

## Evaluation of Double-Decker Traps for Emerald Ash Borer (Coleoptera: Buprestidae)

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J. Econ. Entomol. 104(2): 517–531 (2011); DOI: 10.1603/EC10254

**ABSTRACT** Improved detection tools are needed for the emerald ash borer, *Agilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive forest insect from Asia that has killed millions of ash (*Fraxinus* spp.) trees in North America since its discovery in Michigan in 2002. We evaluated attraction of adult *A. planipennis* to artificial traps incorporating visual (e.g., height, color, silhouette) and olfactory cues (e.g., host volatiles) at field sites in Michigan. We developed a double-decker trap consisting of a 3-m-tall polyvinyl pipe with two purple prisms attached near the top. In 2006, we compared *A. planipennis* attraction to double-decker traps baited with various combinations of manuka oil (containing sesquiterpenes present in ash bark), a blend of four ash leaf volatiles (leaf blend), and a rough texture to simulate bark. Significantly more *A. planipennis* were captured per trap when traps without the rough texture were baited with the leaf blend and manuka oil lures than on traps with texture and manuka oil but no leaf blend. In 2007, we also tested single prism traps set 1.5 m above ground and tower traps, similar to double-decker traps but 6 m tall. Double-decker traps baited with the leaf blend and manuka oil, with or without the addition of ash leaf and bark extracts, captured significantly more *A. planipennis* than similarly baited single prism traps, tower traps, or unbaited double-decker traps. A baited double-decker trap captured *A. planipennis* at a field site that was not previously known to be infested, representing the first detection event using artificial traps and lures. In 2008, we compared purple or green double-decker traps, single prisms suspended 3–5 m above ground in the ash canopy (canopy traps), and large flat purple traps (billboard traps). Significantly more *A. planipennis* were captured in purple versus green traps, baited traps versus unbaited traps, and double-decker versus canopy traps, whereas billboard traps were intermediate. At sites with very low *A. planipennis* densities, more *A. planipennis* were captured on baited double-decker traps than on other traps and a higher percentage of the baited double-decker traps captured beetles than any other trap design. In all 3 yr, peak *A. planipennis* activity occurred during late June to mid-July, corresponding to 800–1,200 growing degree-days base 10°C (DD<sub>10</sub>). Nearly all (95%) beetles were captured by the end of July at ≈1400 DD<sub>10</sub>.

**KEY WORDS** detection tools, green leaf volatiles, manuka oil, invasive forest pest, double-decker trap

The emerald ash borer, *Agilus planipennis* Fairmaire (Coleoptera: Buprestidae), native to Asia, has become the most damaging invasive forest insect in North America. First discovered in southeastern Michigan in 2002, *A. planipennis* has killed tens of millions of ash (*Fraxinus* spp.) trees in Michigan, at least 14 additional states, and the Canadian provinces of Ontario and Quebec, Canada (EAB INFO 2011). Adult *A. planipennis* emergence begins in late spring at 230–260 degree-days base 10°C (DD<sub>10</sub>) (Cappaert et al. 2005) and continues into midsummer. Adults feed on ash leaves throughout their 3–6-wk lifetime but cause

negligible damage. Females oviposit into cracks and crevices on the main bole and branches of live ash trees (Bauer et al. 2004). Larvae tunnel into the cambium and feed in serpentine-shaped galleries that disrupt the vascular system of the tree, eventually resulting in tree mortality (Poland and McCullough 2006). Mature prepupal larvae overwinter in thick outer bark on large trees or in the outer sapwood and then pupate the following spring. Adult beetles chew out through the bark leaving characteristic D-shaped exit holes. Although most *A. planipennis* have a 1-yr life cycle, some require ≥2 yr to complete development (Cappaert et al. 2005, Tluczek 2009). On relatively large trees, *A. planipennis* attacks typically begin in the upper canopy (Cappaert et al. 2005) where exit holes are difficult to see. Other external symptoms, including crown thinning, dieback, and epicormic shoots, are generally not evident until *A. planipennis* densities build to moderate or high densities, usually ≥3 yr after initial infestation.

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Effective methods to detect and monitor newly established populations of *A. planipennis* are critically needed by regulatory and resource management agencies. Although *A. planipennis* may use short range or contact pheromones in mate location (Lelito et al. 2009, Pureswaran and Poland 2009, Silk et al. 2009), there is no evidence that these beetles use long-range sex or aggregation pheromones (Lelito et al. 2007, Rodriguez-Saona et al. 2007). Many operational detection programs, therefore, have relied on ground surveys to identify trees with external symptoms of *A. planipennis* attack (de Groot et al. 2006, Lyons et al. 2007). Because external symptoms are rarely present when *A. planipennis* densities are low, visual surveys are unlikely to detect recent infestations. Other survey efforts incorporate girdled ash trees to attract *A. planipennis*, followed by debarking to locate larval galleries (McCullough et al. 2009a,b). Although girdled trees led to the detection of numerous low-density infestations (Rauscher 2006, Hunt 2007), debarking is labor intensive and suitable trees may not be available, especially for large-scale survey efforts.

An effective, efficient trap and lure for *A. planipennis* detection remains a high priority for scientists and regulatory officials. Recent studies showed that adult *A. planipennis* are attracted to specific shades of purple and green (Francese et al. 2005, Crook et al. 2009, Francese et al. 2010) and volatiles associated with ash leaves (Poland et al. 2005, 2006; de Groot et al. 2008, Grant et al. 2010) and bark (Crook et al. 2008). Purple prism traps (three-sided traps constructed of purple coroplast, 60 by 40 cm on each side) baited with lures containing *cis*-3-hexenol alone or blended with other alcohol or aldehyde green leaf volatiles were attractive to *A. planipennis*, particularly males (Crook et al. 2008; de Groot et al. 2008, Grant et al. 2010). Manuka oil, a natural oil distillate that contains antennally active sesquiterpenes also present in ash bark, significantly increased attraction of *A. planipennis* to purple traps (Crook et al. 2008). In 2008, the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS) implemented area-wide detection surveys using purple prism traps suspended in the canopy of ash trees and baited with manuka oil lures. More than 60,000 traps were deployed in 48 states and 10 new *A. planipennis* infestations were identified in 2008 (Crook and Mastro 2010).

Greater understanding of the visual, olfactory and environmental cues that influence *A. planipennis* host selection behavior could lead to improved traps and lures. Electrophysiological retinogram recordings revealed that beetles respond to wavelengths in the UV, purple, blue, and green ranges (Crook et al. 2009), and small differences in reflectance can affect attraction (Francese et al. 2010). Crook et al. (2009) suspended prism traps of various colors 13 m and 1.5 m above ground in a heavily infested stand. Significantly more *A. planipennis* were captured on dark green traps at the 13-m height than on other colors. When traps were 1.5 m in height, the standard purple and a darker shade of purple captured the most beetles but differences among colors were not significant. Other factors may

also affect the visual response of *A. planipennis*. For example, in a plantation, attack densities were higher on rough-barked than on smooth-barked green ash (*Fraxinus pennsylvanica* Marshall) trees (Anulewicz et al. 2008). Adults are also more likely to colonize open-grown trees compared with trees grown under full or partial shade (McCullough et al. 2009a,b).

Several studies have shown host plant stress resulting from girdling or other factors consistently increases *A. planipennis* attraction (McCullough et al. 2009a,b; Siegert et al. 2010). Attraction to stressed trees may reflect changes in volatiles emitted by ash foliage (Poland et al. 2005, 2006; de Groot et al. 2008; Grant et al. 2010) and bark (Crook et al. 2008) and differences in light reflectance or other visual cues perceived by *A. planipennis* beetles (Bartels et al. 2008, Crook et al. 2009, Francese et al. 2010). Volatile emissions from Manchurian ash (*Fraxinus mandshurica* Rupr.) seedlings were enhanced when subjected to *A. planipennis* leaf feeding or exposure to methyl jasmonate (a signal of physiological stress in plants) and were attractive to female beetles (Rodriguez-Saona et al. 2006). Blends of green leaf volatiles identified from foliage of green ash and white ash (*Fraxinus americana* L.) including *cis*-3-hexenol were attractive to *A. planipennis* (de Groot et al. 2008, Grant et al. 2010).

From 2006 to 2008, we conducted a series of field experiments to compare *A. planipennis* attraction to trap designs and lures incorporating multiple attractive stimuli. Specifically, we evaluated *A. planipennis* attraction to 1) visual silhouettes created by prism panels mounted on poles at different heights; 2) olfactory cues, including an ash leaf volatile blend, manuka oil, ethanol, and extracts of ash leaves and bark; 3) tactile cues representing rough bark texture; 4) purple or green panels or panels with visual enhancements; and 5) traps placed in the canopy of ash trees or mounted on poles and exposed to full sun.

## Materials and Methods

We evaluated different trap designs and lure combinations in field studies conducted at six, eight, and nine different sites in 2006, 2007, and 2008, respectively (Table 1). Infestations of *A. planipennis* in the sites ranged from undetected or very low to relatively high based on the extent of canopy dieback, evidence of woodpecker predation on *A. planipennis* larvae and observations of adult beetle activity.

**Trap Designs.** We developed a trap, hereafter called the “double-decker trap,” incorporating known visual, olfactory, and tactile cues likely to attract *A. planipennis* beetles. Each trap consisted of a 3-m-tall polyvinyl chloride (PVC) pipe (10 cm in diameter). A three-sided prism panel constructed from purple corrugated plastic (4 mm in thickness; Harbor Sales Inc., Sudlersville, MD), 60 cm in height by 40 cm in width on each side, was attached to the top of the PVC pipe (3 m in height) with cable ties. A second prism panel was similarly attached to the PVC pipe 60 cm below the top panel (1.8 m in height). Both purple panels were coated with clear Pestick (Hummert Interna-

**Table 1.** Field sites used for *A. planipennis* trapping experiments in Michigan, 2006–2008

Exp	Site location	Site description	Estimated <i>A. planipennis</i> infestation level <sup>a</sup>	No. replicates
1	MSU: Michigan State University Campus, Ingham Co.	Railroad right-of-way bordered by wooded areas	Moderate to high	10
1	Coleman: Coleman Rd., Isabella County	Perimeter of wooded areas surrounding a factory	Low	5
1	Wstn: Weigh Station, Interstate-96, Livingston Co.	Edge of wooded area at weigh station	Moderate	5
1	SL: Seven Lakes State Recreation Area, Oakland Co.	Openings and perimeter of wooded areas	Moderate	5
1	M52: Interstate-96 and State Rd. 52 intersection, Ingham Co.	Openings among ash trees in cloverleaf rights-of-way	Moderate to high	10
1	AA: Interstate-94 and Ann Arbor-Saline Rd. intersection, Washtenaw Co.	Openings among ash trees in cloverleaf rights-of-way	Low to moderate	5
2	SL: Seven Lakes State Recreation Area, Oakland Co.	Openings and perimeter of wooded areas	Moderate to high	6
2	SodFm: Sod Farm, Livingston Co.	Perimeter of mature trees in abandoned plantation (5 blocks); Berm in open field >300 m from any ash tree (2 blocks)	Moderate	7
2	M52: Interstate-96 and State Rd. 52 intersection, Ingham Co.	Openings among ash trees in cloverleaf rights-of-way	High	4
2	Wstn: Weigh Station, Interstate-96, Livingston, Co.	Edge of wooded area at weigh station	High	2
2	Fen: Fenner Nature Center, Ingham Co.	Openings and perimeter of wooded areas	Moderate	4
2	HortFm: Horticulture Farm, Michigan State University Campus, Ingham Co.	Perimeter of woodlots	Moderate	6
2	KelFor: W.K. Kellogg Experimental Forest, Kalamazoo Co.	Perimeter of mature ash plantations	Previously undetected	2
3	Brchfld: Burchfield Park, Ingham Co.	Edge of wooded areas	Moderate	3
3	SplyHol: Sleepy Hollow State Park, Shiawassee Co.	Openings and along edges of wooded areas	Moderate	3
3	RvrBnd: Riverbend Natural Area, Ingham Co.	Openings in wooded areas	Moderate	2
3	BearLk: Bear Lake Natural Area, Ingham Co.	Perimeter and openings in wooded areas	Moderate	2
3	GrdWds: Grand Woods Park, Ingham Co.	Perimeter of wooded areas	Moderate	2
3	Fen: Fenner Nature Center, Ingham Co.	Openings and along edges of wooded areas	Moderate to high	2
3	ColRd: Collins Rd. Entomology Farm, Michigan State University, Ingham Co.	Perimeter of mature ash plantation	Moderate	2
3	Ionia: Ionia State Park, Ionia Co.	Openings and perimeter of wooded areas	Low	2
3	KelFor: W.K. Kellogg Experimental Forest, Kalamazoo Co.	Perimeter of mature ash plantations	Low	2

<sup>a</sup> *A. planipennis* infestation level was visually estimated based on the presence and abundance of canopy dieback, exit holes, woodpecker attacks, bark splits, and epicormic shoots. Low populations were characterized by no canopy dieback, few or no woodpecker attacks in the upper branches, no exit holes on lower branches or main trunk, and no bark splits or epicormic shoots. Moderate populations were characterized by 10–20% canopy dieback on some trees, few woodpecker attacks and exit holes, and few epicormic shoots. Sites with high populations had trees with ≈30% canopy dieback, abundant woodpecker damage, exit holes visible on lower branches and the main bole, many bark splits, and epicormic shoots.

tional, Earth City, MO). Traps were installed by sliding the PVC pipe over a T-post (1.5 m in height) that was set into the ground to provide support. No additional supports were required. The height and shape of the double-decker trap were intended to produce a silhouette that would be similar to a small tree and highly apparent to *A. planipennis* beetles.

We also developed a taller version of the double-decker trap referred to as “tower trap.” They were constructed by connecting two 3-m-tall PVC pipes together vertically, creating a 6-m tall pole. One pur-

ple prism panel was placed on the top PVC pipe at 6 m and the second panel was 4.8 m above ground. A rope and pulley system was devised, enabling us to raise the panels into place and to lower them when the traps were checked for insects. The lower PVC pipe was firmly attached to a T-post set into the ground and the tower traps were stabilized with three tensioned cables.

In addition, we designed and tested large flat purple panels mounted on poles, hereafter referred to as “billboard traps.” Billboard traps were constructed from a large flat panel (120 by 120 cm) of the same

purple coroplast used for the prism panels (4 mm thick, Harbor Sales Inc.). The flat panel was fastened with cable ties to a 1.8-m-tall PVC pipe (10 cm in diameter) that was slid over a T-post set into the ground for support. Each billboard trap was divided into four quadrants (60 by 60 cm) and different visual enhancements were randomly assigned to each quadrant. Visual enhancements included 1) a circle (30 cm in diameter) of green glitter (metallic green fine glitter, Premo Sculpey, Elk Grove Village, IL) in the center of the quadrant; 2) 25 shiny green or magenta metallic stickers (Sticko Metallic Stickers, EK Success Ltd., Clifton, NJ), each 2.5 cm in diameter, arranged in a 5 by 5 grid pattern at 10-cm spacing; 3) three dead female *A. planipennis* beetles pinned to the coroplast in a triangle 2.5 cm apart in the center of the quadrant; and 4) blank control with no visual enhancement.

Finally, we tested single panel traps and canopy traps. Single panel traps consisted of a single three-sided prism panel attached with wire to a rebar pole  $\approx 1.5$  m above ground. Canopy traps consisted of single prism panels suspended in the canopy of ash trees.

**Experiment 1.** The double-decker traps were evaluated from 15 June to 6 September 2006 at six field sites selected to represent very low to moderate to relatively high *A. planipennis* densities (Table 1). In total, 40 blocks, each consisting of four double-decker traps (see treatments below), were established across the six sites using a randomized complete block design (160 traps total). Traps were set in the open  $\approx 3$ –5 m from the edge of a woodlot or stand with ash trees and spaced  $\approx 15$  m apart.

We evaluated whether olfactory cues consisting of lures with a blend of ash leaf volatiles and/or manuka oil, and a tactile cue consisting of a rough texture to simulate bark, enhanced *A. planipennis* attraction. The leaf volatile blend included *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, BC, Canada). Manuka oil (provided by D. Crook, USDA-APHIS) was dispensed from a cluster of five 0.4-ml polyethylene snap cap tubes (Thermo Fisher Scientific, Waltham, MA) at a total combined release rate of 10 mg/d. Bubble caps with the leaf volatiles were attached to the lower edge of the top prism panel and manuka oil lures were hung from the lower edge of the bottom panel. A rough-bark texture was simulated by mixing fragrance-free kitty litter (Purina Tidy Cats, St. Louis, MO) into purple paint tinted to match the purple coroplast (Sherwin Williams Ltd., Richardson, TX), and applying it to the panels on both prisms. Each block was comprised of four double-decker traps treated with 1) the leaf volatile blend, manuka oil, and texture; 2) the leaf volatile blend and manuka oil, but no texture; 3) texture and the leaf volatile blend but no manuka oil; and 4) texture and manuka oil but no leaf volatile blend.

**Experiment 2.** We compared *A. planipennis* attraction to three different trap designs and different olfactory lures from 31 May to 10 August 2007. Trap designs included the double-decker traps, tower traps,

and single purple panel traps suspended 1.5 m above ground from rebar poles.

The study consisted of 31 blocks, each with five treatments (155 traps total), laid out in randomized complete blocks at eight field sites (Table 1). Each block included 1) unbaited double-decker trap; 2) double-decker trap with the top panel baited with the blend of four leaf volatiles and the bottom panel baited with manuka oil as in 2006; 3) double-decker trap with the top panel baited with the leaf blend plus an ash leaf extract and the bottom panel baited with manuka oil plus an ash bark extract; 4) tower trap with the top panel baited with the leaf blend plus an ash leaf extract and the bottom panel baited with manuka oil plus an ash bark extract; and 5) single panel trap baited with the leaf blend plus an ash leaf extract hung from the top of the trap and manuka oil plus an ash bark extract hung from the bottom of the trap. This design allowed us to compare three different trap designs (double-decker, tower and single panel) baited with the same lure combination and to compare different lure combinations in one trap design (the double-decker trap).

Ash leaf extracts were placed on the traps at the start of the experiment, 31 May 2007, and then replaced with fresh extracts on 29 June 2007 at which time bark extracts also were included. Extraction procedures followed Grant and Langevin (1995). To prepare the leaf extracts, ash foliage was collected from the mid-crown of several green ash trees by using pole pruners on 28 May 2007. Leaves were stripped from the branches and twigs, weighed, macerated in a Waring blender for 2 min with 95% ethanol ( $\approx 500$  g/liter), and then filtered and concentrated by evaporation in a fume hood for 48 h. The residue was extracted in hexane then pipetted into 15-ml low density polyethylene bottles (10 ml per bottle, Con Tech International, Delta, BC, Canada). Similarly, ash leaves and bark were collected from trees on 25 June 2007. Leaf and bark extracts were prepared on 26–28 June 2007 following the same procedures described above. Bark was chopped into small pieces ( $\approx 1$  cm<sup>3</sup>) before maceration in the Waring blender. Release rates for the leaf (12 mg/d) and bark (10 mg/d) extracts were determined gravimetrically in the laboratory at  $22 \pm 2^\circ\text{C}$ .

**Experiment 3.** Beginning in 2008, USDA-APHIS implemented areawide *A. planipennis* detection programs that specified use of single purple prism panels suspended in the canopy of ash trees and baited with manuka oil (USDA-APHIS 2010). We conducted a study from 29 May to 25 September 2008 to compare different trap designs, colors, and trap placement to the standard trap design used in the APHIS detection survey program. The trap designs included double-decker traps made with either purple or green panels, purple billboard traps, and purple or green canopy traps (single prism panels suspended  $\approx 3$ –5 m above ground in the lower canopy of mature ash trees as specified by USDA-APHIS guidelines). The light green double-decker and canopy traps (540 nm, Crook et al. 2009) were identical to their purple counterparts except that they were constructed from light green coroplast (Great Lakes IPM, Inc., Vestaburg, MI).

We established 20 replicates of nine treatments (180 traps total) laid out in randomized complete blocks at nine field sites where *A. planipennis* population densities ranged from very low to moderately high (Table 1). Each block included 1) unbaited purple double-decker trap; 2) purple double-decker trap baited with the four component leaf volatile blend, ethanol, and manuka oil; 3) unbaited green double-decker trap; 4) green double-decker trap baited with the leaf volatile blend, ethanol, and manuka oil; 5) unbaited purple canopy trap; 6) purple canopy trap baited with manuka oil (i.e., the standard trap used by the USDA-APHIS detection program); 7) unbaited green canopy trap; 8) green canopy trap baited with manuka oil; and 9) billboard trap baited with the leaf volatile blend, manuka oil, and ethanol. The leaf volatile blend was the same four component blend used in experiments 1 and 2, as described above. Manuka oil was released from pouches at 50 mg/d (Synergy Semiochemicals Corp., Burnaby, BC, Canada). Ethanol was released from ultrahigh release lures (800 mg/d, Con Tech International, Inc.). Manuka oil lures were hung from the lower edge of canopy traps and the bottom panel of double-decker traps. Lures with the leaf volatile blend and ethanol were hung from the lower edge of the top panel on double-decker traps. All of the lures were hung together in a cluster at the center of the billboard traps.

For all three experiments, traps were checked weekly through August or until no beetles were captured for 2 wk in a row. All *A. planipennis* adults were removed with forceps, placed in labeled resealable plastic bags and returned to the laboratory. Beetles were transferred to labeled petri dishes containing Histo-Clear II (National Diagnostics, Atlanta, GA) to remove Pestick. Species identification was confirmed and *A. planipennis* adults were sexed (experiments 1 and 2) based on the shape of the abdomen and the presence or absence of the characteristic patch of long hairs found on the underside of the head and thorax of males (Rodriguez-Saona et al. 2007).

**Statistical Analysis.** The number of *A. planipennis* captured was related to degree-day accumulations for each site and year. Weather data and growing degree-days above 10°C ( $DD_{10}$ ) were obtained from the Michigan Automated Weather Network (MAWN) reporting station located nearest each site (MSU MAWN 2009). Trap catch data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) and residual plots. Total numbers of *A. planipennis* captured were transformed by  $\ln(y+1)$  to normalize the data. Differences in numbers of beetles captured per trap and per panel among sites and treatments were tested as unplanned comparisons and multiple comparison tests were applied only when overall analysis of variance (ANOVA) was significant ( $P < 0.05$ ). ANOVAs were performed to determine significant effects of site, treatment, and site  $\times$  treatment interaction by using SAS statistical software (PROC GLM, SAS Institute 2003). In experiment 3, the main effects of trap type, color, and bait were analyzed by three-way ANOVA. When significant differences oc-

curred, the Tukey-Kramer least significant means test was used to evaluate treatment differences. The proportion of females captured in 2006 and 2007 was compared among treatments and weeks by ANOVA after arcsine square-root transformation. In experiment 2, four traps that were inadvertently set up near heavily infested trees that displayed severe symptoms by late July were determined to be significant outliers using Grubb's outlier test (Grubbs and Beck 1972) following the extreme studentized deviate method (GraphPad Software, San Diego, CA). To eliminate any bias in results due to the proximity of large sources of emerging *A. planipennis* adults, the entire replicate was eliminated from the analysis in each case. Therefore, four replicates were removed, including one from SL, SodFm, Wstn, and Fen (see Tables 3 and 4 for expanded abbreviations), leaving 27 replicates for analysis. All analyses were conducted at the  $P < 0.05$  level of significance.

## Results

**Experiment 1.** Overall, we captured 4,067 *A. planipennis* in total on the 140 traps in 2006. Total number of captured beetles varied among sites (Fig. 1A); the M52 site (with 10 blocks), Wstn and SL (each with five blocks) sites accounted for 43, 20 and 19%, respectively, of all captures. Relatively few beetles were captured at the AA and Coleman sites (each with five blocks), which accounted for only 6% and 1%, respectively of the total capture. Differences in the numbers of *A. planipennis* captured among sites reflected differences in *A. planipennis* population levels at the sites, but also variation in the number of traps (blocks) set up at each site. Mean numbers of *A. planipennis* captured per trap were significantly higher at the M52, Wstn and SL sites than at the MSU, AA, and Coleman sites ( $F = 55.5$ ;  $df = 5, 154$ ;  $P < 0.0001$ ) (Fig. 1B). At the AA and Coleman sites where *A. planipennis* population densities were very low, beetle captures generally started later and ended sooner than at sites with higher *A. planipennis* densities. The last *A. planipennis* was captured on 18 July at the AA and Coleman sites; whereas, beetles were captured until eight Aug at the other sites (Fig. 1A).

The pattern of *A. planipennis* responses to the different treatments was similar at all sites. For all sites combined, traps baited with both the leaf blend and manuka oil, but without texture captured the most *A. planipennis* (Fig. 2A). Traps baited with manuka oil alone and coated with texture caught significantly fewer *A. planipennis* than traps baited with both manuka oil and the leaf blend without texture. Traps coated with texture and baited with either the leaf blend alone or the leaf blend and manuka oil caught an intermediate number of *A. planipennis*. The number of beetles captured on upper and lower panels was similar (Fig. 2B and C). Traps baited with manuka oil and coated with texture caught significantly fewer *A. planipennis* on the upper panels than any other treatment (Fig. 2B). There were no significant differences

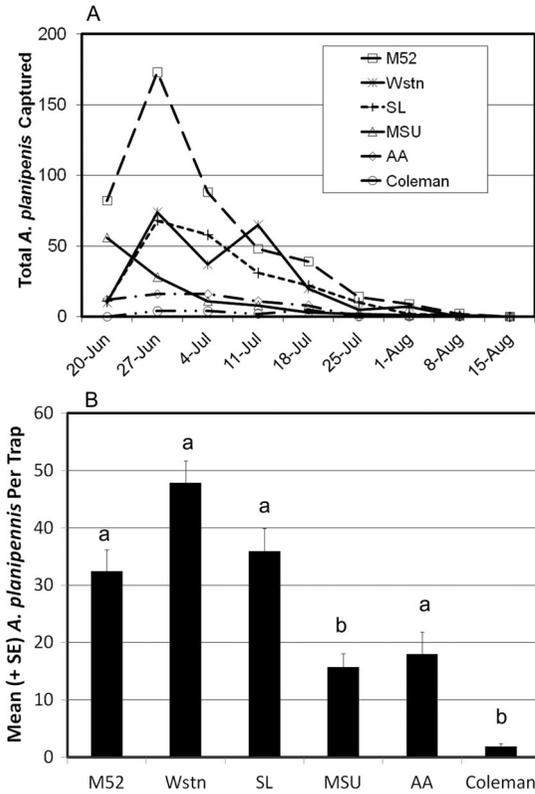


Fig. 1. Total number of *A. planipennis* captured per week (A) and mean (+SE) number captured per trap (B) at six sites in Michigan in experiment 1 in 2006. Field sites were located at Interstate-96 and Michigan State Rd. 52 (M52); a highway weigh station (Wstn); Seven Lakes State Park (SL); the Michigan State University campus (MSU); Interstate-94 in Ann Arbor (AA); and near Coleman, MI (Coleman). Bars topped with the same letter are not significantly different; Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

among treatments in the number of *A. planipennis* captured on the lower panels (Fig. 2C).

All of the traps captured some *A. planipennis*, except at the Coleman site, which had the lowest *A. planipennis* population density. At that site, 100% of the traps baited with leaf blend and manuka oil but with no texture captured *A. planipennis*, whereas 80% of the textured traps baited with leaf blend and manuka oil captured *A. planipennis*. When either the leaf blend or manuka oil was omitted, 60% of the traps captured *A. planipennis* in both cases.

The sex ratio of captured *A. planipennis* did not differ significantly among treatments across sites and collection dates ( $N = 40$ ;  $F = 7.75$ ;  $df = 3, 136$ ;  $P = 0.16$ ). Overall, 42% of the beetles captured were female and the proportion of females captured tended to decrease over time (Table 2). On average, the proportion of females captured was  $0.42 \pm 0.04$  (SE) on traps with leaf blend, manuka oil and texture,  $0.43 \pm 0.03$  on traps with leaf blend and texture,  $0.39 \pm 0.04$  on traps with manuka oil and texture, and  $0.47 \pm 0.04$  on traps with leaf blend and manuka oil.

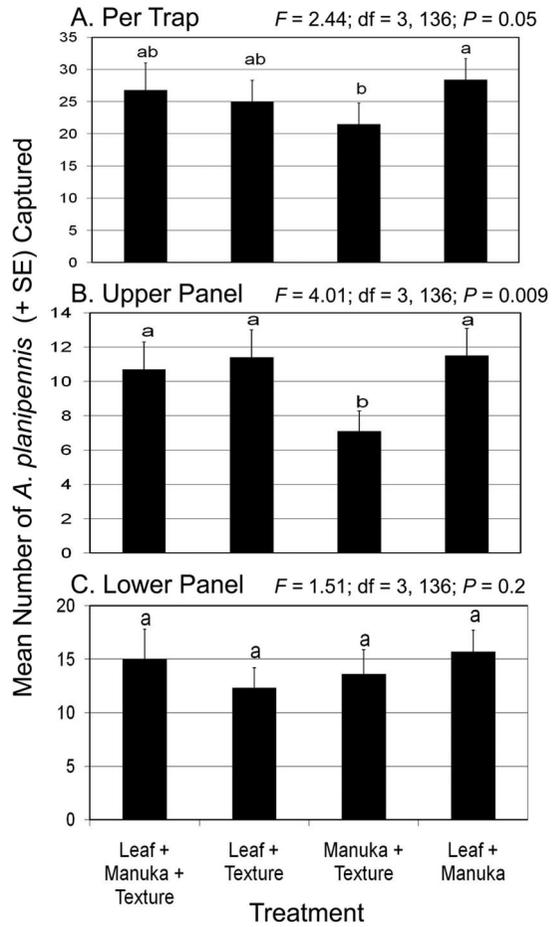


Fig. 2. Mean number ( $\pm$ SE) of *A. planipennis* captured per trap (A), on upper panels (B), and on lower panels (C) of double-decker traps baited with a four-component leaf blend (Leaf), manuka oil (Manuka), and coated with a rough texture (Texture) to simulate bark. Traps were deployed at six sites in Michigan in experiment 1 in 2006 ( $N = 40$ ). Bars topped with the same letter are not significantly different, Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

**Experiment 2.** We collected 3,884 *A. planipennis* in total from the 135 traps used in the analysis in 2007. Relatively high numbers of beetles were captured at the SL, SodFm, MSU, and M52 field sites (Fig. 3A). On average, more beetles were captured per trap at the SL site than at the M52 site, whereas beetle captures at the SodFm and MSU sites were intermediate. The mean number of *A. planipennis* captured at the Fen, HortFm, and Wstn sites were lower than at the SL, SodFm, MSU, and M52 sites. Traps at the KelFor site captured fewer *A. planipennis*, on average, than at any other site ( $F = 26.56$ ;  $df = 7, 126$ ;  $P < 0.0001$ ) (Fig. 3B). At the SL, SodFm, MSU, and M52 sites, which accounted for 92% of the total capture, activity of *A. planipennis* peaked in late June, similar to 2006, but beetle activity continued at the other sites throughout the season (Fig. 3A).

**Table 2.** Mean ( $\pm$  SE) proportion of female *A. planipennis* captured in double-decker traps by date at six sites in Michigan in experiment 1 in 2006 and at eight sites in Michigan in experiment 2 in 2007

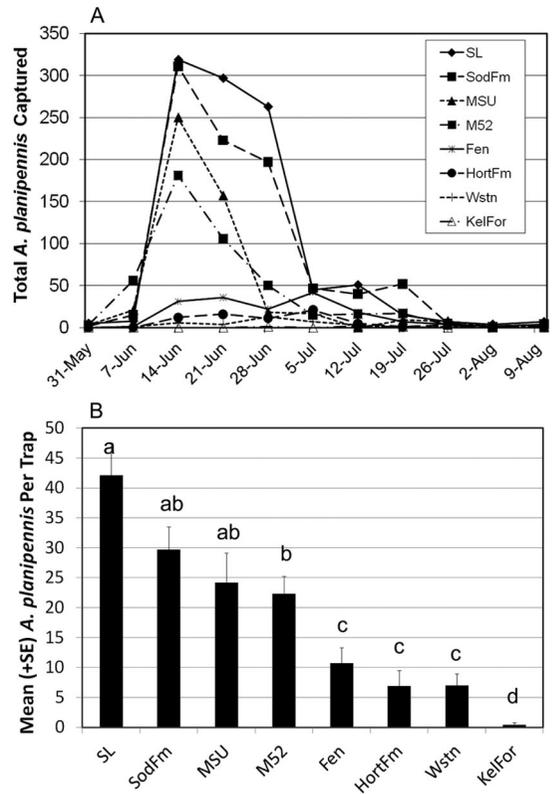
Date	N <sup>a</sup>	Proportion of female <i>A. planipennis</i> captured <sup>b</sup>
<b>Exp 1</b>		
20 June	38	0.51 $\pm$ 0.03a
27 June	28	0.47 $\pm$ 0.03a
4 July	31	0.46 $\pm$ 0.03ab
11 July	30	0.39 $\pm$ 0.03b
18 July	23	0.32 $\pm$ 0.03b
25 July	23	0.43 $\pm$ 0.05ab
1 Aug.	11	0.35 $\pm$ 0.07b
8 Aug.	8	0.28 $\pm$ 0.10b
ANOVA Statistics		F = 5.98; df = 7, 145; P = 0.0005
<b>Exp 2</b>		
31 May	18	0.41 $\pm$ 0.09ab
7 June	25	0.45 $\pm$ 0.04ab
14 June	25	0.62 $\pm$ 0.05a
21 June	24	0.59 $\pm$ 0.04a
28 June	24	0.51 $\pm$ 0.06a
5 July	21	0.37 $\pm$ 0.06b
12 July	22	0.36 $\pm$ 0.06b
19 July	12	0.21 $\pm$ 0.08b
26 July	4	0.38 $\pm$ 0.12ab
2 Aug.	6	0.33 $\pm$ 0.16ab
10 Aug.	8	0.28 $\pm$ 0.16b
ANOVA Statistics		F = 3.68; df = 10, 154; P = 0.0002

<sup>a</sup> Number of replicates in which traps for all treatments combined captured beetles.

<sup>b</sup> Means followed by the same letter within an experiment are not significantly different, Tukey–Kramer multiple comparison test on data transformed by arcsine  $\sqrt{(y)}$ ,  $P > 0.05$ .

The double-decker traps baited with the leaf blend and manuka oil lures, with or without ash leaf and bark extracts, captured more *A. planipennis* than the other trap–lure combinations (Fig. 4A). Addition of the leaf and bark extracts did not significantly increase *A. planipennis* capture compared with traps baited with only the leaf blend and manuka oil (Fig. 4A–D). There was little difference in *A. planipennis* captures between the upper and lower panels of any of the traps (Fig. 4B and C). The average number of *A. planipennis* captured was similar on the upper panels (6 m in height) of tower traps baited with leaf blend, manuka oil, and ash extracts and the upper panels (3 m in height) of unbaited double-decker traps (Fig. 4B). Average *A. planipennis* capture was similar among lower panels (2 m in height) of unbaited double-decker traps, the single panel traps (1.5 m in height) baited with leaf blend, manuka oil, and ash extracts and lower panels (4.8 m in height) on the tower traps baited with leaf blend, manuka oil, and ash extracts (Fig. 4C). Individual panels on the double-decker traps baited with leaf blend and manuka oil, with or without ash extracts, captured significantly more *A. planipennis* per panel than individual panels of any of the other traps (Fig. 4D).

With the exception of the KelFor site, all of the traps at all of the sites captured *A. planipennis*. At the KelFor site, however, *A. planipennis* were captured only on the baited double-decker trap.



**Fig. 3.** Total number of *A. planipennis* captured per week (A) and mean (+SE) number captured per trap (B) at eight field sites in Michigan in experiment 2 in 2007. Field sites were located at Seven Lakes State Park (SL), a sod farm (SodFm), the Michigan State University campus (MSU), Interstate-96 and Michigan State Rd. 52 (M52), Fenner Nature Center (Fen), the MSU Horticulture Farm (HortFm), a highway weigh station (Wstn), and the Kellogg Experimental Forest (KelFor). Bars topped with the same letter are not significantly different; Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

At the KelFor site where *A. planipennis* was not known to be established, a baited double-decker trap captured four *A. planipennis* in total between 28 June and 5 July. This represented the first recorded “detection event” of *A. planipennis* with traps and lures. No *A. planipennis* were captured on the sticky bands on the two girdled ash trees roughly 150 m away from this trap. These trees were felled in September, debarked, and found to be uninfested.

At the SodFm site, one block of traps was installed on a slight (1 m in height) berm at the edge of a field. The traps were  $\geq 300$  m away from the nearest ash tree. Despite this distance, we captured 67 *A. planipennis* in total on two double-decker traps baited with leaf blend and manuka oil (one with ash extracts and one without), 25 *A. planipennis* on the tower trap baited with leaf blend, manuka oil and ash extracts, 15 *A. planipennis* on the unbaited double-decker trap and three *A. planipennis* on the single panel trap baited with leaf blend, manuka oil, and ash extracts. These

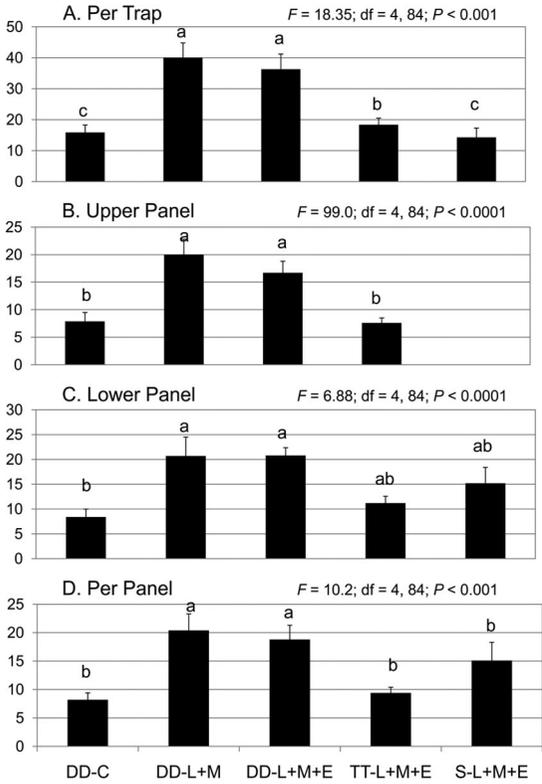


Fig. 4. Mean number ( $\pm$ SE) of *A. planipennis* captured per trap (A), on upper panels (B), on lower panels (C), and per panel (D) of double-decker (DD) traps, tower traps (TT), or single (S) panel traps baited with a four component leaf blend (L), manuka oil (M), and extracts (E) of ash leaf and bark or unbaited control (C). Traps were deployed at eight sites in Michigan in experiment 2 in 2007 ( $N = 27$ ). Bars topped with the same letter are not significantly different, Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

results show that the traps effectively attracted dispersing *A. planipennis* from at least 300 m away.

For all sites and weeks combined, the proportion of females captured was higher ( $0.66 \pm 0.05$ ) on single panel traps baited with leaf blend, manuka oil and ash extracts than on double-decker traps with any bait treatment or tower traps baited with leaf blend, manuka oil and ash extracts (range,  $0.45 \pm 0.05$ – $0.49 \pm 0.03$ ;  $F = 5.34$ ;  $df = 4, 95$ ;  $P = 0.0006$ ). Sex ratio varied by collection date, and the proportion of females captured tended to decrease over time for all sites and treatments combined (Table 2).

**Experiment 3.** We collected 4,218 beetles in total from the 180 traps in 2008. High numbers of beetles were captured at the SplyHol, Brchfld, and Fen sites; moderate numbers were captured at BearLk, RvrBnd, and GrdWds sites; and very few beetles were captured at the KelFor and Ionia sites (Fig. 5A). The mean number of beetles captured per trap was significantly higher at the Fen site than at the SplyHol, BearLk, RvrBnd, and GrdWds sites, whereas mean trap captures at the ColRd and Brchfld sites were intermedi-

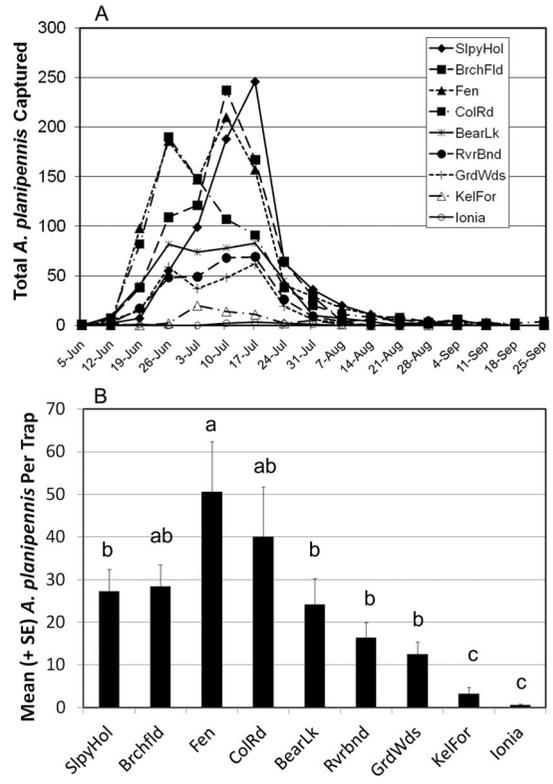
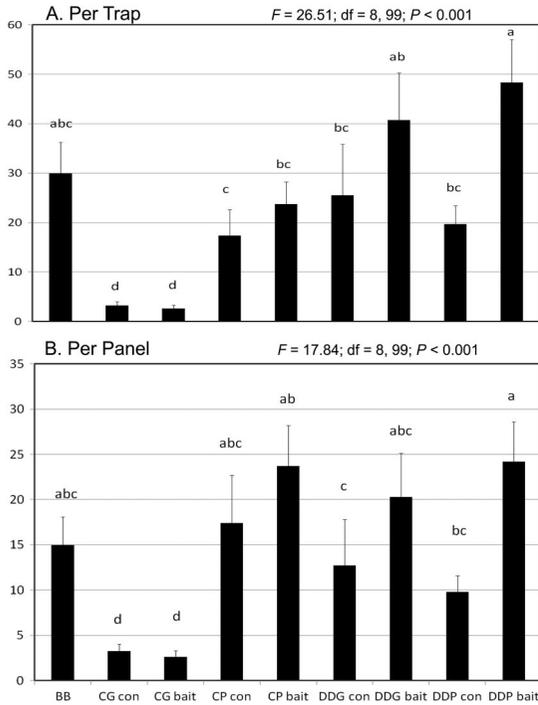


Fig. 5. Total number of *A. planipennis* captured per week (A) and mean number ( $\pm$ SE) captured per trap (B) at nine sites in Michigan in experiment 3 in 2008. Field sites were located at Sleepy Hollow State Park (SplyHol), Burchfield Park (Brchfld), Fenner Nature Center (Fen), Collins Rd. on the MSU campus (ColRd), Bear Lake Natural Area (BearLk), Riverbend Park (RvrBnd), Grand Woods Park (GrdWds), Kellogg Experimental Forest (KelFor), and Ionia State Park (Ionia). Bars topped with the same letter are not significantly different; Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

ate. Mean trap captures at the Ionia and KelFor sites were significantly lower than at any other site ( $F = 16.94$ ;  $df = 8, 171$ ;  $P < 0.001$ ) (Fig. 5B). At the sites with moderate and high captures, beetle activity was somewhat variable but peaked between 26 June and 17 July (Fig. 5). At several sites (e.g., Brchfld, Fen, BearLk, and GrdWds), there was a notable drop in beetle captures during the week of 3 July, probably due to stormy weather and heavy rain during that week (i.e., 6.3 cm precipitation on 2 July 2008, MSU MAWN 2009). At the KelFor and Ionia sites, *A. planipennis* captures were very low throughout the season but were highest during the weeks of 3 and 10 July. After 24 July, trap catches tapered off dramatically and few beetles were captured over the next 8 wk.

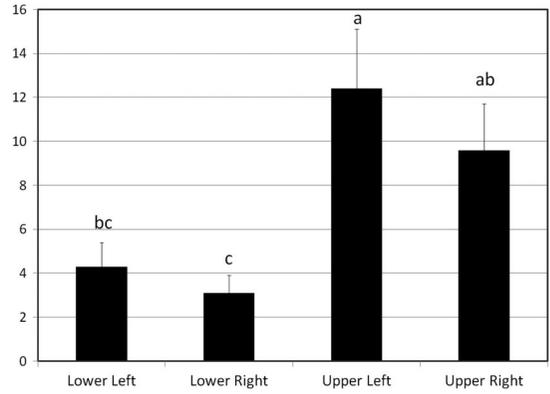
There was a significant effect of trap type, color and baiting status (regardless of lure type) on the number of *A. planipennis* captured per panel on the different traps. Overall, double-decker traps (mean  $\pm$  SE =  $16.8 \pm 2.2$ ; purple or green, baited or unbaited) captured significantly more *A. planipennis* than canopy



**Fig. 6.** Mean number ( $\pm$ SE) of *A. planipennis* captured per trap (A) and per panel (B) of double-decker (DD) traps, canopy traps (C), or billboard (BB) traps. Billboard traps were purple and baited with a four component leaf blend, manuka oil, and ethanol. Canopy traps and double-decker traps were either purple (P) or green (G) and were either baited (bait) or unbaited controls (con). Traps were deployed at nine sites in Michigan in experiment 3 in 2008 ( $N = 20$ ). Bars topped with the same letter are not significantly different; Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

traps ( $11.7 \pm 2.0$ ; purple or green, baited or unbaited), whereas the number captured on billboard traps was intermediate ( $15.0 \pm 3.2$ ; purple, baited) ( $F = 4.79$ ;  $df = 2, 175$ ;  $P = 0.0095$ ). For all trap types and lures combined, purple traps ( $18.0 \pm 1.8$ ) captured more *A. planipennis* than green traps ( $9.7 \pm 1.9$ ) ( $F = 20.93$ ;  $df = 1, 175$ ;  $P < 0.0001$ ) and baited traps (regardless of lure type) captured more ( $17.2 \pm 1.9$ ) *A. planipennis* than unbaited traps ( $10.8 \pm 1.9$ ) ( $F = 5.35$ ;  $df = 1, 175$ ;  $P = 0.02$ ).

The baited purple double-decker traps captured the most *A. planipennis*, accounting for 22.9% of the total catch, whereas green canopy traps (including baited or unbaited traps), captured the least, accounting for only 2.7% of the total catch (Fig. 6A). Baited purple double-decker traps captured significantly more *A. planipennis* than unbaited purple or green double-decker traps and baited or unbaited canopy traps of either color. *A. planipennis* captured on baited green double-decker traps and billboard traps were intermediate between captures on baited purple double-decker traps and purple canopy traps. Green canopy traps captured significantly fewer *A. planipennis* than any other trap type (Fig. 6A). With the exception of



**Fig. 7.** Mean number ( $\pm$ SE) of *A. planipennis* captured in the four quadrants of purple billboard traps baited with a four-component leaf blend, manuka oil, and ethanol. Traps were deployed at nine sites in Michigan in experiment 3 in 2008 ( $N = 20$ ). Bars topped with the same letter are not significantly different, Tukey–Kramer multiple comparison test on data transformed by  $\log(y + 1)$ ,  $P > 0.05$ .

the KelFor and Ionia sites where captures were very low, 100% of the double-decker traps (purple or green, baited or unbaited), the baited purple canopy traps, and billboard traps captured at least one beetle. Overall, 86% of the baited green canopy traps, 95% of the unbaited green canopy traps, and 95% of the unbaited purple canopy traps captured beetles.

When *A. planipennis* captures were standardized per panel, the pattern of responses remained similar (Fig. 6B). Baited purple double-decker traps captured significantly more *A. planipennis* per panel than unbaited green or purple double-decker traps and baited or unbaited green canopy traps. *A. planipennis* captures on green canopy traps (baited or unbaited) were significantly lower than any other treatment (Fig. 6B).

None of the visual enhancements including green glitter, iridescent stickers or pinned female beetles added to the purple billboard traps affected *A. planipennis* captures ( $F = 0.03$ ;  $df = 3, 76$ ;  $P = 0.99$ ). Significantly more *A. planipennis* were captured in the upper quadrants of the billboard traps compared with the lower quadrants, regardless of which visual enhancement was present in the quadrants (Fig. 7).

At the KelFor and Ionia field sites, very few beetles were captured and there were no visible signs of *A. planipennis* on any ash trees at either site. We captured 57 beetles at the KelFor site and 10 beetles at the Ionia site, representing 1.4 and 0.2% of the total capture at all sites, respectively. Of the 57 beetles captured at the KelFor site, 49% were on baited purple double-decker traps, 78% were on baited traps, and 82% were on double-decker traps (baited or unbaited of either color). All of the purple double-decker traps (baited or unbaited), baited purple canopy traps, and baited green double-decker traps captured *A. planipennis*. In contrast, 50% of the unbaited purple canopy traps, the baited green double-decker traps, and the baited green canopy traps captured *A. planipennis*. None of

**Table 3.** Cumulative percentage of *A. planipennis* captured by date and accumulated degree days base 10°C (DD<sub>10</sub>) at six field sites in Michigan in experiment 1 in 2006

Date	Cumulative % of <i>A. planipennis</i> captured								All
	DD <sub>10</sub> <sup>a</sup>	M52	Wstn	SL	MSU	AA	DD <sub>10</sub> <sup>a</sup>	Coleman	
20 June	773	18.0	4.6	5.4	50.9	19.0	810	0	16.1
27 June	895	56.0	38.5	38.9	76.4	44.4	932	26.7	50.2
4 July	1036	75.4	55.5	67.5	86.4	69.8	1077	53.3	70.3
11 July	1162	85.9	85.3	82.8	93.6	87.3	1208	66.7	85.8
18 July	1339	94.5	94.5	93.6	96.4	100	1384	100	94.9
25 July	1487	97.6	96.8	98.5	98.2	100	1530	100	97.8
1 Aug.	1682	99.5	100	99.5	99.1	100	1724	100	99.6
8 Aug.	1845	100	100	100	100	100	1886	100	100

Field sites were located at Interstate-96 and Michigan State Rd. 52 (M52); a weigh station on Interstate-96 near Brighton, MI (Wstn); Seven Lakes State Park (SL); the Michigan State University campus (MSU); Interstate-94 in Ann Arbor (AA); and near Coleman, MI (Coleman).

<sup>a</sup> Degree-day data were acquired from the Michigan Automated Weather Station closest to each site (MAWN 2009). Field sites in columns to the right of a degree-day column had the same accumulated degree-days.

the unbaited green canopy traps or billboard traps captured any beetles.

Of the 10 *A. planipennis* captured at the Ionia site, seven were on baited double-decker traps and three were on billboard traps. None of the canopy traps (green or purple, baited or unbaited) nor any of the unbaited double-decker traps captured any beetles, but 50% of the baited double-decker traps (purple or green), and 50% the billboard traps captured at least one *A. planipennis*.

**Trap Captures by DD.** In 2006 (experiment 1), *A. planipennis* flight peaked in most sites (excluding Coleman) during the week of 20–27 June, corresponding to 895 accumulated dDD<sub>10</sub>, when 50.2% of all the *A. planipennis* adults were captured (Table 3). By 11 July, corresponding to 1162 accumulated DD<sub>10</sub> and by 18 July, corresponding to 1339 accumulated DD<sub>10</sub>, 86 and 95% of the beetles had been captured, respectively. The last date on which a beetle was captured was eight Aug., corresponding to 1845 DD<sub>10</sub> (Table 3).

In 2007 (experiment 2), 84% of the beetles had been captured by 28 June, corresponding to an accumulation of 1044–1081 DD<sub>10</sub> at sites with moderate to high infestations (SodFm, MSU, M52, and SL), whereas

95% of the beetles were captured by 12 July, when 1327–1359 DD<sub>10</sub> had accumulated (Table 4). Fewer beetles were captured at the other sites, but beetle activity generally continued throughout the season (Fig. 3). At the KelFor site, with an extremely low EAB infestation, the few beetles captured were caught during a very short window at the end of the flight peak between 28 June to 5 July (Table 4).

In 2008 (experiment 3), *A. planipennis* activity began to peak by 26 June when 25.2% of the beetles had been captured and 817–856 DD<sub>10</sub> had accumulated (Table 5). By 17 July, 85% of the beetles had been captured, corresponding to 1214–1273 accumulated DD<sub>10</sub> and by 31 July, 95% of the beetles had been captured and DD<sub>10</sub> had reached 1497–1580 (Table 5).

## Discussion

From 2006 to 2008, we evaluated six different trap designs for *A. planipennis* detection in 17 different sites that represented a range of conditions and *A. planipennis* densities. The baited, purple double-decker traps were consistently more effective than any of the other trap designs at capturing beetles, particularly at

**Table 4.** Cumulative percentage of *A. planipennis* captured by date and degree-day base (10°C; DD<sub>10</sub>) at eight field sites in Michigan in experiment 2 in 2007

Date	Cumulative % of <i>A. planipennis</i> captured											All
	DD <sub>10</sub> <sup>a</sup>	SL	DD <sub>10</sub> <sup>2</sup>	SodFm	MSU	M52	Fen	HortFm	Wstn	DD <sub>10</sub> <sup>a</sup>	KelFor	
31 May	500	0.5	544	0.2	0.6	0.7	0	0	0	611	0	0.4
7 June	611	1.3	652	1.9	4.9	13.2	0.6	0	2.9	722	0	3.7
14 June	751	32.5	787	36.6	56.5	53.7	19.2	17.4	20	856	0	39.2
21 June	906	61.6	947	61.4	88.9	77.4	40.7	40.6	31.4	1018	0	66.1
28 June	1044	87.4	1081	83.4	92.6	88.6	53.9	56.5	68.6	1158	25	84.4
5 July	1156	91.8	1190	88.6	96.1	91.9	79.0	87.0	88.6	1258	25	90.6
12 July	1327	96.8	1359	93.1	96.1	99.3	89.8	95.6	97.1	1427	100	94.9
19 July	1454	98.2	1481	98.9	97.9	99.3	94.0	95.6	97.1	1553	100	98.2
26 July	1567	98.9	1596	99.4	99.6	100	96.4	100	100	1667	100	99.2
2 Aug.	1744	99.3	1642	99.6	99.8	100	97.6	100	100	1846	100	99.4
10 Aug.	1933	100	1839	100	100	100	100	100	100	2048	100	100

Field sites were located at Seven Lakes State Park (SL); Sod Farm in Brighton, MI (SodFm); the Michigan State University campus (MSU); Interstate-96 and Michigan State Rd. 52 (M52); Fenner Nature Center (Fen); the Horticulture Farm at MSU (HortFm); a weigh station on Interstate-96 near Brighton, MI (Wstn); and the Kellogg Experimental Forest (KelFor).

<sup>a</sup> Degree-day data were acquired from the Michigan Automated Weather Station closest to each site (MAWN 2009). Field sites in columns to the right of a degree-day column had the same accumulated degree-days.

**Table 5.** Cumulative percentage of *A. planipennis* captured by date and degree-day base (10°C; DD<sub>10</sub>) at nine field sites in Michigan in experiment 3 in 2008

Date	Cumulative % of <i>A. planipennis</i> captured													
	DD <sub>10</sub> <sup>a</sup>	SlypHol	DD <sub>10</sub> <sup>a</sup>	BrchFld	Fen	ColRd	BearLk	RivBnd	GrWds	DD <sub>10</sub> <sup>a</sup>	Ionia	DD <sub>10</sub> <sup>a</sup>	KelFor	All
5 June	440	0	464	0	0	0.1	0	0	0	414	0	486	0	0.02
12 June	604	0.3	629	0.9	0	1.0	2.1	0.7	2.0	566	3.5	649	0	0.8
19 June	707	1.2	734	5.7	10.8	12.3	11.5	6.4	7.8	663	3.5	755	10.0	7.9
26 June	817	8.7	856	19.3	31.2	38.7	30.3	22.6	31.0	777	7.0	811	10.0	25.2
3 July	936	22.1	980	34.5	47.3	59.2	47.4	39.2	45.5	891	42.1	935	10.0	41.7
10 July	1061	47.6	1115	64.3	70.4	74.1	65.3	62.2	64.3	1019	66.7	1069	30.0	64.3
17 July	1214	81.0	1273	85.3	87.6	86.7	84.4	85.5	88.6	1167	86.0	1225	60.0	85.3
24 July	1355	89.6	1424	93.3	92.1	92.0	94.7	94.3	96.1	1303	89.5	1372	80.0	92.5
31 July	1497	94.4	1580	96.9	95.3	94.7	97.0	97.3	98.4	1440	98.2	1530	90.0	95.9
7 Aug.	1635	97.2	1736	97.5	97.1	96.3	98.6	98.6	99.2	1584	100	1682	90.0	97.5
14 Aug	1721	98.6	1842	98.2	98.4	97.4	99.5	98.6	99.2	1685	100	1787	90.0	98.4
21 Aug.	1837	98.9	1970	98.9	98.9	98.5	99.9	98.6	99.2	1810	100	1918	90.0	98.9
28 Aug.	1957	99.2	2101	99.2	99.5	98.9	100	100	100	1931	100	2052	100	99.4
4 Sept.	2078	99.9	2240	100	99.7	98.9	100	100	100	2060	100	2195	100	99.7
11 Sept.	2144	100	2316	100	100	99.2	100	100	100	2125	100	2249	100	99.9
18 Sept.	2227	100	2407	100	100	99.4	100	100	100	2209	100	2344	100	99.9
25 Sept.	2304	100	2502	100	100	100	100	100	100	2298	100	2441	100	100

Field sites were located at Sleepy Hollow State Park (SlypHol), Burchfield Park (Brchfld), Fenner Nature Center (Fen), Collins Rd. on the MSU campus (ColRd), Bear Lake Natural Area (BearLk), River Bend Park (RvrBnd), Grand Woods Park (GrdWds), the Kellogg Experimental Forest (KelFor), and Ionia State Park (Ionia).

<sup>a</sup> Degree-day data were acquired from the Michigan Automated Weather Station closest to each site (MAWN 2009). Field sites in columns to the right of a degree-day column had the same accumulated degree-days.

sites with very low beetle densities. Several features of the double-decker traps, including their placement in full sun, the vertical silhouette, and the purple color of the panels likely contribute to *A. planipennis* attraction. Previous studies have shown that *A. planipennis* adults respond to visual stimuli, including color and hyperspectral signatures associated with stressed trees (Francese et al. 2005, Crook et al. 2009, Francese et al. 2010). Beetles are more active in sunny conditions (Yu 1992) and are more likely to colonize open-grown trees than shaded trees (McCullough et al. 2009a, 2009b). Placing the baited double-decker traps in the open takes advantage of this preference for sun. Moreover, the baited double-decker traps provide beetles with a distinct point source of host-related volatiles. In contrast, when single prism traps are placed in the canopies of ash trees, volatiles from the lures are mixed with and may be overwhelmed by volatiles emitted from the live ash trees, particularly if ash trees are stressed by *A. planipennis* feeding or other problems. In addition, traps suspended in the canopies are obscured by foliage and often not visible from more than a few meters away, potentially negating the advantage of incorporating color into the traps.

The size, silhouette, and apparency of the double-decker traps are intrinsic aspects of its design, but even when we considered beetle captures by individual panels, the double-decker traps performed better than the other traps. Individual panels on the purple double-decker traps captured more *A. planipennis* than purple single panel traps baited with the same lures in experiment 2 and also captured more beetles than green canopy traps in experiment 3. Tower traps, where the upper panels were 6 m above ground, may have simply been too high to attract or capture flying *A. planipennis*. Captures were especially low on the upper panels of the tower trap and fewer *A. planipen-*

*nis* overall were captured on tower traps compared with double-decker traps, where the upper panels were only 3 m above ground. Similarly, few beetles were captured on the lower quadrants of the billboard traps, which may have been too low or herbaceous vegetation may have interfered with beetle captures. In addition, although the billboard traps provided a large, broad trapping surface area, the wide, rectangular silhouette did not resemble a tree.

Trap color consistently seems to play a role in *A. planipennis* attraction. Francese et al. (2005) evaluated eight different trap colors using four-sided box traps set at 1.8 and 6.1 m in height. Purple traps captured significantly more beetles than any of the other colors at both heights. Crook et al. (2009) suspended prism traps that were dark and light green and dark and light purple 1.5 and 13 m above ground in the canopy of large ash trees. Although differences were not always significant, dark purple traps captured the most beetles at 1.5 m, whereas dark green traps captured the most beetles at 13 m. Additional prism traps of various shades of blue, purple, green or red set 1.5 m in height also were evaluated (Crook et al. 2009). Light green and dark purple traps captured the most beetles, whereas dark blue traps captured the least. In experiment 3, we found both green and purple baited double-decker traps attracted *A. planipennis* but overall, purple traps captured more beetles than green traps. This result was largely due to the green canopy traps suspended ≈3–5 m high in the canopy of ash trees. They captured very few beetles and were less attractive than any other trap and lure combination. Marshall et al. (2010a) also reported that purple traps captured more beetles per day than green traps at sites with relatively low densities of *A. planipennis*. In that study, 80% of purple traps captured at least one beetle compared with <50% of green traps. Similarly, *A.*

*planipennis* were captured on 95.2% of purple double-deckers, 81.0% of purple prism traps (6 m in height in ash canopies) but only 66.7% of the green prism traps set 13 m in height (Marshall et al. 2010b). Overall, it seems that purple traps are more attractive and are more likely to capture at least one *A. planipennis* than green traps at heights  $\leq 6$  m above ground. Green traps placed 13 m above ground also capture beetles (Crook et al. 2009, Marshall et al. 2010a), but the difficulty of setting traps at this height and the lack of tall, accessible ash trees in some areas may limit their use in operational programs.

The combination of ash leaf volatiles and manuka oil consistently increased attraction of *A. planipennis* to purple double-decker traps compared with unbaited traps, but trap texture did not. In experiment 1, omitting the leaf volatile blend significantly reduced *A. planipennis* captures, but omitting the manuka oil lure did not, indicating that the ash leaf volatiles were an important factor in *A. planipennis* attraction. Of the four leaf volatiles included in the blend, *cis*-3-hexenol alone or blended with the other leaf volatiles, seems to be primarily responsible for *A. planipennis* attraction (de Groot et al. 2008, Grant et al. 2010). Overall, traps baited with both lures but with no texture captured the most beetles in experiment 1. The rough surface on the traps with "texture" was difficult to coat with Pestick, which reduced adhesion of the beetles and probably interfered with *A. planipennis* capture. Although bark roughness may be involved in postlanding acceptance of oviposition sites (Anulewicz et al. 2008), it is probably not an important factor in long-range *A. planipennis* attraction.

Adding ash leaf and bark extracts to traps already baited with the ash leaf blend and manuka oil lures did not enhance *A. planipennis* attraction in experiment 2. Although the extracts contained additional host volatiles, the leaf blend and manuka oil lures contained the specific volatile compounds that elicited the highest electrophysiological and behavioral activity for *A. planipennis* (Rodriguez-Saona et al. 2006, de Groot et al. 2008, Crook et al. 2008, Grant et al. 2010). Furthermore, the overall release rate of the ash extracts was probably too low to provide a significant source of other potentially attractive compounds present in ash leaf or bark volatiles. Ethanol, a volatile frequently emitted by stressed hardwood trees (Kelsey 2001), was implicated in attraction of the native *Agrilus bilineatus* (Weber) to girdled oak (*Quercus* spp.) trees (Haack and Benjamin 1982). In experiment 3, ethanol was included with the leaf blend and manuka oil on baited green or purple double-decker traps and on the purple billboard traps, but not on the purple or green canopy traps. The presence of ethanol may have contributed to the increased attraction of double-decker or billboard traps compared with the canopy traps; however, in other studies, we found that ethanol lures did not consistently increase attraction of *A. planipennis* to traps baited with the leaf blend and manuka oil (T.M.P. et al., unpublished data).

Results from areas where *A. planipennis* densities are low and trees exhibit no symptoms of infestation

are most relevant for identifying trap designs and lures for early detection. Our studies were designed to include sites where *A. planipennis* populations ranged from very low to relatively high; but to date, most other efforts have been conducted in sites with high *A. planipennis* densities to ensure relatively high trap catches for statistical comparisons. Ash trees with *A. planipennis* feeding damage emit stress-related volatiles (Rodriguez-Saona et al. 2006), and their canopies thin and decline, affecting foliar light reflectance and light penetration through the canopy. In one study where purple prism traps captured an average of  $244 \pm 108$  beetles per trap in one site and  $118 \pm 61$  beetles per trap in a second site, it was noted that ash trees in both sites exhibited moderate to severe dieback from *A. planipennis* damage (Lelito et al. 2008). Crook et al. (2008) trapped beetles from 12 June to 19 July and reported a mean weekly capture rate of  $>100$  beetles per prism trap when traps were 13 m in height and  $>60$  beetles per trap at 1.5 m in height. Crook et al. (2009) also captured  $>100$  beetles per trap between 29 May and 18 July on green and dark purple prism traps set 13 m in height in this site. Canopy dieback ranged from 35 to 50% (D. Crook, personal communication), indicating the site was heavily infested. Marshall et al. (2009) distinguished between sites with low and high *A. planipennis* densities based on the total number of beetles captured on prism traps and trap trees during the summer. Ash canopy dieback, however, averaged 28.7 and 21.7% in sites they designated as high-density and low-density sites, respectively.

Each of our experiments included sites where *A. planipennis* densities were very low and no visible symptoms of infestation were apparent on any ash trees in the vicinity. For example, in experiments 1–3, we captured an average of 12.7, 13.3, and 14.3 beetles, respectively, per panel across sites. In our low-density sites where trees exhibited no symptoms of infestation, we captured an average of 1.9 beetles at the Coleman site in experiment 1; 6.9, 7.0, and 0.4 beetles at the HortFm, Wstn, and KelFor sites, respectively, in experiment 2; and 3.1 and 0.6 beetles at the KelFor and Ionia sites, respectively, in experiment 3. Similarly, Marshall et al. (2010a) captured a mean total of 64 adults over the entire flight season at low-density sites compared with  $>300$  adults at high-density sites. Results from low-density sites are likely to be more applicable for *A. planipennis* detection than studies in sites where trees are obviously infested and declining. Additional studies to assess how adult *A. planipennis* behavior and response to visual or olfactory cues vary with population density could help to determine how to best incorporate color or related visual cues into trap designs.

Seasonal patterns of *A. planipennis* flight activity were generally similar among sites and years. In experiment 1 in 2006, traps were set up in mid-June and  $>16\%$  of the total *A. planipennis* captured for the season were collected when traps were initially checked on 20 June, corresponding to 770–810 accumulated DD<sub>10</sub>. At the AA and Coleman sites where *A. planipennis* density was very low, captures were con-

centrated during a shorter window of time that at the other sites, probably because so few beetles were present as flight activity declined at the end of the season. In experiment 2 in 2007 and experiment 3 in 2008, traps were set up by late May. In both years, the first few *A. planipennis* beetles were captured  $\approx 410$ – $550$  DD<sub>10</sub>. In all 3 yr, *A. planipennis* flight activity peaked in late June to mid-July, corresponding to an accumulation of 800–1200 DD<sub>10</sub>. By 1200 DD<sub>10</sub>, 85% of the beetles had been captured, and 95% were captured by 1400–1500 DD<sub>10</sub>. Similarly, the number of *A. planipennis* adults captured on sticky bands affixed to girdled ash trap trees peaked in late June or early July, corresponding to 925–1065 DD<sub>10</sub>, in studies conducted from 2003 to 2007 in other Michigan sites (McCullough et al. 2009a,b). In trapping studies in 2006–2008, 5% of beetles were captured at 542 DD<sub>10</sub>, 50% at DD<sub>10</sub> 761, and 95% by DD<sub>10</sub> 1068 (Francesse et al. 2010).

In operational survey programs, traps do not necessarily need to capture the first *A. planipennis* beetles, but they should be in place before peak flight activity. Setting traps by 700 DD<sub>10</sub>, for example, which generally corresponds to early June in southern Michigan, would ensure that traps would be present when adult *A. planipennis* are most abundant. Traps should remain in place at least until the peak flight activity ends. In our sites,  $\geq 95\%$  of the beetles were captured by  $\approx 1400$  DD<sub>10</sub>, corresponding to the end of July. Crook et al. (2009) stated median trap catch occurred  $\approx 23$ – $29$  d after the study began on 29 May, corresponding to a median date of 21–27 June, depending on trap color and placement height. In other studies, traps were deployed for  $< 6$  wk (Francesse et al. 2005, Crook et al. 2008).

The ratio of female-to-male *A. planipennis* captured was significantly higher on single panel traps, which were only 1.5 m above ground, than on double-decker or tower traps in experiment 2, where panels were 1.8 and 3 m above ground or 4.8 and 6 m above ground, respectively. Male *A. planipennis* tend to hover near the canopies of ash trees (Lelito et al. 2007, Rodriguez-Saona et al. 2007), which might have accounted for relatively high captures of males on the higher panels. There were no differences in sex ratios of captured beetles among the different double-decker trap treatments in either experiment 1 or 2, although overall, the proportion of females captured tended to decrease over time. Females may become more occupied with oviposition later in the season and may be less likely to fly around and become captured on traps.

Some operational issues should be considered if traps are to be used programmatically for *A. planipennis* detection or monitoring. Pestick was reapplied to several traps after heavy rains and occasionally when an accumulation of flies or other insects obscured a panel and had to be scraped off. We noticed that *A. planipennis* occasionally fell off the panels, especially after heavy rains. Checking traps at least every two weeks would be desirable during peak *A. planipennis* activity periods, if resources permit.

Both the double-decker traps and the canopy traps had advantages and disadvantages. The single prism canopy traps distributed by APHIS from 2008 to 2010 for areawide *A. planipennis* surveys are relatively inexpensive, whereas the double-decker traps require a T-post, a PVC pipe, zip ties, and two prism panels (McCullough and Poland 2009). The T-posts and pipe, however, can be used for multiple years, decreasing annual costs. Guidelines developed by USDA–APHIS require survey crews to use a throw-line and rope or a telescoping pole that extends to 7.0 m to hang traps in the canopy of ash trees that are preferably at least 20 cm in diameter at breast height (USDA–APHIS 2010). Trap placement in the canopy can sometimes be difficult depending on tree density and terrain. In addition, large ash trees may be unavailable or inaccessible in many areas. Canopy traps must occasionally be scraped and Pestick reapplied due to the accumulation of leaves and debris from the tree. The double-decker traps were more resistant to severe weather than the canopy traps. In 2008, for example, severe storms and heavy winds occurred during the first week of June at some of our sites, and a tornado was recorded in Ingham Co. (MSU MAWN 2009, Tornadohistoryproject 2010). Maximum windspeeds ranged from 51.4 to 67.6 km/h from 6 to 8 June 2008, compared with daily maximum windspeeds that averaged 28.9 km/h during the rest of the month (MSU MAWN 2009). Numerous large trees at some of our study sites broke or were blown over during these storms. On 9 June when traps were checked, 20% (16/80) of the canopy traps in our sites had blown out of the trees and were damaged, whereas only 5% (1/20) of the billboard traps and 3.8% (3/80) of the double-decker traps sustained damage.

Opportunity costs associated with inaccurate results from *A. planipennis* detection surveys, i.e., zero captures in a site with an established *A. planipennis* infestation, should be recognized. Strategies that incorporate multiple detection methods may be ideal. Inexpensive canopy traps may be appropriate for systematic sampling across large areas, whereas double-decker traps may be most appropriate for high risk sites such as campgrounds. Girdled detection trees (McCullough et al. 2009a,b), along with visual surveys in late winter or early spring to identify ash trees with woodpecker damage, also can be incorporated into survey activities, especially in high risk sites. Integrating multiple methods for *A. planipennis* detection is likely to increase the effectiveness and efficient allocation of resources available for surveys.

### Acknowledgments

We thank Jacob Baker, Tara Dell, Stephanie Bloomer, James Wieferich, Keali Chambers, Bob McDonald, Andrew Tluczek, Chenin Limback, Ben Schmitt, Erin Burkett, Sarah Smith (all of MSU) and Alison Barc, Tina Ciaramitaro and Stephen Burr (U.S. Forest Service), who assisted with field studies in 2006–2008. Cooperation from the Michigan Department of Agriculture, Ingham County Parks, the Michigan Department of Natural Resources, and the Michigan Depart-

ment of Transportation is gratefully acknowledged. Comments provided by Deepa Pureswaran, Damon Crook, and two anonymous reviewers helped to improve this manuscript. Funding for this project was provided by the USDA Forest Service, Special Technology Development Program and the Northeastern Area Forest Health Protection programs.

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Received 7 July 2010; accepted 5 February 2011.