to which we applied a 'dry' adhesive provided by ChemTica International S.A. After melting the adhesive at 100°C and applying it to the cards with a paint scraper, the sticky substance cooled to result in a surface tackier to the touch than Tangle-Trap. To half the sticky card traps, we pinned a single, dead adult female EAB visual decoy, and the remaining control traps were left blank. Both sets of traps were wrapped in wax paper and stored until use. When deployed, the sticky card traps were affixed with metal twist ties over several ash leaflets using holes punched in each corner of the card. Once the sticky cards were tied to leaves, we sprayed the dead EAB pinned to the centre of each trap with spray-on Tangle-Trap to ensure the EAB decoy was covered in adhesive.

Four ash trees separated by 20–25 m were selected that had visible EAB exit holes and living foliage, as described for the previous experiment. At both 2 and 4 m heights, we placed the following traps: (i) one EAB-baited sticky leaf, (ii) one control sticky leaf lacking an EAB, (iii) one sticky card with an EAB affixed, and (iv) one control sticky card. In each tree, we also hung one unbaited purple prism trap from a rope over a branch at 4 m height.

We placed all traps associated with this experiment into trees on 6 and 7 June 2008 and subsequently replaced all traps on 20 June 2008. We checked all traps for the presence of adult EAB and similar-sized insects twice weekly, recording the number and gender of adult EAB captured. We

checked all traps and removed them from the ash trees on 7 and 8 July 2008. This experiment was performed at two EAB-infested sites, one near the town of Pinckney in Washtenaw County, Michigan, and the other near the town of Williamston in Ingham County, Michigan.

Effect of manuka oil volatiles as point sources or tree sources

Manuka oil dispensers were provided as thin plastic packets by ChemTica International in Costa Rica. The dispensers had been measured to release 25 mg/day of the total ensemble of manuka oil volatiles for 45 days. We located six trees at each of the locations used in these experiments, which had visible EAB exit holes while still retaining some healthy foliage. Within each site, we assigned three classes of trees that differed with respect to the placement of the supplemental odour lures directly at traps (point source) versus merely somewhere in the same tree (remote source) (fig. 2).

All traps were hung at 4 m in the tree, and all treated trees were separated by between 20 and 25 m. All volatile lure packets were rotated randomly between traps weekly. The experiment was duplicated in two EAB-infested forest/agricultural edge sites in Ingham and Washtenaw counties in south-eastern Michigan. Traps were deployed on 9 and 10 June 2008 and were checked for EAB as above twice weekly through 6 July 2008.

Effect of phoebe oil volatiles as point sources or tree sources

Phoebe oil dispensers were also deployed as thin plastic packets obtained from ChemTica International that released 25 mg/day of the ensemble of phoebe oil volatiles (Crook et al. 2008). The set-up for this experiment was identical to that used in the manuka oil lure experiment outlined above (fig. 2), except that the prism traps in this experiment were green rather than purple and the volatile lures emitted phoebe oil volatiles. Sites included a forested area and an agricultural forest-edge habitat near Ingham County, Michigan. All traps were placed into ash trees on 9 and 10 June 2008 and were checked twice weekly through 7 July 2008.

Statistical analyses

For each experiment, the mean and standard error for number of males and females caught by each

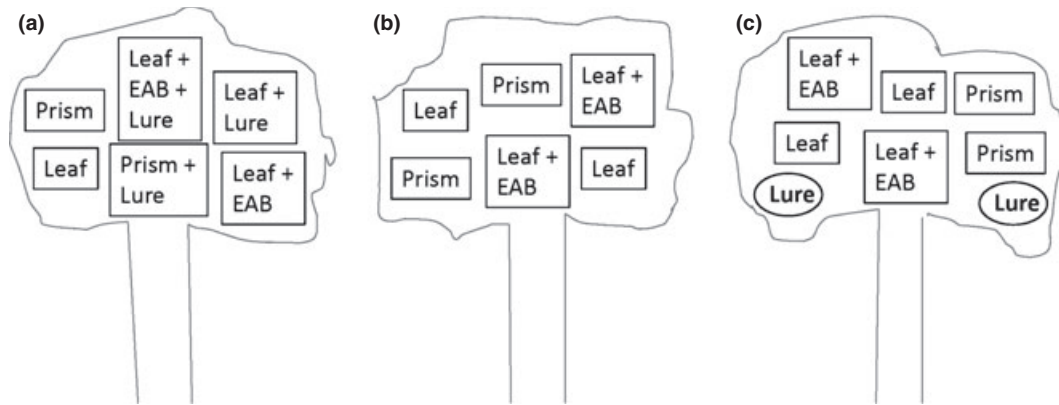


Fig. 2 Lure and trap arrangement for volatile lure experiments. Type A trees had the following point-source treatments: (i) a purple prism trap with a manuka oil lure hung from the centre, (ii) an emerald ash borer (EAB) decoy-baited sticky leaf trap with a manuka oil lure hung from the petiole of the leaf and (iii) a leaf trap with no visual decoy and a manuka oil lure hung from the petiole. Type A trees also had remote-source treatments lacking odour lures including (iv) a purple prism trap, (v) an EAB decoy-baited leaf trap and (vi) a leaf trap with no visual decoy. Type B trees had (i) a point-source manuka-baited purple prism trap and non-volatile-baited traps (remote source) including (ii) a purple prism trap, (iii) two EAB decoy-baited sticky leaf traps and (iv) two sticky leaf traps lacking an EAB decoy. To make the total manuka oil volatile emission of the tree similar to that of type A trees, each type B tree also contained two additional manuka oil lures that were hung from the petioles of leaves at least 1 m from all other traps. Type C trees received no manuka oil lures and contained two purple prism traps, two EAB decoy-baited sticky leaf traps and two sticky leaf traps lacking an EAB lure.

trap treatment were computed. We also computed the trap captures for each treatment as a surface-area-adjusted quantity of beetles/m²/day, for the first two experiments, in which a number of different trap designs were employed, to provide some indication of how relatively effective the traps are at providing an attractive substrate for landing. However, no attempt is made to generate statistical models capable of testing hypotheses of differences between traps at a per-area scale. The large number of empty trap captures in certain treatments caused substantial deviation from the distributive assumptions necessary for analysis of variance using either total trap captures or area-adjusted trap captures.

Most comparisons were based on the per-trap detection rate at each trap check, which is meaningful from a management perspective if the goal is to track the expanding host range of this invasive species. Furthermore, modelling the data as a binomial function whereby traps are deemed successful or not is a more statistically coherent mechanism for comparing a variety of traps that might differ greatly in numbers of beetles caught, or consistency of such catches. We employed PROC GENMOD in SAS (Version 9.2, SAS Institute, 2006) to fit logit link functions for generalized linear models to the data. This procedure allows the evaluation of experimental factors in a manner analogous to ANOVA, using likelihood ratio tests. The LSMEANS option in SAS provides Wald chi-square tests for comparing differ-

ences in detection rates between individual trap types. When such multiple individual comparisons were made between traps within an experiment, a Bonferroni correction was employed to conservatively test for differences in detection rate at a 0.05 experimental error rate. All statistical comparisons of trap designs for the first two experiments were made using this procedure.

For the prism traps within the manuka and phoebe oil experiments, the detection rates were uniformly near 100%. However, it was still desirable to assess the effects of these odours on trap captures. Thus, in this case only, ANOVA was employed using Proc GLM in SAS to analyse differences in the total numbers of males and females caught given different odour treatments. In these experiments, the other leaf-based traps, with or without visual lures, again experienced a wide range of detection rates, so the data were analysed separately using the binomial model described above.

Results

Visual cue test

Whole-beetle visual decoys were the most effective visual lures for the sticky leaf traps, followed by two elytra placed side by side, although the mean numbers captured on the sticky leaf traps were lower than those on the large prism traps (fig. 3a).

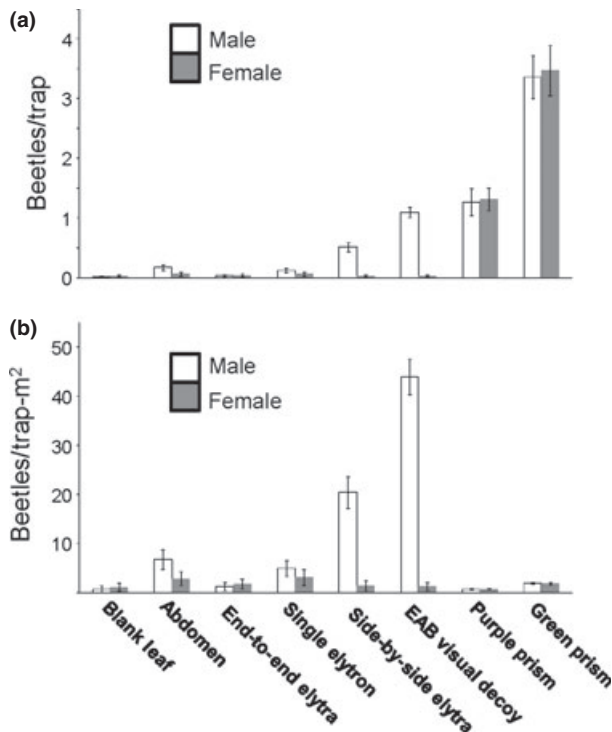


Fig. 3 Mean capture (\pm SE) of Emerald ash borer in sticky leaf traps containing various visual cues from the treatments shown in fig. 1 and from purple or green prism traps. (a) Mean captures per trap at each trap-check interval. (b) Mean captures per trap at each trap check adjusted for the surface area.

However, when the data were examined on a per-trap-surface-area basis, the sticky leaf traps with either the whole-body EAB decoy or the side-by-side elytra were more effective in trapping EAB than the large prism traps (fig. 3b). For these visual-decoy-based sticky leaf traps, the trap captures were strongly male biased (fig. 3b).

We compared the detection rates of the various traps, which are the percentage of deployed traps that captured at least one EAB during these experiments (fig. 4), and there was a significant effect of trap type, regardless of whether males, females or all beetles are considered (Table 1). These results further indicate that a single ash leaflet containing a visual whole-beetle-decoy lure did not have a significantly lower detection rate than purple or green prism traps when males only (fig. 4a) or all beetles (fig. 4b) are considered. This high detection rate for sticky leaves with visual decoys did not extend to females, which were detected at a significantly lower rate by all the leaf-based traps when compared with the prism traps (fig. 4a).

An adult flight period effect on detection as shown in Table 1 was common in all the analyses of

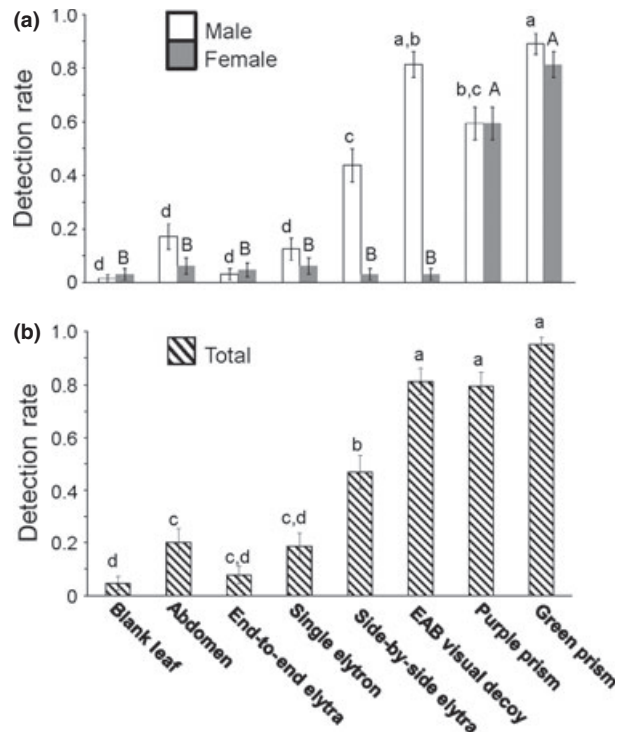


Fig. 4 Rate of detection of at least one beetle (\pm SE) of the various traps in the visual cue test at each trap check interval. (a) Trap detection rates of male vs. female emerald ash borer (EAB). (b) Trap detection rates for EAB irrespective of gender. Within each subfigure, treatments having no letters in common indicate significantly different proportions (Wald chi-square, Bonferroni correction, $\alpha = 0.05$). In a, comparisons for males use lower case and for females upper case. There were 64 replicates per trap type.

detection ability. In this case, it was reflective of low detection rates at the first trap check (19%), followed by more consistent rates near 50% for the remaining periods. Such a time period effect where all traps were less effective at the first trap check was common in all the experiments and was not further described and explored.

Green plastic sticky cards

Although whole-beetle visual decoys placed on green plastic sticky cards were not as good at capturing EAB as the sticky leaf traps at either a 2 or 4 m height in the tree (fig. 5a), they did capture large numbers of EAB males. The whole-beetle-visual-decoy sticky leaf trap deployed at 4m captured similar numbers of EAB males as the purple prism traps at that height. This high capture rate was not evident on sticky leaves without the visual decoy (fig. 5a). Purple prism traps also captured as many

Table 1 Summary of experimental effects on the detection rates observed in trap captures during visual cue test

Effect	d.f.	Male		Female		Total	
		χ^2	P	χ^2	P	χ^2	P
Tree	7	8.7	0.28	13.4	0.06	7.1	0.42
Period	7	47.9	<0.0001	33.0	<0.0001	46.9	<0.0001
Trap	7	292.8	<0.0001	261.4	<0.0001	304.6	<0.0001

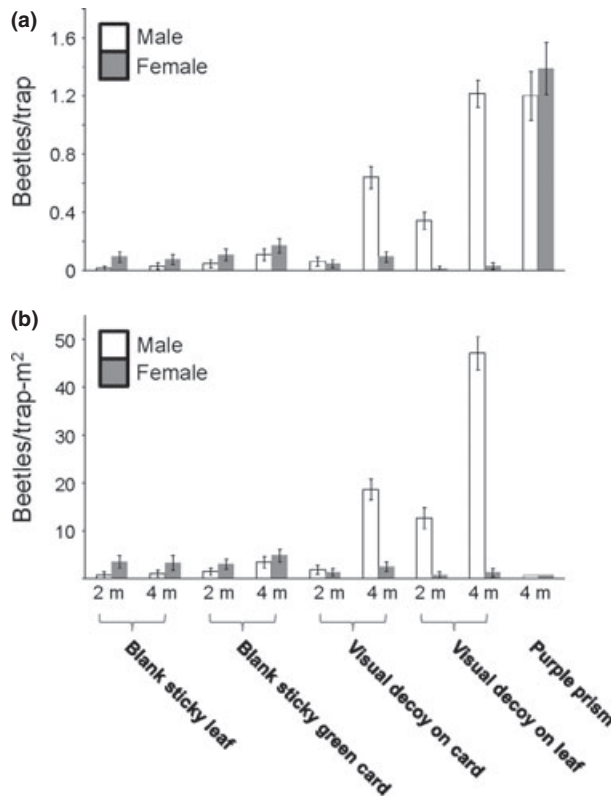


Fig. 5 Captures of beetles in the sticky green card experiment. (a) Mean captures per trap at each trap check. (b) Mean captures per trap at each trap check adjusted for the trap surface areas.

females as males, while females were essentially absent in the EAB decoy-baited leaf traps (fig. 5a). When considered from a trap-area-adjusted perspective, the EAB-baited green card traps at 4 m and the EAB-baited leaf traps at either height caught beetles at a far greater rate than any of the other traps (fig. 5b).

As in the first experiment, prism traps again provided a very high rate of detection when considering males alone, females alone, or their pooled capture (fig. 6b). Sticky leaf traps with EAB visual decoy lures at the 4m height had similarly high rates of

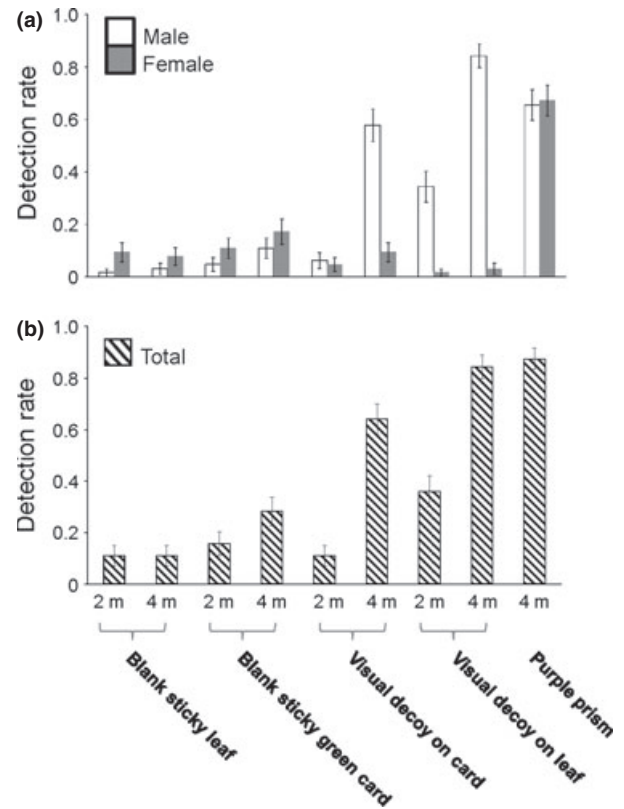


Fig. 6 Proportion \pm SE of (a) male and female and (b) total per-trap positive detection of beetles at each trap check for the green card experiment. There were 64 replicates per trap type.

detecting EAB as the prism traps (fig. 6b), due nearly entirely to their high male detection rates (fig. 6a). The detection rates of the sticky green cards with visual decoys were somewhat lower than the prism and sticky leaf traps at the 4 m height (fig. 6b), and again these cards captured mostly males (fig. 6a).

We used a $2 \times 2 \times 2$ factorial design that excluded the prism traps to explore the specific effects of decoy presence, trap surface, and trap height and their interaction on detection ability (Table 2, fig. 6). For the detection of males and overall detection rate, there

Table 2 Summary of experimental effects on the detection rates observed in trap captures during the green card experiment

Effect	d.f.	Male		Female		Total	
		χ^2	P	χ^2	P	χ^2	P
Tree	7	8.9	0.26	10.8	0.15	5.3	0.62
Period	7	37.1	<0.0001	34.0	<0.0001	42.3	<0.0001
Emerald ash borer decoy	1	98.7	<0.0001	10.1	0.002	59.6	<0.0001
Leaf vs card Surface	1	21.2	<0.0001	0.8	0.38	23.6	<0.0001
Trap height	1	31.3	<0.0001	2.2	0.14	46.2	<0.0001
Decoy*surface	1	0.6	0.46	6.1	0.01	1.6	0.20
Decoy*height	1	6.9	0.009	0.8	0.37	22.1	<0.0001
Surface*height	1	0.3	0.62	0.7	0.42	0.2	0.64

were strongly significant positive effects of the presence of visual decoys, leaf vs. plastic trap surfaces and trap height. For female detection, EAB decoys had a significant negative effect (Table 2, fig. 6a).

Effect of manuka oil volatiles as point or remote sources within trees

The emission of manuka oil increased the capture of EAB in both the purple prism traps and sticky leaf traps (fig. 7a). It was again clear that the prism traps caught the greatest total numbers of beetles, followed by manuka-baited visual decoy sticky leaf traps and then non-manuka-baited visual decoy sticky leaf traps. Manuka oil emission sources had a significant effect on the total numbers of both males and females caught in purple prism traps. The manuka lure needed to be located at the trap itself to observe a significant increase in comparison with control traps. Placing the dispenser in the tree away from the trap did not increase the trap capture significantly.

For comparing the effectiveness of leaf traps, under different odour treatments, it was again most illustratively appropriate to consider a binomial model for positive detection rate (Tables 3 and 4). Visual decoy traps containing both the visual decoy lures and emitting manuka oil volatiles had increased detection ability compared with the non-manuka controls (fig. 7c). Like the prism traps, the detection rate became nearly 100% (fig. 7b) in manuka oil and decoy-baited leaf traps. The capture of males in the decoy-baited sticky leaf traps when manuka oil was emitted remotely was intermediate, and not statistically significantly different from the point source or control treatments (fig. 7b). For sticky leaf traps containing no visual decoy, manuka oil volatile emission increased the capture of only female EAB and only when the manuka

oil volatiles were point source rather than remote-source emissions (fig. 7c).

Effect of phoebe oil volatiles as point sources or tree sources

As in the manuka oil experiment, the emission of phoebe oil increased the capture of EAB in both the (now green) prism traps and in the sticky leaf traps (fig. 8a). It was again clear that the prism traps caught a far greater total number of beetles, followed by visual-decoy-baited sticky leaf traps. There were significant effects of the placement of phoebe oil volatiles on green prism trap captures. The phoebe oil emission source had to be located at the trap itself in order for it to significantly increase green prism trap capture compared with the non-phoebe control traps. Placing the dispenser in the tree away from the trap had no such effect. The addition of phoebe oil volatiles at the trap itself more than doubled the captures in comparison with non-phoebe controls. The capture of male EAB in sticky leaf traps containing visual decoy lures and emitting phoebe oil volatiles was likewise more than doubled compared with the non-phoebe controls.

All of the main effects and most of the specific comparisons involving trap and odour treatments had significant effects on detection rate, with the exception of remote-source odour placement versus odourless controls (Table 4). The visual-decoy-baited leaf traps with a point-source manuka lure and all prism traps have similar detection rates near 100% (fig. 8c). When phoebe oil was emitted within the trees but away from the traps, detection rates and captures of EAB in the visual-decoy-sticky-leaf traps still increased significantly in comparison with the non-phoebe control trees (fig. 8c). Again, the detection

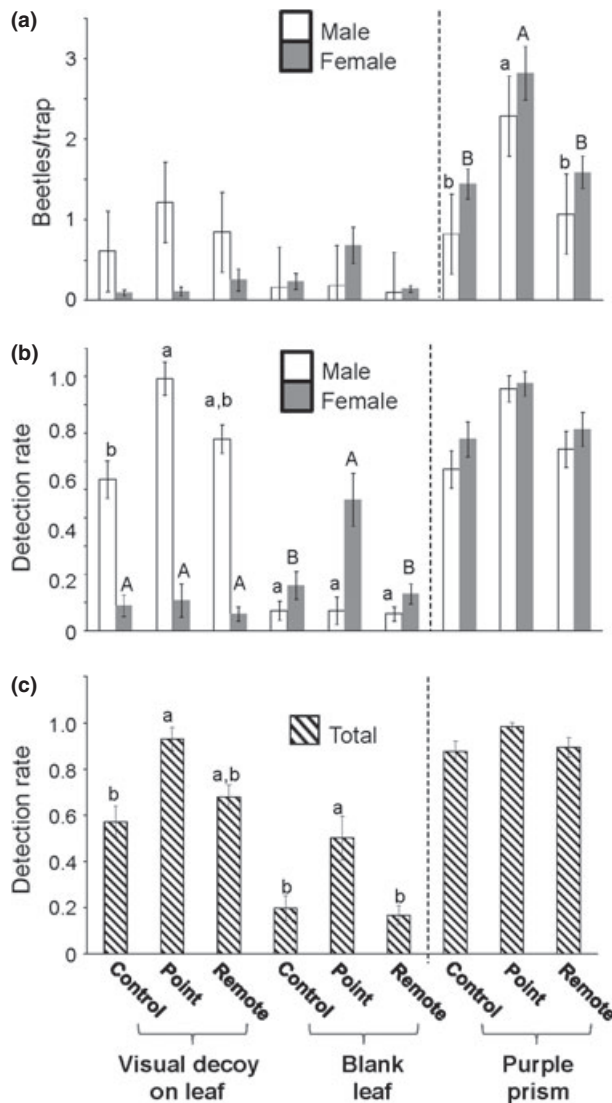


Fig. 7 Captures of beetles in the manuka oil experiment from each combination of the 3x3 factorial design including trap type and odour placement. (a) Mean captures per trap at each trap check. For the prism traps only, ANOVA was performed (Effects: Site – $F = 0.74$, $d.f. = 1$, $P = 0.39$; Period – $F = 7.9$, $d.f. = 6$, $P < 0.0001$; Odour – $F = 21.9$, $d.f. = 2$, $P < 0.0001$). (b) Trap detection rates ($\pm SE$) of male vs. female EAB in each of the 9 treatment types. (c) Trap detection rates for EAB in each of the 9 treatment types irrespective of gender. Within each subfigure, within each trap type, treatments having no letters in common indicate significantly different proportions (for part a, Tukey's correction $\alpha = 0.05$; for parts b and c, Wald chi-square, Bonferroni correction, $\alpha = 0.05$). In parts a and b, comparisons for males use lower case and for females upper case. There were, respectively, 56, 28, 84, 56, 28, 84, 56, 56 and 56 replicates of each odour \times trap combination.

rates for the decoy-baited treatments were heavily determined by detection of males rather than females (fig. 8b). As in the manuka oil experiment,

Table 3 Summary of experimental effects on the detection rate observed in trap captures during the manuka oil lure experiment

Effect	d.f.	Male		Female		Total	
		χ^2	P	χ^2	P	χ^2	P
Site	1	2.2	0.14	0.02	0.90	0.05	0.82
Period	6	11.2	0.08	14.7	0.02	3.4	0.75
Trap type	2	183.7	<0.0001	187.0	<0.0001	141.0	<0.0001
Decoys vs. leaves	1	80.0	<0.0001	10.7	0.001	43.8	<0.0001
Decoys vs. prism	1	0.15	0.70	104.1	<0.0001	9.9	0.0002
Leaves vs. prism	1	86.5	<0.0001	75.4	<0.0001	72.5	<0.0001
Manuka lure source	2	7.4	0.03	10.1	0.006	27.4	<0.0001
Point vs. remote	1	5.7	0.02	11.2	0.008	14.1	0.0002
Point vs. control	1	8.6	0.003	8.0	0.005	16.3	<0.0001
Remote vs. control	1	0.72	0.40	0.30	0.58	0.28	0.6
Trap type * manuka	4	3.9	0.42	3.3	0.51	1.7	0.79

Table 4 Summary of experimental effects on the detection rate observed in trap captures during the phoebe oil lure experiment

Effect	d.f.	Male		Female		Total	
		χ^2	P	χ^2	P	χ^2	P
Site	1	0.1	0.75	3.1	0.08	0.3	0.56
Period	7	21.9	0.003	25.3	0.007	7.4	0.39
Trap type	2	315.7	<0.0001	316.3	<0.0001	154.0	<0.0001
Decoys vs. leaves	1	83.5	<0.0001	14.9	0.001	39.6	<0.0001
Decoys vs. prism	1	293.8	<0.0001	97.1	<0.0001	285.9	<0.0001
Leaves vs. prism	1	613.5	<0.0001	75.1	<0.0001	378.0	<0.0001
Phoebe lure source	2	14.9	0.0006	32.7	<0.0001	6.9	0.03
Point vs. remote	1	180.5	<0.0001	19.0	<0.0001	450.0	<0.0001
Point vs. control	1	194.5	<0.0001	19.7	<0.0001	13.6	0.0002
Remote vs. control	1	1.42	0.23	0.64	0.42	457.0	<0.0001
Trap type * phoebe	4	7.2	0.13	0.8	0.94	4.4	0.36

for sticky leaf traps containing no visual decoy, phoebe oil volatile emission increased the capture of female EAB and not males. This increase occurred when the phoebe oil volatiles were emitted at the

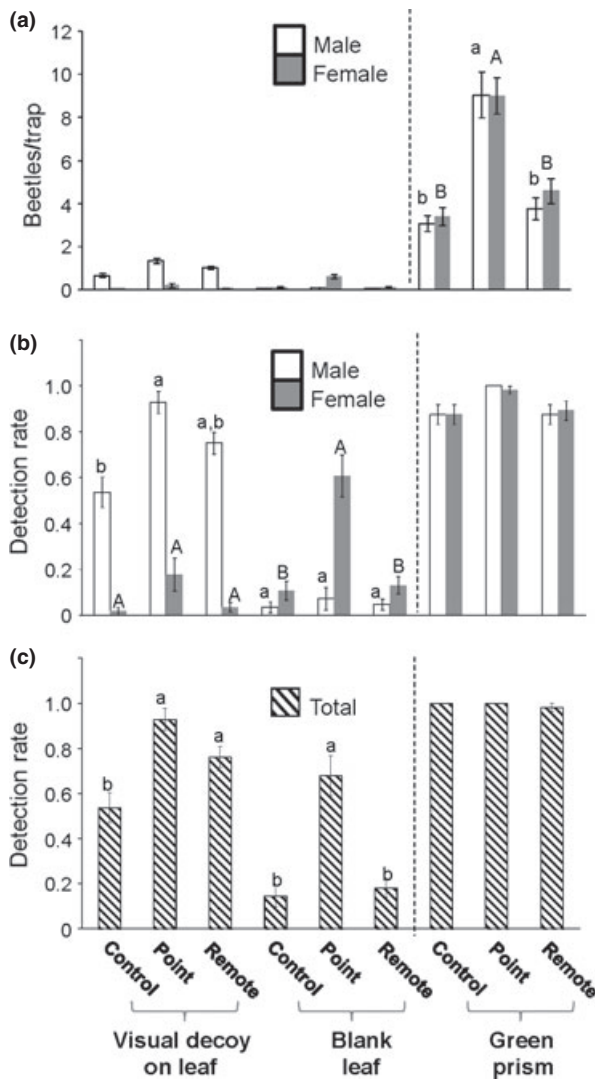


Fig. 8 Captures of beetles in the phoebe oil experiment from each combination of the 3 × 3 factorial design including trap type and odour placement. (a) Mean captures per trap at each trap check. For the prism traps only, ANOVA was performed (Effects: Site – F = 0.03, d.f. = 1, P = 0.86; Period – F = 14.3, d.f. = 7, P < 0.0001; Odour – F = 32.5, d.f. = 2, P < 0.0001). (b) Detection rates (±SE) at each trap check interval of male vs. female emerald ash borer (EAB) in each of the nine treatment types. (c) Detection rates at each trap check interval for EAB in each of the nine treatment types irrespective of gender. Within each subfigure, within each trap type, treatments having no letters in common indicate significantly different proportions (for part a, Tukey’s correction $\alpha = 0.05$; for parts b and c, Wald chi-square, Bonferroni correction, $\alpha = 0.05$). In parts a and b, comparisons for males use lower case and for females upper case. There were, respectively, 56, 28, 84, 56, 28, 84, 56, 56 and 56 replicates of each odour × trap combination.

sticky leaf itself, but not when the volatiles were emitted remotely from within the tree, away from the visually blank sticky leaf (fig. 8a,b).

Discussion

The results from these experiments confirm and extend findings from previous studies showing that EAB males can be trapped highly effectively by using a dead EAB visual decoy pinned to a terminal ash leaflet (Lelito et al. 2008). This trap effectively exploits the mate-finding behaviour of males, which fly around the tree canopy, locate females visually and perform a rapid aerial descent to pounce onto the back of the female resting on an ash leaflet (Lelito et al. 2007). Here, we have now demonstrated that the detection rates of these decoy-baited sticky leaf traps can be as high as either purple or green large visually unbaited prism traps.

It is noteworthy that the per-area detection trap captures on prism traps were similar to that on non-visually baited smaller leaf or green card traps. Adding the decoy to smaller traps makes them clearly much more highly attractive on a per-area scale, exploiting the biological process of mate attraction. Thus, the behavioural basis for the effectiveness of the visual decoy traps is clear. Furthermore, because the per-area detection rate of smaller green surfaces was similar to that of the larger green prism traps, we can infer that captures of beetles on the larger traps were likely not functioning as visual lures to attract more beetles.

Lelito et al. (2007) observed that males were not attracted to pinned, dead-female decoy models whose wings had been splayed apart, while they were to beetles with fully closed elytra covering the abdomen. This previous experiment could not discern, however, the effects of wing orientation versus the prominence of the reddish-coloured abdomen that was exposed when the wings were splayed. Our current results clearly demonstrate the importance of the natural, side-by-side positioning of the elytra themselves, a cue that was effective in capturing beetles, even without the full beetle body (figs 2 and 3). Other elytral arrangements, or the abdomen alone, were completely ineffective.

Emission of the manuka or phoebe volatiles increased equally the captures of both sexes on the purple or green prism traps, confirming similar findings in other studies (Crook et al. 2008; de Groot et al. 2008). Thus, the signals sufficiently exceeded the undoubtedly high background noise of induced volatiles from the trees they were deployed in. While remote placement of odour sources did not generally lead to increases in trap captures, it seems likely that such trees do have more beetles drawn to them, albeit not in the direction of the trap.

However, the visual decoy leaf traps were the exception to this pattern, where a remote source of phoebe oil still led to increases in trap capture and detection rate. Thus, it seems likely that the decoys are able to draw towards them the additional beetles pulled into the tree by the remote odours, while the other traps cannot.

Only male captures were increased by volatiles emitted from the visual-decoy-baited leaf traps, whereas only female captures and female detection rates were increased on sticky leaves emitting manuka or phoebe volatiles, but lacking visual decoys. We interpret the pattern of female capture on blank sticky leaves to mean that females, like males, are attracted to the point source of the volatiles, but they land at the source only if there is no other beetle on it. Thus, for females there is a visually mediated avoidance of the dead EAB decoy. In our experiments, this avoidance response by females to other EAB may have even depressed the capture numbers of females on the blank sticky leaves, because after one capture the leaves would no longer be blank.

In these high-density sites, it should be noted that even traps with relatively low per-trap-check interval rates will have a relatively high per-season detection rate. For example, a 10% detection rate for each half a week would translate to a potential 57% detection rate over eight sampling periods during the season. Nevertheless, the detection rate analyses provide some indications of which traps might be worth evaluating at lower densities. In our experiments, the strong responses of males to our visual decoy traps resulted in detection rates per trap check as high as in the large prism traps. This type of trap takes advantage of the high vagility of patrolling males and as such might offer advantages for detecting the presence of EAB in low-density populations. Because these experiments were performed in heavily infested ash plots in Michigan, it is not clear what the relative performance of each type of trap might be in low population densities. However, it is clear the smaller trap designs were relatively ineffective, displaying a low detection rate per trap check even at these high densities. Thus the data suggest that only designs with visual lures might be able to significantly detect EAB well at low population densities.

Nevertheless, these results are promising for the potential use of visual-decoy-based traps in future detection/monitoring programs for EAB. Because of their small size, distributed arrays of great numbers of such traps could effectively be deployed at low

heights in the trees and attain a high detection ability. The small size could conceivably allow for greater ease of deployment and lower production costs versus the larger prism traps. However, at this time, it is impossible to quantify the differences in operational costs between such smaller visual-decoy-based traps and prism traps. For example, the currently used visual decoys are dead EAB, which, while plentiful in certain areas, would require collection and distribution to those using such traps. At the same time, additional research funding and effort will be required if effective artificial decoys are to be developed.

Furthermore, there is also an obvious need to substitute the living leaf surfaces with an artificial substrate, because the leaves sprayed with tanglefoot deteriorate in a few days owing to phytotoxicity (Lelito et al. 2008; Silk et al. 2011). Our experiments using green plastic sticky cards indicate that decoy traps having synthetic surfaces can be used to detect EAB, but such traps so far are not as effective as the visual decoy sticky leaves. The use of 'dry' adhesive may have contributed to somewhat-lower-than-expected captures in these experiments. Its tackiness seemed to dissipate over just a few days, whereas the tangle-trap adhesive used on the sticky leaves maintained its adhesiveness far longer. Further experiments using improved adhesives on artificial cards or on other leaf-mimicking surfaces are anticipated.

The improvement in the performance of the visual decoy sticky leaf traps by the addition of volatiles further illustrates how the EAB-baited leaf type of trap can become a logical and competitive option to prism traps. We anticipate that improvements in the quality of the volatile emissions, such as by adding the putative lactone pheromone component identified from EAB females (Bartelt et al. 2007) to plant volatile emission sources, might further enhance detection rates of visual-decoy-baited traps, as it has been shown for green prism traps (Silk et al. 2011). Engineering efforts to improve trap longevity, deployment, maintenance and distribution patterns should be continued.

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