Effects of Trap Type, Placement and Ash Distribution on Emerald Ash Borer Captures in a Low Density Site

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ABSTRACT Effective methods for early detection of newly established, low density emerald ash borer (*Agrilus planipennis* Fairmaire) infestations are critically needed in North America. We assessed adult *A. planipennis* captures on four types of traps in a 16-ha site in central Michigan. The site was divided into 16 blocks, each comprised of four 50- by 50-m cells. Green ash trees (*Fraxinus pennsylvanica* Marshall) were inventoried by diameter class and ash phloem area was estimated for each cell. One trap type was randomly assigned to each cell in each block. Because initial sampling showed that *A. planipennis* density was extremely low, infested ash logs were introduced into the center of the site. In total, 87 beetles were captured during the summer. Purple double-decker traps baited with a blend of ash leaf volatiles, Manuka oil, and ethanol captured 65% of all *A. planipennis* beetles. Similarly baited, green double-decker traps captured 18% of the beetles, whereas sticky bands on girdled trees captured 11% of the beetles. Purple traps baited with Manuka oil and suspended in the canopies of live ash trees captured only 5% of the beetles. At least one beetle was captured on 81% of the purple double-decker traps, 56% of the green double-decker traps, 42% of sticky bands, and 25% of the canopy traps. Abundance of ash phloem near traps had no effect on captures and trap location and sun exposure had only weak effects on captures. Twelve girdled and 29 nongirdled trees were felled and sampled in winter. Current-year larvae were present in 100% of the girdled trees and 72% of the nongirdled trees, but larval density was five times higher on girdled than nongirdled trees.

KEY WORDS *Fraxinus*, *Agrilus planipennis*, double-decker traps, girdled trees, Manuka oil

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a secondary pest of ash (*Fraxinus* spp.) in Asia (Yu 1992), has become the most destructive forest insect to invade North America. This phloem-feeding pest was first identified as the cause of widespread ash decline in Detroit, MI and Windsor, Ontario in July 2002 (Cappaert et al. 2005). Since then, *A. planipennis* populations have been found in at least 14 additional states and Quebec, Canada and tens of millions of ash trees have been killed (EAB.info 2011).

Adult *A. planipennis* emergence begins in May or June at ≈450 accumulated degree-days (base 50°*F* = 10°C) (MSU Enviro-Weather 2010). Beetles feed on ash foliage for at least 21 d before females begin laying eggs, but this feeding causes negligible damage. Larvae begin feeding in mid to late summer on phloem and cambium in serpentine galleries that typically score the outer sapwood, disrupting nutrient and water transport. As larval densities build and trees become stressed, foliage thins, epicormic sprouts may appear, branches die, and eventually the tree succumbs. Most *A. planipennis* overwinter as prepupal larvae in thick outer bark or in the outer sapwood, then pupate in spring. In healthy trees with a low *A. planipennis* density, a high proportion of larvae overwinter as early instars, feed for a second summer and emerge the following year, completing a 2-yr life cycle (Cappaert et al. 2005, Siegert et al. 2010, Tluczek et al. 2011).

Effective and efficient methods to detect recently established infestations of *A. planipennis* remain a high priority for resource managers, regulatory officials, arborists, and property owners. Localized outlier infestations resulting from inadvertent transport of infested ash nursery trees, logs, or firewood are typically discovered at least 4–6 yr after establishment, when declining canopies of heavily infested ash trees are noticed. Early detection of new infestations could enable managers to rapidly implement strategies to protect urban trees or slow *A. planipennis* population growth and delay the onset and progression of ash mortality (Katovich and McCullough 2010, Poland and McCullough 2010, Mercader et al. 2011).

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Detection methods for *A. planipennis* have included visual surveys to identify symptomatic trees, girdling and debarking trees, and baited traps. Visual surveys are problematic because ash trees exhibit virtually no external symptoms of infestation until *A. planipennis* densities build to moderate or high levels (Cappaert et al. 2005, Poland and McCullough 2006). Stressed ash trees are highly attractive to adult *A. planipennis* beetles (McCullough et al. 2009a,b; Tluczek et al. 2011) and systematic grids of girdled ash trees have been used to detect and delimit numerous *A. planipennis* infestations in Michigan and other states (Rauscher 2006, Hunt 2007, Poland and McCullough 2010). Debarking girdled trees to locate larval galleries is labor intensive, however, and accessible trees may not always be available (McCullough et al. 2009a,b).

Effective traps and lures that could be efficiently deployed across large areas would significantly enhance *A. planipennis* detection and delineation efforts. Although short-range or contact pheromones may be involved in mating (Bartelt et al. 2007, Lelito et al. 2009, Silk et al. 2009), *A. planipennis* do not appear to produce long-range sex or aggregation pheromones (Lelito et al. 2007, Rodriguez-Saona et al. 2007, Crook and Mastro 2010). Beetles rely instead on a combination of visual and olfactory cues to locate host trees and subsequently identify mates. In electroretinogram studies, beetles responded to specific shades of purple and green (Crook et al. 2009). Several field trials have confirmed *A. planipennis* attraction to purple traps (Francese et al. 2005, Crook et al. 2008, Marshall et al. 2009, Poland et al. 2011). In one study where all traps were, placed 13 m above ground, dark green traps captured significantly more beetles than dark purple traps (Crook et al. 2009). Other colors, including blue, red, orange and white, do not appear to be attractive to *A. planipennis* (Francese et al. 2005, Crook et al. 2009; D.G.M. and T.M.P., unpublished data).

Considerable research has been conducted to identify host volatiles to use in lures to attract adult *A. planipennis* (Rodriguez-Saona et al. 2006, Crook et al. 2008, de Groot et al. 2008, Grant et al. 2010). The most promising attractants tested to date include a blend of leaf volatiles (*cis*-3-hexenol, *trans*-2-hexenol, hexanal, and *trans*-2-hexenal) emitted by stressed ash trees (Poland et al. 2011) and Manuka oil, a commercially available distillate from the New Zealand tea tree (*Leptospermum scoparium* J.R. Forst. & G. Forst.) that contains compounds associated with ash wood and bark. Both the leaf blend and Manuka oil lures attracted *A. planipennis* beetles in laboratory and field studies (Rodriguez-Saona et al. 2006, Crook et al. 2008, de Groot et al. 2008, Grant et al. 2010, Crook and Mastro 2010, Poland et al. 2011). More recently, *A. planipennis* lures have included an 80:20 ratio of Manuka oil and Phoebe oil, a product distilled from the Brazilian walnut tree (*Phoebe porosan*) (Crook and Mastro 2010). Ethanol, emitted by numerous hardwood species experiencing stress (Kelsey 2001, Kelsey and Joseph 2003), is commonly used, either alone or with host volatiles, to attract several phloem- and wood-boring species to traps (Moeck 1970, Haack and Benjamin 1982, Klimetzik et al. 1986, Byers 1992, Dunn and Potter 1991, Erasmus and Chown 1994, Miller 2006).

Three-sided prism traps made from purple or green coroplast coated with Pestick (Hummert International, Earth City, MO) are an effective means to capture adult *A. planipennis* (Francese et al. 2005, Marshall et al. 2009). Purple prism traps, typically suspended from rebar, were used in various field studies to evaluate potential lures (Crook et al. 2008, Francese et al. 2008, Poland et al. 2011). Beginning in 2008, the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (APHIS PPQ) distributed >60,000 purple prism traps annually to state regulatory agencies for *A. planipennis* detection (USDA–APHIS 2010). Survey protocols stipulated that traps be baited with a Manuka oil lure (2008) or 80:20 Manuka oil-Phoebe oil lures (2009, 2010) and suspended in the mid canopy of ash trees (Crook and Mastro 2010, USDA–APHIS 2010).

An alternate trap design, referred to as the double-decker trap, incorporates two purple prism traps attached to a 2.4-m-tall polyvinyl chloride (PVC) pipe and is designed to represent the silhouette of a tree placed in full sun (McCullough and Poland 2009, Poland et al. 2011). Double-decker traps are placed in openings or near the edge of wooded areas to exploit the preference for sunny conditions exhibited by adult *A. planipennis* (Yu 1992; McCullough et al. 2009a,b). Field studies in 2006–2008 showed double-decker traps baited with leaf blend and Manuka oil lures captured *A. planipennis* beetles across a wide range of sites (Poland et al. 2011).

Most field studies to evaluate new lures or trap designs for *A. planipennis* have been conducted in areas where *A. planipennis* populations are at moderate to high densities (Francese et al. 2005, 2008; Lelito et al. 2007; Crook et al. 2008, 2009). In these sites, however, many ash trees exhibit canopy decline and dieback and emit stress-related volatiles, all of which may affect either the visual response of beetles to traps or the olfactory response of beetles to the lures under evaluation. Ideally, traps and lures developed for *A. planipennis* detection should be assessed in sites where *A. planipennis* density is at low levels and few, if any trees are symptomatic or stressed by larval feeding.

We evaluated the effectiveness of four trap designs in a forested setting in central Michigan. We suspected *A. planipennis* was present in this site but symptoms of *A. planipennis* infestation were not apparent on ash trees within or near the study area. Trap designs tested in the study included the purple canopy trap baited with the Manuka oil lure used in national *A. planipennis* detection surveys in 2008, double-decker traps with purple or green panels and baited with the leaf blend and Manuka oil lures, and girdled ash trees. An intensive pretreatment inventory of ash trees on the site enabled us to assess potential relationships between ash distribution and *A. planipennis* captures.

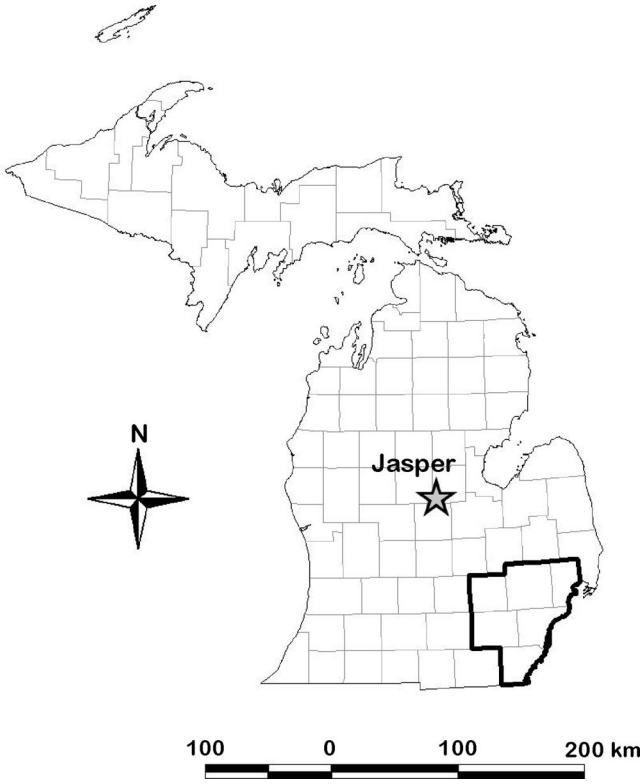

Fig. 1. The study site was located in the Au Sable State Forest, Jasper Township, Midland County, MI. The six counties originally quarantined for *A. planipennis* in southeastern Michigan in 2002–2003 are outlined by the bold line.

Methods

Study Site. The study was conducted in 2008 at a 16.0-ha area of the Au Sable State Forest in Jasper Township, Midland County, MI (Fig. 1). The site included recent clearcut areas comprised of patchy maple (*Acer* spp.) and aspen (*Populus tremuloides* Michx.) regeneration, forested areas with abundant overstory green ash (*Fraxinus pennsylvanica* Marsh.) and forested areas comprised of nonash hardwoods. An 8 by 8 grid consisting of 64 cells, each 50 by 50 m (0.25 ha), was overlaid on the site (Fig. 2a). Geo-referenced coordinates were recorded at each grid cell corner with a Garmin ETrex GPS (Garmin Ltd., Olathe, KS). All ash trees >2.5 cm in diameter at breast height (DBH) were inventoried by size class in January–February 2008 (Fig. 2b). Total area of ash phloem within each grid cell was calculated using methods described in McCullough and Siegert (2007) (Fig. 2c).

Pretreatment *A. planipennis* Survey. To determine whether *A. planipennis* was present and if so, to systematically estimate *A. planipennis* density at the site, we selected and felled an ash tree in alternate grid cells throughout the 16-ha area, excluding cells where no ash occurred. In total, 18 ash trees were felled, measured, and sampled in March 2008. Mean (± SE) DBH of the felled trees was 23.0 ± 1.8 cm (range: 9.0–36.3 cm). Three to seven sample areas, depending on tree size, were marked on the stem and primary branches. Sample areas were evenly spaced between the midtrunk and the upper canopy (>4 cm in diameter) and individual sample areas ranged from 990 to 5,400 cm^2 (average of 2,227 ± 82.4 cm^2). Each area was intensively examined to locate any holes left by emerging *A. planipennis* adults or by woodpeckers preying on *A. planipennis* larvae (Lindell et al. 2008). Sample areas were measured, carefully debarked, and the number and stage of *A. planipennis* larvae were recorded. Larval density was standardized per m^2 of exposed area for each tree.

Adult *A. planipennis* Release. To ensure that a detectable density of *A. planipennis* would be present, we introduced 22 infested ash logs to the site to supplement the ultra-low wild *A. planipennis* population. These logs, averaging (± SE) 20.2 cm ± 1.0 cm in diameter (range: 11.6–29.1 cm) and 60.3 ± 0.5 cm in length (range: 55.0–64.0 cm), were collected from infested ash trees in Livingston Co., MI in May 2008. Each log was examined and existing exit holes or woodpecker holes were marked with staples. Logs were transported to the Jasper site and placed upright in the center of the 16-ha site on 27 May (Fig. 2a). In September 2008, we counted the number of new exit holes on each log and determined that 415 adult *A. planipennis* in total emerged from the infested logs during the summer.

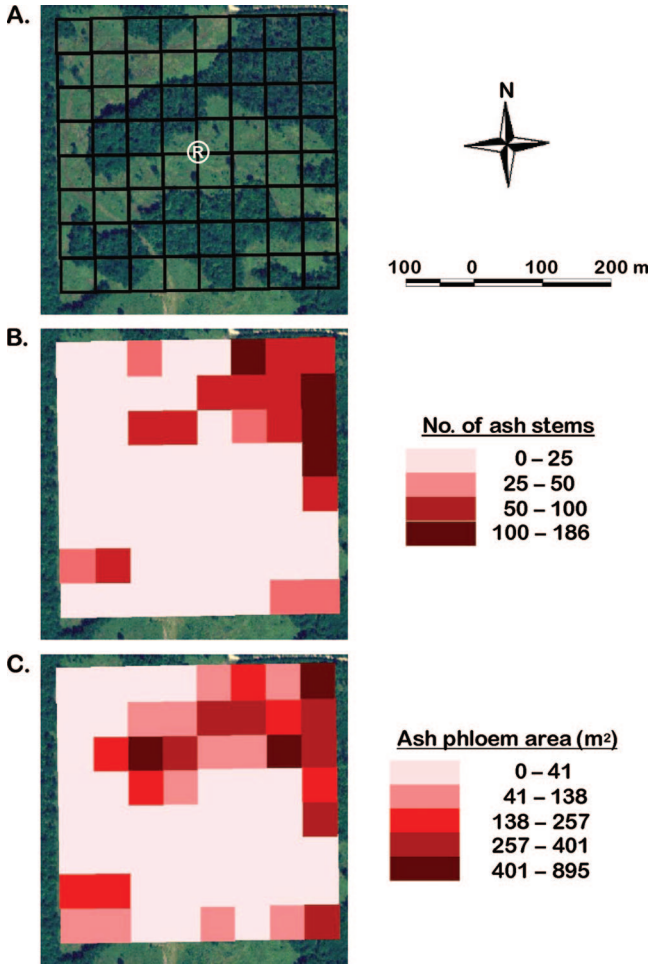

Fig. 2. The Jasper study site overlaid with 50- by 50-m grid cells to indicate (A) distribution of forested and open areas and the location of infested ash logs introduced to release *Agrilus planipennis* beetles (indicated by), (B) abundance of ash trees (>2.5 cm in diameter), and (C) estimated area of ash phloem in each grid cell. Ash trees were inventoried by size class and area of ash phloem in each grid cell was estimated using methods described in McCullough and Siegert (2007).

Experimental Design and Traps. We evaluated four trap designs for adult *A. planipennis* in 2008 including 1) purple canopy traps suspended in the mid to upper canopy of an ash tree and baited with a Manuka oil lure (e.g., the 2008 APHIS program trap); 2) double-decker traps with two purple panels baited with a leaf blend lure and an ethanol lure on the upper panel and a Manuka oil lure on the lower panel; 3) double-decker traps with two green panels baited with the leaf blend, ethanol, and Manuka oil lures; and 4) sticky bands affixed to girdled ash trees. In May 2008, we divided the site into 16 blocks, each consisting of 2 by 2 grid cells (1.0 ha per block) (Fig. 2a). One type of trap was randomly assigned to each grid cell in each block (Fig. 3a). In four blocks where no ash trees occurred, the three artificial traps were randomly assigned to three of the four grid cells; one grid cell was left without a trap. GPS coordinates were recorded for each trap (including girdled trees). We also recorded sun exposure for each trap or trap tree as open (exposed to full sun), fully shaded or growing on an edge of a wooded area and partially shaded.

The canopy trap consisted of a three-sided panel of purple coroplast (4 mm thick, Harbor Sales Inc., Sudlersville, MD), folded into a prism (60 cm tall by 40 cm wide on each side) and secured with zip ties. We coated the outer surface of each face of the prism trap with clear Pestick just before placing traps into trees. A pouch with Manuka oil released at 50 mg/d (Synergy Semiochemicals Corp., Burnaby, B.C., Canada) was attached near the lower edge of the canopy trap. We suspended canopy traps ≥3 m high from branches of ash trees, using guidelines issued by USDA APHIS, which specified traps were to be suspended in the mid canopy of live ash trees. A canopy trap was placed in a total of 11 ash trees growing along the edge of wooded areas near the center of the appropriate grid cell. In five blocks where ash trees did not occur or were inaccessible, canopy traps were suspended ≈2 m above ground from a rebar pole.

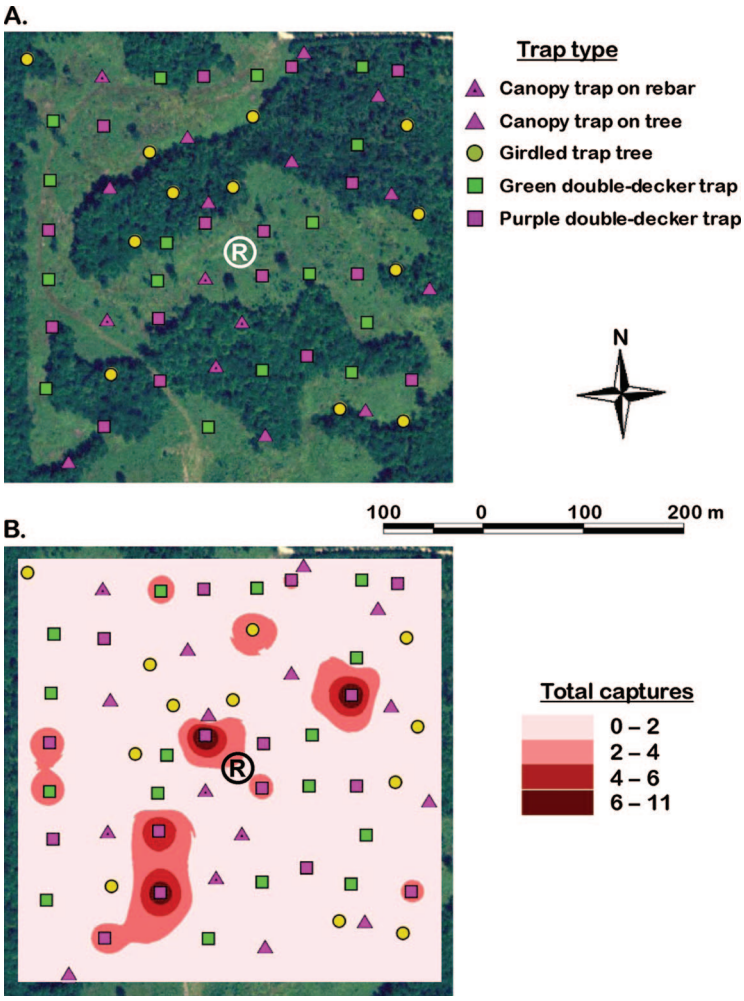

Fig. 3. Location (A) of purple canopy traps, green double-decker traps, purple double-decker traps, girdled trees, and infested ash logs (indicated by) and (B) number of *Agrilus planipennis* beetles captured on traps at the Jasper site in 2008.

Each double-decker trap had one three-sided prism trap, either purple or green, attached with zip ties to the top of a 2.4-m-tall PVC pipe (10 cm in diameter, white), which we slid over a t-post (1.5 m) set in the ground. A second prism trap (of the same color) was attached to the PVC pipe 60 cm below the upper panel (1.8 m high) creating a vertical silhouette that resembled a tree (Fig. 4). Green double-decker traps were identical to their purple counterparts except that the prisms were constructed from light green coroplast (Great Lakes Integrated Pest Management Inc., Vestaburg, MI). The outer surface of all panels of the prism traps on the 16 purple and 16 green double-decker traps were coated with Pestick. A leaf blend lure consisting of *cis*-3-hexenol, *trans*-2-hexenol, *trans*-2-hexenal, and hexanal released separately from bubble caps at 3.7, 3.7, 13, and 13 mg/d, respectively (Contech Enterprises, Inc., formerly Phero Tech, Inc., Delta, B.C., Canada) was suspended from the lower edge of the upper panel. An ultra-high release ethanol lure (800 mg/d; Contech Enterprises, Inc., Delta, B.C., Canada) was suspended from the upper panel. A Manuka oil lure, the same as that used on the canopy traps, was suspended from the lower panel. Double-decker traps were placed in full or nearly full sun, typically near the center of the grid cell, to provide *A. planipennis* beetles with a distinct and readily apparent visual and olfactory focus (McCullough and Poland 2009, Poland et al. 2011).

Ash trees selected for girdling averaged (± SE) 28.6 ± 1.34 cm DBH and ranged from 18.0 to 35.2 cm in DBH. Girdled trees were selected near the center of the appropriate grid cell when possible in 12 blocks where ash occurred. Trees were girdled in early April by removing a band of outer bark and phloem, at least 30 cm wide, around the circumference of the tree, ≈1 m aboveground. A 30-cm-wide band of plastic shrink wrap was wrapped tightly around the tree trunk, 0.5 m above the girdle, and coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) on 29 May.

Traps were installed and baited on 29 May, then checked on 1 July, 9 July, 30 July, 20 August, and 19

Fig. 4. A double-decker trap consists of two three-sided prism traps, either purple or green, attached to a 2.4-m-tall PVC pipe (10 cm diameter), which slides over a t-post set in the ground.

September. All *A. planipennis* were collected, returned to the Forest Entomology Laboratory at Michigan State University, and soaked in Histoclear (National Diagnostics, Atlanta, GA) to remove Pestick (from beetles captured on traps) or ethanol to remove Tanglefoot (from beetles captured on sticky bands). Beetles were examined under a microscope to confirm species identification. Lures and Pestick were replaced on 9 July to ensure traps would remain effective throughout the summer.

Larval Density. In January 2009, the 12 girdled trees were felled and the main trunk and primary branches were bucked into 1-m-long sections, beginning 1 m above the sticky band and continuing down to a diameter of 8 cm. Alternate 1-m-long sections were carefully examined and any holes left by emerging *A. planipennis* adults or woodpeckers preying on *A. planipennis* larvae were recorded. Diameter and length of the sections were measured and surface area calculated. Each section was debarked and number and stage of *A. planipennis* larvae were recorded. Larval density was standardized per m^2 of area sampled per tree.

An additional 29 nongirdled ash trees distributed systematically throughout the site, with an average DBH of 28.3 ± 1.73 cm, were felled and sampled between January and March 2009 (1–2 trees sampled per grid cell where ash occurred). Four to seven 1-m-long sections, distributed from the midtrunk through the canopy, were marked on the upper surface of the trunk and primary branches (>8 cm in diameter). Each section was examined to locate *A. planipennis* exit holes or woodpecker holes. Sections were debarked and larval stage and density were recorded and standardized per m^2 as described above. Coordinates of nongirdled trees were recorded (Garmin ETrex GPS).

Evaluation of Trap Types. To provide a robust and rigorous assessment of potential differences in adult *A. planipennis* captures among the trap types, we report analyses based on three different measures of trap effectiveness. The simplest measure was the total number of beetles captured by each trap type, pooled across traps of the same type. In addition, we examined the number of beetles captured per trap to see whether results were unduly influenced by a few traps capturing high numbers of beetles, whereas the others of the same type caught none. Finally, we examined the probability that a trap was positive, defined as having captured at least one *A. planipennis* during the summer (catch ≥ 1 versus catch = 0).

Although our primary goal was to evaluate trap types, we controlled for other potentially important covariates as well. We hypothesized, for example, that distance between traps and the central *A. planipennis* release point might affect captures, so we controlled for distance (measured in 100-m units) in some models. Similarly, we thought beetles might be more attracted to traps in areas with relatively high amounts of ash phloem, so we controlled for phloem area, measured in 100-m^2 units. Scaling the latter two predictors ensured that their regression coefficients would reflect any effects of meaningful changes in phloem area and distance. We also controlled for sun exposure of traps (open, edge, or shaded) given the preference of adult *A. planipennis* for sunny conditions (Yu 1992; McCullough et al. 2009a,b).

Statistical Analyses. Number of ash stems recorded and area of ash phloem estimated for each grid cell were mapped using the ArcView 3.2 geographic information system (Environmental Systems Research Institute, Redlands, CA). Geo-referenced trap catch data were imported and interpolated with the Spatial Analyst extension using inverse distance weighting of the 12 nearest neighbors to each trap.

Total number of adult *A. planipennis* captured on purple and green double-decker traps, canopy traps, and sticky bands during the summer were determined and a χ^2 goodness-of-fit (GOF) test (Sheskin 2007) was used to test whether total captures differed among the four trap types. We then used additional GOF tests, with a Bonferroni correction ($\alpha = 0.05/6 = 0.008$) to the significance criterion, to conduct pairwise comparisons between the trap types. Finally, we used a GOF test to contrast the number of beetles caught by purple double-decker traps with the combined capture by the other trap types (sticky bands, green traps, and canopy traps).

We used Poisson regression to assess the effect of trap type on the number of *A. planipennis* captured per trap, while controlling for phloem area, distance, and sun exposure. Sticky bands served as the reference group for trap type, whereas traps in partially shaded, edge locations served as the reference group for sun exposure. We used likelihood ratio (LR) tests based on the deviance statistics (D) to examine which pre-

dictors were significantly contributing to model fit. To clarify effects of significant categorical predictors, we applied the procedure of Westfall (1997) to conduct pairwise multiple comparisons. This procedure is more powerful than alternatives such as Bonferroni adjustment, Tukey's honestly significant difference (HSD) test, or Holm's test, while still providing strong control over the family-wise error rate (Bretz et al. 2010). We report the adjusted *P* values for those pairwise comparisons.

We used logistic regression to examine the effect of trap type on the probability of capturing at least one *A. planipennis* during the summer, while controlling for phloem area, distance, and sun exposure. We again used LR tests to identify significant predictors, then applied pairwise multiple comparisons based on the procedure of Westfall (1997) if appropriate. Thus, this analysis mirrored the structure of the Poisson model, but used a dichotomous measure of trap effectiveness instead of counts of captured beetles.

For both the Poisson and logistic regression models, we also used LR tests to examine whether adding interaction terms (one at a time) to the base models would improve model fit. Testing a trap type × phloem area interaction allowed us to see whether the effect of trap type depended on the amount of nearby phloem, whereas testing a trap type × distance interaction allowed us to see whether the trap type effect depended on distance from the release point. We also evaluated a phloem area × distance interaction to see whether the effect of distance on trap effectiveness was moderated by the amount of phloem near the trap. Because no sticky bands were located in full sun, it was not feasible to properly test effects of sun exposure. We conducted analyses with R version 2.12.1 (R Development Core Team 2010). We used version 1.2–5 of the multicomp package (Hothorn et al. 2008) for the pairwise comparisons for the Poisson and logistic regression models.

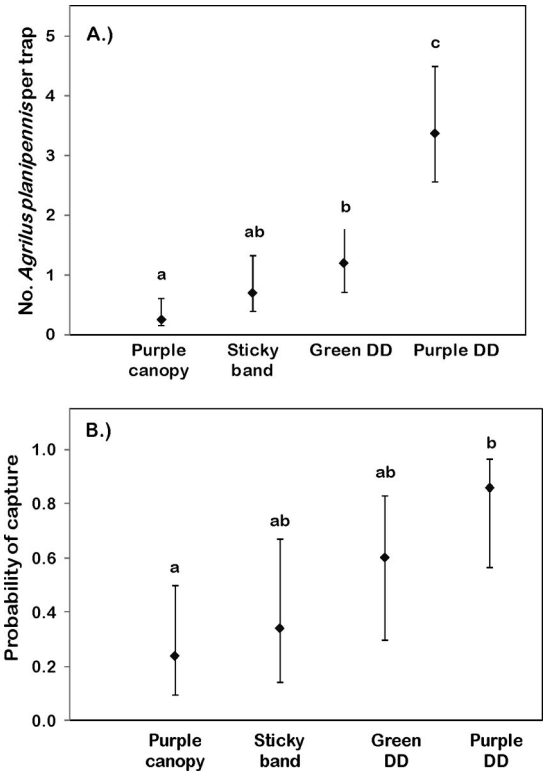

Fig. 5. Mean and 95% confidence intervals for (A) the number of adult *Agrilus planipennis* beetles captured per trap based on the Poisson regression model and (B) the probability of capturing at least one adult *A. planipennis* beetle based on the logistic regression model on purple canopy traps, sticky bands on girdled trees, green double-decker (DD), and purple DD traps. Letters at the top of each panel indicate the results of pairwise comparisons based on the correction of Westfall (1997) for maintaining the family-wise error rate. Trap types that do not share the same letter differed significantly (adjusted $P < 0.05$).

Results

Initial *A. planipennis* Density. In total, 21.6 m^2 of phloem was exposed on the D18 trees sampled in March 2008 before our study began (average of 1.2 ± 0.04 m^2 per tree). Results indicated *A. planipennis* had been present for at least 2–3 yr, but density of current-year larvae was extremely low. We found evidence of *A. planipennis* infestation on only six of the 18 trees. One tree harbored two live *A. planipennis* prepupae and a single *A. planipennis* exit hole, whereas a second tree had two dead larvae. Four other trees had 1–7 larval galleries (density of 0.8–4.7 per m^2) in total, but all of these larvae had been predated by woodpeckers. Total larval density, including predated, dead and live larvae, was 0.83 larvae per m^2 and density of live larvae was 0.08 larvae per m^2. The six trees with current or previous *A. planipennis* infestation averaged 27.4 ± 1.7 cm in DBH (range, 23.6–35.1 cm), whereas the 12 uninfested trees averaged 20.9 ± 2.4 cm in DBH (range: 9.0–36.3 cm).

Ash Inventory. In total, 1,648 ash trees (>2.5-cm DBH) and an estimated 7,322 m^2 of ash phloem occurred in the 16-ha site (Fig. 2c). No ash trees occurred in 25 of the 64 grid cells. In the remaining cells, number of ash stems per grid cell ranged from 1 to 186 (Fig. 2b) and area of ash phloem ranged from 5.5 to 894.8 m^2 per cell (Fig. 2c). Number of ash stems in a grid cell explained <50% of the variability in the estimated area of ash phloem ($Y = 0.1512x + 8.4421$; $r^2 = 0.42$; $P < 0.05$). Most ash trees (72%) were relatively small (2.5–12.5-cm DBH), and collectively accounted for only 10.6% of the total ash phloem. Trees that were 12.5–25 cm in DBH accounted for 16% of the total ash stems and 19.9% of the total phloem. Ash trees of merchantable size (≥25 cm DBH) were relatively uncommon, comprising <2% of the ash inventory, but accounting for 73.5% of the ash phloem on the site.

Adult *A. planipennis* Captures. In total, 87 *A. planipennis* beetles were captured on the four trap types from June through September. Captures peaked in July and 40% of the beetles were caught between 9 and

Table 1. Poisson regression model predicting effects of ash phloem area, distance of traps to the central release point, sun exposure, and trap type on the number of adult *A. planipennis* beetles captured on purple or green double-decker (DD) traps, purple canopy traps, and sticky bands on girdled trees

Effect	Model coefficients			Analysis of deviance[a]			
	Coeff	SE	Z	*df*	AIC	*D*	LRT
Intercept	0.512	0.421	1.217	1			
Phloem area (100 m^2)	−0.023	0.063	−0.366	1	188.71	91.540	0.138
Distance (100 m)	−0.382	0.175	−2.175*	1	193.21	96.045	4.643*
Sun exposure				2	194.33	99.158	7.756*
Edge (RG)							
Open	−0.766	0.283	−2.712*				
Shaded	−0.160	0.267	−0.599				
Trap type				3	250.15	156.980	65.578***
Sticky band (RG)							
Canopy trap	−1.123	0.598	−1.877				
Green DD	0.543	0.427	1.272				
Purple DD	1.604	0.358	4.481***				
Residual				52	190.57	91.402	

[a] Analysis of deviance indicates whether removing each effect would significantly decrease model fit. Coeff = estimated coefficient, SE = standard error of the coefficient, Z = Wald test *z*-score for the coefficient. *D* = Deviance, LRT = Likelihood ratio test associated with dropping the effect, RG = reference group. For a null model with only an intercept term, $df = 59$, AIC = 252.75, and $D = 167.58$. *, $p < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

30 July, corresponding to ≈525 and 768 accumulated degree-days (base 10°C) (MSU Enviro-Weather 2010). Overall, 23, 68, and 9% of beetles were captured in June, July, and from August to mid-September, respectively.

Of the 60 total traps on the site, 31 traps (52%) were positive and captured at least one *A. planipennis* beetle. Thirteen of the 16 purple double-decker traps (81%) and nine of the 16 green double-decker green traps (56%) were positive. Five of the 12 sticky bands on girdled trees (42%) were positive. Four of the 16 canopy traps (25%), including one of the five traps suspended from rebar, each captured a single beetle. Purple double-decker traps captured 57 *A. planipennis*, representing 65% of the total beetle capture. The green double-decker traps, sticky bands and purple canopy traps captured 16, 10, and four *A. planipennis*, representing 18%, 11%, and 5% of the total captures, respectively (Fig. 5a). At least one beetle was captured on purple double-decker traps in all five sampling periods during the summer. Green double-decker traps, canopy traps and sticky bands captured beetles during the first three sampling periods but none of these traps captured a beetle after 1 August.

χ^2 GOF Tests. Total number of *A. planipennis* captured by the four types of traps were clearly unequal [$\chi^2(3) = 79.48$; $P < 0.001$]. Pairwise comparisons showed the purple double-decker traps captured more beetles than the canopy traps [$\chi^2(1) = 46.05$; $P < 0.001$]; the sticky bands [$\chi^2(1) = 32.97$; $P < 0.001$]; and the green double-deckers [$\chi^2(3) = 23.03$; $P < 0.001$]. Number of beetles captured on the canopy traps and the green double-decker traps did not differ from the sticky bands [$\chi^2(1) = 2.57$; $P = 0.11$ and $\chi^2(1) = 1.38$; $P = 0.24$, respectively], but green double-decker traps captured more beetles than the canopy traps [$\chi^2(3) = 7.20$; $P = 0.007$]. An additional follow-up test showed the purple double-decker traps captured more beetles than all three other types of traps combined [$\chi^2(3) = 8.38$; $P = 0.004$].

Poisson Regression. Results from a Poisson regression model to predict the number of adult *A. planipennis* captured by each type of trap showed trap type strongly influenced the number of beetles captured (LR = 65.578; $P < 0.001$) (Table 1). On average, beetle captures per trap were 3- to 15-fold higher on purple double-decker traps than on the other trap types (Fig. 5a). Pairwise comparisons indicated purple double-decker traps ($M = 3.35$) captured more *A. planipennis* per trap than canopy traps ($M = 0.22$; $P < 0.001$); sticky bands ($M = 0.67$; $P < 0.001$); and green double-decker traps ($M = 1.16$; $P < 0.001$) (Table 2). Number of *A. planipennis* captured per trap on sticky bands did not differ from captures on canopy traps ($P = 0.060$) or the green double-decker traps ($P = 0.203$). Green double-decker traps captured more beetles per trap than the canopy traps ($P = 0.009$). These results both confirm the GOF test results and extend them by demonstrating the importance of trap type even after controlling for other covariates.

Table 2. Pairwise multiple comparisons of the mean log counts of adult *A. planipennis* captured on purple or green double-decker (DD) traps, purple canopy traps, and sticky bands on girdled trees and by traps at each level of sun exposure, based on the Poisson regression model

Comparison	Estimate	SE	Z
Trap type			
Sticky bands vs canopy	1.123	0.598	1.877
Green DD vs canopy	1.666	0.567	2.937**
Purple DD vs canopy	2.723	0.520	5.248***
Green DD vs sticky bands	0.543	0.427	1.272
Purple DD vs sticky bands	1.604	0.358	4.481***
Purple DD vs green DD	1.061	0.290	3.658***
Sun exposure			
Open vs edge	−0.766	0.283	−2.712*
Shaded vs edge	−0.160	0.267	−0.599
Shaded vs open	0.606	0.324	1.872

We report adjusted *P* values based on applying the correction of Westfall (1997) to maintain the family-wise error rate at 0.05. * Adjusted $p < 0.05$; ** adjusted $P < 0.01$; *** adjusted $P < 0.001$.

Table 3. Logistic regression model predicting effects of ash phloem area, distance of traps to the central release point, sun exposure, and trap type on the probability of capturing at least one adult *A. planipennis* beetle on purple or green double-decker (DD) traps, purple canopy traps, and sticky bands on girdled trees

Effect	Model coefficients			Analysis of deviance[a]			
	Coef	SE	Z	*df*	AIC	*D*	LRT
Intercept	−0.404	1.102	−0.366	1			
Phloem area (100 m^2)	0.147	0.200	0.736	1	83.040	69.040	0.575
Distance (100 m)	0.088	0.512	0.171	1	82.495	68.495	0.864
Sun exposure				2	83.484	71.484	0.221
Edge (RG)							
Open	−0.528	0.857	−0.616				
Shaded	−1.344	0.803	−1.673				
Trap type				3	90.246	80.246	11.781**
Sticky band (RG)							
Canopy trap	−0.501	0.881	−0.568				
Green DD	1.000	0.966	1.036				
Purple DD	2.238	1.006	2.226*				
Residual				52	68.465	84.465	

[a] Analysis of deviance indicates whether removing each effect would significantly decrease model fit. Coef = estimated coefficient, SE = standard error of the coefficient, Z = Wald test *z*-score for the coefficient. *D* = Deviance, LRT = Likelihood ratio test associated with dropping the effect, RG = reference group. For a null model with only an intercept term, *df* = 59, AIC = 85.11, and *D* = 83.11. *, $p < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Phloem area had no discernible influence on the number of *A. planipennis* beetles captured per trap (LR = 0.138; $P = 0.711$), while distance had a weak negative effect (LR = 4.643; $P = 0.031$) (Table 1). Traps in grid cells closest to the release point (31 m away) caught an average of 1.42 beetles per trap, whereas those in grid cells farthest from the release point (285 m away) caught an average of 0.54 beetles (a difference of less than one beetle). Sun exposure also had a weak effect on beetle captures per trap (LR = 7.756; $P = 0.021$). Traps in the open ($M = 0.58$) captured fewer beetles per trap than partially shaded traps located along or near the edge of wooded areas ($M = 1.18$; $P = 0.018$), but did not differ from fully shaded traps ($M = 1.01$; $P = 0.061$) (Table 2). There was no difference in the number of beetles captured per trap between partially and fully shaded traps ($P = 0.549$) (Table 2).

The three interaction terms we tried adding to the base model failed to improve model fit. Nonsignificant trap type × phloem area (LR = 0.403; df = 3; $P = 0.940$) and trap type × distance (LR = 6.537; df = 3; $P = 0.088$) interactions indicate the effect of trap type did not vary as a function of either the amount of ash phloem within the grid cell or the proximity of traps to the release point. The phloem area × distance interaction also was not significant (LR = 0.766; df = 1; $P = 0.382$), indicating the distance effect did not depend on the amount of ash phloem within the respective grid cell.

Logistic Regression. Results from the logistic regression to predict whether each trap was positive (e.g., captured ≥ 1 adult *A. planipennis*) showed trap type affected the probability of beetle captures (LR = 11.781; df = 3; $P = 0.008$) (Table 3). The probability of catching at least one beetle spanned a wide range of values for the four trap types (83% for purple double-decker traps, 59% for green double-decker traps, 35% for sticky bands and 24% for canopy traps), but there was considerable sampling error around those estimates (Fig. 5b). Although the purple double-decker traps outperformed the sticky bands ($\beta = 2.238$; z = 2.226; $P = 0.026$) (Table 3), this difference was only marginally significant (adjusted $P = 0.067$) in the pairwise comparisons (Table 4). Only two trap types clearly differed in effectiveness after correcting for conducting multiple tests: purple double-decker traps were more likely to capture at least one beetle than the canopy traps (adjusted $P = 0.016$).

Spatial distribution of the 31 positive traps shows *A. planipennis* were captured across much of the site, including in areas where little or no ash occurred (Fig. 3b), consistent with results showing area of ash phloem had little effect on the probability that traps were positive (LR = 0.575; $P = 0.448$) (Table 3). Distance of positive traps to the central release point ranged from 30.7 to 285.0 m (average 155.0 ± 61.4 m) and distance was unrelated to the probability of capturing one or more beetles (LR = 0.029; $P = 0.864$). Only two of the four traps within 50 m of the central release point captured *A. planipennis*, whereas six traps that were at least 200 m from the central release point captured at least one beetle (Fig. 3b).

Table 4. Pairwise multiple comparisons of the probability of capturing at least one adult *A. planipennis* on purple or green double-decker (DD) traps, purple canopy traps, and sticky bands on girdled trees based on the logistic regression model

Comparison	Estimate	SE	Z
Trap type			
Sticky bands vs canopy	0.501	0.881	0.568
Green DD vs canopy	1.501	0.871	1.724
Purple DD vs canopy	2.739	0.927	2.954*
Green DD vs sticky bands	1.000	0.966	1.036
Purple DD vs sticky bands	2.238	1.006	2.226
Purple DD vs green DD	1.238	0.866	1.429

We report adjusted *P* values based on applying the correction of Westfall (1997) to maintain the family-wise error rate at 0.05. * Adjusted $p < 0.05$.

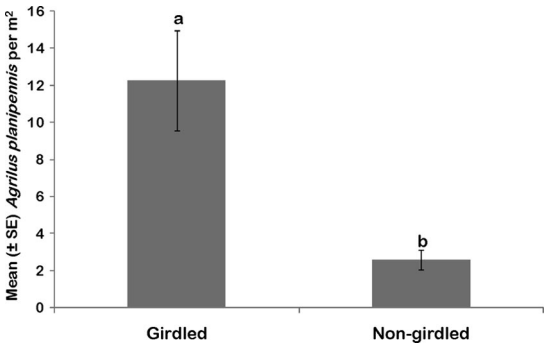

Fig. 6. Mean (±SE) number of *Agrilus planipennis* larvae per m^2 of ash phloem on girdled (n = 12) and nongirdled (n = 29) ash trees felled and sampled between January and March 2009. Letters above bars indicate statistically significant differences ($P < 0.05$).

Sun exposure of the traps also had only a weak effect on the probability of catching one or more *A. planipennis* beetles (LR = 3.019; P = 0.221) (Table 3). Six of the 19 traps (32%) that were fully exposed to sun and ten of the 24 traps (40%) near an edge and partially exposed to sun captured *A. planipennis.* Five of the 17 traps (28%) that were partly or fully shaded by other trees were positive.

The interaction terms again failed to improve model fit. The lack of support for trap type × phloem area (LR = 1.784; df = 3; P = 0.618) and trap type × distance (LR = 0.457; df = 3; P = 0.928) interactions indicate that the effect of trap type did not vary as a function of either the area of ash phloem in the grid cell or the distance between the trap and the release point. A phloem area × distance interaction also was not significant (LR = 0.026; df = 1; P = 0.873).

Larval Density on Girdled and Nongirdled Trees. We examined and exposed a total of 74.0 m^2 and 70.6 m^2 of phloem on the 12 girdled and 29 nongirdled trees, respectively. All 12 girdled trees were colonized by ovipositing females in 2008. We recorded 863 current-year larvae on the girdled trees, in total. Current-year larvae were present on 21 of the 29 nongirdled trees (72%) and we recorded 177 current-year larvae, in total. On 17 of the 21 infested nongirdled trees, we found ≤4 larvae. Larval density ranged from 1.3 to 26.5 larvae per m^2 on the 12 girdled trees, compared with 0.4–11.0 larvae per m^2 on the 21 nongirdled but infested trees. A t-test, with degrees of freedom adjusted to account for unequal sample size and variance (Ruxton 2006), showed larval density was significantly higher on girdled trees than on the nongirdled trees (t = 3.49; df = 11; P = 0.002), a difference of approximately five-fold (Fig. 6). On the seven girdled trees that captured zero adult beetles on sticky bands, larval densities ranged from 1.3 to 18.9 larvae per m^2. Nongirdled trees with and without *A. planipennis* larvae averaged (± SE) 27.8 ± 1.8 cm in DBH (range: 9.2–41.3 cm) and 29.5 ± 4.2 cm in DBH (range: 10.4–45.3 cm), respectively.

Discussion

Evaluation of traps and lures for detection or delineation of *A. planipennis* infestations should ideally occur in sites with low or very low densities of *A. planipennis.* As *A. planipennis* populations build and trees begin to experience stress from larval galleries, the visual and olfactory signals that affect beetle behavior and host selection change. Foliage on girdled branches yellows, canopies thin, and branches begin to die, altering visual cues. Hyperspectral signatures collected from foliage of injured or declining ash detected changes in reflectance likely to be perceived by beetles but not visible to human observers (Bartels et al. 2008). Activity of adult *A. planipennis* is consistently higher in sunny conditions than in dark, shady areas (Yu 1992; McCullough et al. 2009a,b) and when foliage thins and branches die, light penetration through the canopy increases. Moreover, as *A. planipennis* densities build within sites, a higher proportion of trees emit stress-related volatiles (Rodriguez-Saona et al. 2006), potentially competing with or overwhelming lures on traps. Differential attraction of *A. planipennis* to girdled versus nongirdled trees declines as overall *A. planipennis* density increases and nongirdled trees become stressed by larval feeding (Mercader et al. 2011).

Many studies designed to evaluate *A. planipennis* traps or lures, however, have been conducted in sites with moderate to high *A. planipennis* infestations. Although relatively high numbers of beetles are captured on individual traps, visible symptoms of infestation are already apparent in these areas and detection traps would be unnecessary. For example, an average of 244 ± 108 and 118 ± 61 beetles per trap were captured on prism traps suspended in ash trees in two sites, but canopy dieback was moderate to severe in these sites (Lelito et al. 2007). In other studies, weekly captures averaged >100 beetles per trap and >60 beetles per trap on green or purple prism traps placed 13 m and 1.5 m above ground, respectively, in sites where canopy dieback ranged from 35 to 50% (Crook et al. 2008 2009). Marshall et al. (2009) used total beetle captures over the summer to distinguish between low and high density sites, but external symptoms were already present in these sites. Canopy dieback averaged 29% and 22% in the sites designated as high density and low density, respectively, suggesting *A. planipennis* populations in this study were at least at moderate densities.

Our study was conducted in a site with an ultra-low density of *A. planipennis* with no external symptoms of infestation apparent, consistent with conditions in areas where detection or delineation would be appropriate. When trees were sampled before the study began, overall larval density was <0.1 larvae per m^2 and the most heavily infested tree had <5 larvae per m^2. As a basis for comparison, canopy dieback of ≈20% is generally associated with ≥20 *A. planipennis* per m^2 (Anulewicz et al. 2007) and on average, 105 *A. planipennis* beetles can develop per m^2 of phloem on trees ≥13 cm in DBH (McCullough and Siegert 2007). In our sites, even after emergence of the 2008 cohort of

adult *A. planipennis* and the release of 415 additional beetles in summer 2008, larval density on the nongirdled trees sampled in fall 2008 averaged only 2.5 larvae per m^2.

Purple double-decker traps, which incorporate several visual and olfactory cues used by adult *A. planipennis*, were substantially more effective at capturing adult *A. planipennis* than the other trap types included in the study. Both total and per-trap beetle captures were higher, a greater proportion of the traps were positive (compared with canopy traps and sticky bands), and beetles were captured over a longer time period on purple double-deckers than on green double-deckers, canopy traps, and sticky bands. Even accounting for the greater trapping area on the double-decker traps, the average number of beetles captured per trap was 3- to 12-fold higher on the purple double-decker traps than on other traps. Similarly, in sites with relatively low *A. planipennis* densities, Marshall et al. (2010a,b) reported at least one beetle was captured on 95% of purple double-deckers, 81% of purple prism traps placed 6 m high in ash canopies, and 67% of green prism traps set 13 m high. Poland et al. (2011) evaluated various traps and lures across a range of *A. planipennis* infestation levels. At sites with very low *A. planipennis* densities, purple double-decker traps baited with the leaf blend and Manuka oil lures captured more beetles and a higher proportion of traps were positive than other trap designs.

Our results indicate beetles likely responded to distinct visual and olfactory cues associated with the different trap designs. The shade of purple used for panels on double-decker traps and canopy traps has been shown to be attractive to *A. planipennis* beetles both in electroretinograph studies (Crook et al. 2009) and several field trials (Crook et al. 2008; Francese et al. 2010; Marshall et al. 2010a, b). Double-decker traps are placed near edges or in open areas, where they are visually apparent and provide a readily discernable point source of volatiles for beetles. In contrast, prism traps suspended in the canopies of ash trees may be visually obscured and volatiles emitted from lures may be masked or overwhelmed by volatiles from surrounding trees. Double-deckers in our study were baited with a leaf blend lure containing *cis*-3-hexanol, a compound that may be especially attractive to *A. planipennis* males, as well as the Manuka oil lure, which appears to be particularly attractive to female beetles (Poland et al. 2011). Results from recent studies, however, showed more *A. planipennis* were captured on double-decker traps than canopy traps, even when the same trap color and lures were used (Poland et al. 2011). Ethanol reportedly attracted the native *Agrilus bilineatus* (Weber) to girdled oak (*Quercus* spp.) trees (Haack and Benjamin 1982) and ethanol lures, such as those on the double-decker traps, are often used to attract cerambycid and scolytid bark beetles to traps, usually in combination with host volatiles (Moeck 1970, Klimetzik et al. 1986, Byers 1992, Dunn and Potter 1991, Erasmus and Chown 1994, Miller 2006). We cannot assess whether the ethanol lures on double-decker traps contributed to *A. planipennis* attraction in this site, but in other studies, ethanol lures had no consistent effect on *A. planipennis* captures by traps already baited with *cis*-3-hexanol and Manuka oil (Poland et al. 2011).

We originally hypothesized that beetle captures would be greatest in areas with abundant ash phloem or on traps close to the central point where beetles emerged from the infested logs. Previous work in sites with low *A. planipennis* density showed beetle dispersal was directed toward areas with relatively abundant ash phloem in the vicinity of the emergence point of the adults (Siegert et al. 2010). Dispersal studies in two other sites indicated most *A. planipennis* eggs were laid on ash trees growing within 100 m of the point where adult beetles emerged (Mercader et al. 2009). In our study, however, beetle captures were not related to the abundance of ash phloem. We cannot exclude the possibility that a few immigrant beetles from surrounding areas may have been captured on our traps, but it seems clear that many beetles that emerged from the introduced ash logs or infested ash trees on the site bypassed ash trees on their way to purple double-decker traps. Beetle captures were only weakly related to the distance between traps and the infested logs that were placed in the center of the site, again suggesting that beetles actively flew to traps.

Although artificial traps have many advantages compared with girdled trees, debarking girdled trees to look for *A. planipennis* larvae remains a highly effective option for detection surveys. In our study, 100% of the girdled trees had *A. planipennis* larvae when trees were debarked. Sticky bands on girdled trees were not highly effective at trapping adult *A. planipennis.* Sticky bands captured beetles on less than half of the girdled trees and no more than four beetles were captured on any sticky band, regardless of larval density on the tree. Other studies have similarly noted the need to debark trees and look for larvae if girdled trees are employed for *A. planipennis* detection or delimitation (McCullough 2009a,b; Katovich and McCullough 2010; Tluczek et al. 2011). Although sticky bands have largely been abandoned, grids of girdled trees were used to detect numerous low density *A. planipennis* infestations in Michigan and Ohio (Rauscher 2006, Hunt 2007, Poland and McCullough 2010) and girdled trees continue to be used in some operational programs (SLAMEAB.info 2011). We also noted that larvae were present on 72% of the nongirdled trees that were felled and debarked from January to March 2009, an efficiency rate only slightly lower than that of the purple double-decker traps. Larval densities were very low on the nongirdled trees, however, making it difficult for inexperienced surveyors to determine whether trees were positive.

Advantages and disadvantages are associated with each of the trapping options evaluated in our study. Costs of double-decker traps, which require two prism panels, PVC pipe, and a t-post, are higher than costs for the single-prism canopy traps. The PVC pipe and t-post can be reused for several years, however, decreasing per trap costs over time. Moreover, the process of installing and checking double-decker traps

was at least as efficient as using a long pole and hook to lower and raise canopy traps into trees. Double-decker traps were less likely to be lost or damaged by high winds and storms than canopy traps (Poland et al. 2011) or to be obscured by ash leaves or other debris. We debarked at least half of the surface area of the girdled trees to estimate larval density, which was a fairly labor-intensive process, particularly with large, thick-barked trees. For operational programs such as the SLAM (SLow Ash Mortality) pilot project, survey guidelines stipulate girdled detection trees should be 10–20 cm in DBH (SLAMEAB.info 2011), which facilitates efficient debarking. In terms of time allocation, our experience has shown that the time needed to girdle and debark trees <20 cm DBH is not appreciably different than the time required to install traps in spring, check traps and replace lures in midseason, then retrieve traps in fall.

Opportunity costs, i.e., the probability of not detecting an *A. planipennis* infestation, must also be considered when developing protocols for detection surveys. Employing multiple trapping options may be an ideal scenario for many programs. Canopy traps will likely continue to be used for large-scale surveys, but employing purple double-decker traps may be highly appropriate for areas such as campgrounds or sawmills where risks of *A. planipennis* introduction are high. Similarly, in forested areas, a combination of girdled trees, purple double-decker traps, and canopy traps may be ideal.

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