

Comparison Of Trap Types and Colors for Capturing Emerald Ash Borer Adults at Different Population Densities

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ABSTRACT Results of numerous trials to evaluate artificial trap designs and lures for detection of *Agrilus planipennis* Fairmaire, the emerald ash borer, have yielded inconsistent results, possibly because of different *A. planipennis* population densities in the field sites. In 2010 and 2011, we compared 1) green canopy traps, 2) purple canopy traps, 3) green double-decker traps, and 4) purple double-decker traps in sites representing a range of *A. planipennis* infestation levels. Traps were baited with *cis*-3-hexenol in both years, plus an 80:20 mixture of Manuka and Phoebe oil (2010) or Manuka oil alone (2011). Condition of trees bearing canopy traps, *A. planipennis* infestation level of trees in the vicinity of traps, and number of *A. planipennis* captured per trap differed among sites in both years. Overall in both years, more females, males, and beetles of both sexes were captured on double-decker traps than canopy traps, and more beetles of both sexes (2010) or females (2011) were captured on purple traps than green traps. In 2010, detection rates were higher for purple (100%) and green double-decker traps (100%) than for purple (82%) or green canopy traps (64%) at sites with very low to low *A. planipennis* infestation levels. Captures of *A. planipennis* on canopy traps consistently increased with the infestation level of the canopy trap-bearing trees. Differences among trap types were most pronounced at sites with low *A. planipennis* densities, where more beetles were captured on purple double-decker traps than on green canopy traps in both years.

KEY WORDS double-decker trap, canopy trap, *cis*-3-hexenol, Manuka oil, Phoebe oil

Detection of low-density populations of *Agrilus planipennis* Fairmaire, the emerald ash borer, remains a challenge for regulatory officials, foresters, and arborists in North America. Visual surveys to identify infested trees or delimit infestations are problematic. Colonization typically begins in the upper canopy (Cappaert et al. 2005), where D-shaped exit holes left by emerging *A. planipennis* adults or larger holes left by woodpeckers preying on late stage larvae are difficult to spot. Other symptoms including canopy thinning or dieback, epicormic shoots, and bark splits are rarely evident until population levels have increased and multiple generations of adults have already dispersed (Poland and McCullough 2006).

Ash trees girdled in spring then debarked in fall or winter to locate larvae have been used operationally to detect *A. planipennis*, monitor distribution, and assess density (Rauscher 2006, Hunt 2007, Mercader et al. 2013). Adult beetles are attracted to volatiles emitted by stressed ash (Rodriguez-Saona et al. 2006) and preferentially oviposit on girdled trees (McCullough et al. 2009a,b). Although girdled trees are the most

effective tool for *A. planipennis* detection (McCullough et al. 2011, Mercader et al. 2013), debarking trees to locate larval galleries can be labor-intensive and costly. Moreover, suitable trees may not always be available or accessible, especially in urban areas or when multiyear surveys are needed.

Considerable research has been invested in efforts to develop effective artificial traps and lures to attract and capture *A. planipennis* adults. Adult beetles, which are active throughout the summer, feed on ash foliage during their 3- to 6-wk life span (Cappaert et al. 2005). Close range or contact pheromones are involved in mating behavior (Lelito et al. 2009, Pureswaran and Poland 2009, Silk et al. 2009), and the female-produced *cis*-lactone (Bartelt et al. 2007) may increase attraction to host volatiles in the field (Silk et al. 2011, Ryall et al. 2012). Artificial trap designs incorporate visual cues that affect adult *A. planipennis* behavior. In China and North America, adult beetles are more active in sunny than shady locations, and trees that are fully or partially exposed to sun are consistently colonized before shaded trees (Yu 1992; McCullough et al. 2009a,b). Beetles also distinguish color, and electroretinogram assays have shown both male and female *A. planipennis* adults are sensitive to specific wavelengths of violet and green light (Crook et al. 2009), while females are sensitive to red ranges of the spectrum.

Ash volatiles, including green leaf volatiles and bark sesquiterpenes, elicit antennal responses by adult bee-

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ties and have been used in lures to attract adult *A. planipennis* beetles to artificial traps. In field studies, traps baited with the leaf alcohol *cis*-3-hexenol alone or combined with other green leaf volatiles (Rodríguez-Saona et al. 2006, de Groot et al. 2008, Grant et al. 2010) and natural tree oils including Manuka oil (from the New Zealand tea tree, *Leptospermum scoparium* J. R. and G. Forst (Myrtaceae)) and Phoebe oil (from the Brazilian walnut tree, *Phoebe porosa* Mez (Lauraceae)) were attractive to *A. planipennis* (Crook et al. 2008; de Groot et al. 2008; Grant et al. 2010, 2011; Poland et al. 2011). Since 2008, the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS) has used purple prisms suspended in the canopy of ash trees for large scale detection surveys (USDA–APHIS 2013). Lures used to bait traps have varied from year to year; Manuka oil alone and an 80:20 blend of Manuka and Phoebe oil have been used in past years, while Manuka oil and *cis*-3-hexenol lures are currently used.

Field studies to evaluate composition of lures, and trap design, color, and placement, however, have not yielded consistent results (Crook and Mastro 2010). Studies conducted in moderately to severely infested ash stands reported that prism traps, either baited with Manuka oil and green leaf volatiles or left unbaited, captured more beetles when placed at a height of 13 m in the canopy than when placed at a height of 1.5 m (Crook et al. 2008), and green prisms captured more beetles than purple prisms at a height of 13 m but not at 1.5 m (Crook et al. 2009). Poland et al. (2011) evaluated several trap designs at multiple field sites with *A. planipennis* infestations that ranged from very light to heavy. Double-decker traps, which consisted of a 3-m-tall PVC pipe with either two purple or green prisms attached near the top baited with green leaf volatiles and Manuka oil captured more beetles than similar but taller (6 m tall) tower traps or than purple or green prism traps hung in the canopy of ash trees. In a large area (16.2 ha), where *A. planipennis* density was extremely low, baited purple double-decker traps captured more beetles than baited green double-decker traps or purple prism traps in the canopy of ash trees (McCullough et al. 2011). The detection rate, that is, the percentage of traps that captured at least one beetle, was also higher for purple double-decker traps (81%) compared with green double-decker traps (56%) or purple canopy traps (25%). Similarly, in sites with low infestations, Marshall et al. (2010) reported at least one beetle was captured on 95% of purple double-decker traps baited with Manuka and Phoebe oil and on 81% of similarly baited purple prism traps hung at a height of 6 m in ash trees compared with 67% of similarly baited green prism traps hung at a height of 13 m. Although green prism traps captured the highest number of *A. planipennis* adults, they had the lowest detection rates because they were significantly influenced by the individual ash trees in which they were hung, and the number of beetles captured was not significantly different from the purple prism traps.

Inconsistencies in field study results may arise from different *A. planipennis* population densities in the

sites where traps were tested. At moderate to high *A. planipennis* densities, volatiles emitted by heavily infested, stressed trees may overwhelm or compete with volatiles emitted from lures (McCullough et al. 2011, Poland et al. 2011). Light availability increases as canopy dieback or thinning progresses, and hyperspectral signatures of stressed trees vary from those of healthy trees (Bartels et al. 2008), potentially affecting beetle response to trap color or placement. Our objective was to compare promising trap designs and lures for *A. planipennis* at a range of field sites representing low to moderate or heavy infestations.

Methods

We conducted similar trapping experiments in 2010 and 2011 to compare *A. planipennis* attraction to traps of different designs and colors, baited with host volatiles. In both years, experiments were conducted at sites selected to represent a range of *A. planipennis* infestation levels from low to moderate or high (Tables 1, 2). Low infestation sites were characterized by healthy ash trees with no visible *A. planipennis* exit holes on the bole or branches, no epicormic shoots, and little canopy dieback or thinning. At sites with moderate infestations, some trees in the vicinity of the traps were symptomatic, with up to 35% canopy dieback, a few woodpecker attacks on the upper portion of the trunk or leader and occasional epicormic shoots. At sites with heavy infestations, most trees were clearly infested with >35% canopy dieback, abundant woodpecker attacks, and epicormic shoots on the trunk and large branches.

Field sites used in 2010 included Sleepy Hollow State Park in Clinton Co., Burchfield Park near Holt in Ingham Co., Michigan State University's (MSU) Bear Lake Natural Area near East Lansing in Ingham Co., MSU's W.K. Kellogg Experimental Forest in Kalamazoo Co., Ionia State Recreation area in Ionia County, and Jasper Township in the Au Sable State Forest in Midland Co. In 2011, we used sites in Kellogg Experimental Forest, Ionia State Recreation Area and Jasper Township, along with two new sites—Charlton Park in Barry Co. and Portland State Game Area near the town of Portland in Ionia Co.

Traps were set up in randomized complete blocks with two to eight blocks per site, and a total of 21 blocks at six sites in 2010 and 20 blocks at five sites in 2011. Each block included the following four trap designs (i.e., treatments): 1) green canopy trap, 2) purple canopy trap, 3) green double-decker trap, and 4) purple double-decker trap. Canopy traps consisted of three-sided prisms (60 cm in height \times 40 cm in width on each side) made of corrugated plastic and hung from a branch, typically at a midcanopy height of ash trees (mean tree and trap heights \pm SE were 11.7 ± 0.5 m and 4.3 ± 0.19 m, respectively, in 2010, and 14.5 ± 0.6 m and 6.1 ± 0.4 m, respectively, in 2011). A double-decker trap consisted of a 3-m-tall PVC pipe (10 cm diameter) slid over a T-post in the ground to provide support. Two prisms were attached to the PVC pipe, first one at the top and the other 60 cm

Table 1. Mean (\pm SE) ratings for transparency, dieback, woodpecker attacks, epicormic shoots, and overall *A. planipennis* infestation level of trees bearing canopy traps, and *A. planipennis* infestation level of trees in the vicinity (within 10 m) of each trap at field sites used for *A. planipennis* trapping in 2010 in Michigan

Site	County location	Canopy trap tree transparency ^a	Canopy trap tree dieback ^b	Canopy trap tree woodpecker attack ^c	Canopy trap tree epicormic shoots ^d	Infestation level of canopy trap tree ^e	Infestation level of trees in vicinity ^e
Sleepy Hollow	Clinton	1.5 \pm 0.3	1.2 \pm 0.2	1.7 \pm 0.4ab ^f	1.5 \pm 0.7	1.9 \pm 0.4ab	3.6 \pm 0.1a
Burchfield	Ingham	1.3 \pm 0.3	1.3 \pm 0.3	1.6 \pm 0.4ab	1.6 \pm 0.4	2.2 \pm 0.4b	4.0 \pm 0a
Bear Lake	Ingham	1.8 \pm 0.5	1.3 \pm 0.3	3.0 \pm 0a	2.5 \pm 0.5	3.1 \pm 0.2a	4.0 \pm 0a
Kellogg	Kalamazoo	2.3 \pm 0.8	2.3 \pm 0.8	1.0 \pm 0b	1.5 \pm 0.5	2.2 \pm 0.4b	4.0 \pm 0a
Jasper	Midland	2.0 \pm 0.3	2.3 \pm 0.4	2.0 \pm 0.3ab	1.9 \pm 0.2	2.2 \pm 0.3ab	2.5 \pm 0.2b
Ionia	Ionia	1.0 \pm 0	1.0 \pm 0	1.0 \pm 0b	1.0 \pm 0	1.31 \pm 0.03b	2.0 \pm 0b
χ^2		3.63	6.12	3.50	3.22	12.12	19.07
df		5	5	5	5	5	5
P		0.6	0.3	0.01	0.6	0.03	0.002

^a Transparency ratings: 1) <10% healthy full canopy; 2) 11–35%; 3) 36–65%; 4) >65% weak and dying crown.

^b Dieback ratings: 1) <10% dying branches; 2) 11–35%; 3) 36–65%; 4) >65% dying branches.

^c Woodpecker attacks: 1) no woodpecker attacks; 2) 1–5 woodpecker attacks; 3) >5 woodpecker attacks.

^d Epicormic shoots: 1) no epicormic shoots; 2) 1–5 epicormic shoots; 3) >5 epicormic shoots.

^e *A. planipennis* infestation level on canopy trap tree or on trees within 10 m of each trap: 1) very light, no to few symptoms; 2) light, few symptoms; 3) moderate, symptoms very evident; 4) heavy, high degree to extreme level of symptoms.

^f Within each column, means followed by the same letter are not significantly different, logistic regression on ordinal data, $P > 0.05$.

below the first (McCullough and Poland 2009). Double-decker traps were placed in full or nearly full sun along the edge or in openings of the wooded areas. Prism surfaces were coated with clear Pestick insect trapping glue (Hummert International, Earth City, MO). In 2010, green prisms were made from light green ($\lambda = 540$ nm, peak reflectance 64%) corrugated plastic (Great Lakes Integrated Pest Management, Vestaburg, MI). In 2011, a darker shade of green ($\lambda = 540$ nm, peak reflectance 49%) was used because it was reported to be more attractive to *A. planipennis* (Francese et al. 2010). Purple corrugated plastic (Harbor Sales Inc., Suddlersville, MD) was used in both years.

Within each year, all traps were baited with the same lure combination. In 2010, lures consisted of *cis*-3-hexenol released at 3.7 mg/d from two bubble caps for a combined released rate of 7.4 mg/d (Con-tech Enterprises Inc., Delta, BC), and an 80:20 blend of Manuka and Phoebe oils released from pouches at 50 mg/d (Synergy Semiochemicals Inc., Burnaby, BC). In 2011, the *cis*-3-hexenol lure was used again; however, Phoebe oil was unavailable, and thus the

second lure consisted of Manuka oil released from pouches at 50 mg/d (Synergy Semiochemicals Inc., Burnaby, BC). Release rates for all lures were determined by the manufacturers in the laboratory at an ambient temperature. The *cis*-3-hexenol lures were hung from the top panel of double-decker traps or from the top edge of canopy traps. An 80:20 blend of Manuka and Phoebe oil or Manuka oil lures were hung from the bottom panel on double-decker traps or the bottom edge of canopy traps.

Traps were set up between 24 and 28 May in 2010, and between 25 May and 2 June in 2011. Traps within the blocks were separated by at least 15 m, and the blocks were at least 30 m apart. Although symptoms of *A. planipennis* were evident on some trees at moderately infested sites and on many trees at heavily infested sites, we avoided placing double-decker traps adjacent to or hanging canopy traps in heavily infested trees. Both double-decker traps, set in the open ≈ 3 m from the edge of the stand, and canopy traps, hung from accessible branches on the open side of trees, were along the open edge of the stand and exposed to

Table 2. Mean (\pm SE) ratings for transparency, dieback, woodpecker attacks, epicormic shoots, and overall *A. planipennis* infestation level of trees bearing canopy traps, and *A. planipennis* infestation level of trees in the vicinity (within 10 m) of each trap at field sites used for *A. planipennis* trapping in 2011 in Michigan

Site	County location	Canopy trap tree transparency ^a	Canopy trap tree dieback ^b	Canopy trap tree woodpecker attack ^c	Canopy trap tree epicormic shoots ^d	Infestation level of canopy trap tree ^e	Infestation level of trees in vicinity ^e
Kellogg	Kalamazoo	2.8 \pm 0.5a ^f	3.0 \pm 0.4a	2.8 \pm 0.3a	2.8 \pm 0.3a	3.2 \pm 0.5a	4.0 \pm 0a
Charlton	Eaton	2.2 \pm 0.3ab	2.3 \pm 0.4ab	2.3 \pm 0.3ab	2.0 \pm 0.7abc	2.2 \pm 0.5ab	3.0 \pm 0bc
Portland	Ionia	1.3 \pm 0.1bc	1.2 \pm 0.1c	1.4 \pm 0.2bc	1.1 \pm 0.1bc	1.0 \pm 0.2bc	2.9 \pm 0.2b
Ionia	Ionia	1.1 \pm 0.1c	1.0 \pm 0c	1.0 \pm 0c	1.0 \pm 0c	0.7 \pm 0.1c	2.0 \pm 0c
Jasper	Midland	1.4 \pm 0.3bc	1.9 \pm 0.3bc	2.0 \pm 0.3ab	2.0 \pm 0.4abc	1.9 \pm 0.4ab	4.0 \pm 0a
χ^2		13.73	12.32	8.79	8.39	12.37	61.1
df		4	4	4	4	4	4
P		0.0008	0.01	0.05	0.05	0.02	<0.0001

^a Transparency ratings: 1) <10% healthy full canopy; 2) 11–35%; 3) 36–65%; 4) >65% weak and dying crown.

^b Dieback ratings: 1) <10% dying branches; 2) 11–35%; 3) 36–65%; 4) >65% dying branches.

^c Woodpecker attacks: 1) no woodpecker attacks; 2) 1–5 woodpecker attacks; 3) >5 woodpecker attacks.

^d Epicormic shoots: 1) no epicormic shoots; 2) 1–5 epicormic shoots; 3) >5 epicormic shoots.

^e *A. planipennis* infestation level on canopy trap tree or on trees within 10 m of each trap: 1) very light, no to few symptoms; 2) light, few symptoms; 3) moderate, symptoms very evident; 4) heavy, high degree to extreme level of symptoms.

^f Within each column, means followed by the same letter are not significantly different, logistic regression on ordinal data, $P > 0.05$.

sunlight. Traps were checked every alternate week to collect the captured *A. planipennis* adults. Lures were replaced in late June in both years to ensure adequate release rates throughout the *A. planipennis* adult activity period. Experiments ended on 9 September 2010 and 30 August 2011, after at least 1 wk of zero captures. Beetles recovered from traps were soaked in Histo-Clear II (National Diagnostics, Atlanta, GA) to remove Pestick, and then counted and sexed.

We assessed canopy conditions at all sites in late July of both years when *A. planipennis* symptoms were readily apparent. The general level of *A. planipennis* infestation in the vicinity of each double-decker and canopy trap was visually estimated by assigning an overall ranking for all ash trees within 10 m of each trap to indicate the level of beetle pressure likely to influence trap capture. The number of trees within the vicinity of each trap varied somewhat among sites, ranging from 4 to 6 trees (e.g., traps in sites with widely spaced ash) and from 8 to 12 trees (e.g., traps along the edge of woodlots or plantations). In some cases, ash trees could have influenced two adjacent traps, and therefore ratings of those trees were recorded for both traps. The infestation level in the vicinity of the traps (hereafter referred to as "the infestation level of trees in the vicinity") was ranked as follows: 1) very light, if almost no symptoms were visible on any of the trees; 2) light, if there were a few visible symptoms on some of the trees; 3) moderate, if many trees had at least a few woodpecker attacks and epicormic shoots but <35% canopy dieback; and 4) heavy, if most trees had abundant woodpecker damage, epicormic shoots, obvious exit holes on the trunk, and substantial canopy dieback (>35%).

For the individual trees bearing canopy traps, we used a clinometer to measure the height of the tree and of the trap. We also visually estimated canopy transparency and dieback on these trees, each of which were categorized separately as 1) <10, 2) 11–35, 3) 36–65%, and 4) >65% transparency or dieback. Abundance of woodpecker attacks and epicormic shoots on the canopy trap-bearing trees were assigned a rank of 1) if no woodpecker attacks or epicormic shoots were observed, 2) if 1–5 woodpecker attacks or epicormic shoots were observed, and 3) when >5 woodpecker attacks or epicormic shoots were present. Scores assigned for canopy transparency and dieback, and abundance of woodpecker attacks and epicormic shoots were averaged to yield a mean infestation level (a continuous variable used for correlation analysis and hereafter referred to as "the mean infestation level of canopy trap tree") for each trap-bearing tree, and then rounded to the nearest integer to give an overall tree condition score (a nominal variable used for ANOVA, and hereafter referred to as "canopy trap tree condition score").

Canopy conditions were compared among sites each year. Categorical ratings for canopy transparency and dieback, woodpecker attacks, epicormic shoots, canopy trap tree condition score (for trees bearing canopy traps), and the rating for overall *A. planipennis* infestation level of trees in the vicinity (i.e., within 10 m of each trap) were compared among sites using logistic regression analysis for ordinal data (PROC LO-

GISTIC), and repeated using each site as the reference group to compare differences among all sites.

Trap catch data were tested for normality (PROC UNIVARIATE). Total numbers of *A. planipennis* males, females, and both sexes captured per trap were transformed by $\ln(y + 1)$ to normalize the data which was confirmed by testing residuals after ANOVA. For each year, differences in the numbers of males, females, or beetles of both sexes captured per trap among sites were compared using the one-way ANOVA, followed by the Tukey HSD test if ANOVA results were significant (PROC GLM, PROC MEANS TUKEY).

Numbers of beetles captured per trap for all sites combined were compared by trap color (green vs. purple) and trap type (canopy vs. double-decker) using a 2 by 2 factorial analysis with color and type as factors as well as their interaction. To compare the differences in beetle captures among all four treatments (both levels of both factors), we used a mixed linear model (PROC MIXED) with treatment as a fixed effect and site, replicate within site, and *A. planipennis* infestation level on trees in the vicinity as random effects, followed by the Tukey–Kramer multiple comparison procedure (PROC MEANS TUKEY). Similarly, numbers of beetles captured per trap were compared by trap color and trap type for each site separately, using a 2 by 2 factorial model with color, type, and the interaction as factors. Differences in beetle captures among the four treatments were compared using a mixed linear model with treatment as a fixed effect and replicate and *A. planipennis* infestation level on trees in the vicinity as random effects followed by the Tukey–Kramer multiple comparison procedure (PROC MIXED, PROC MEANS TUKEY). For all sites combined, beetle captures per trap were compared by the infestation level of trees in the vicinity of the traps using the one-way ANOVA (PROC GLM), followed by the Tukey HSD test (PROC MEANS TUKEY). Numbers of beetles captured per canopy trap were compared by the canopy trap tree condition score for the specific trap-bearing tree using the one-way ANOVA (PROC GLM) followed by the Tukey HSD test (PROC MEANS TUKEY). Linear associations between the number of beetles captured on canopy traps and the mean infestation level of canopy trap trees were evaluated using correlation analysis (PROC CORR). All analyses were performed using the SAS version 9.1 for Windows statistical package (SAS Institute 2003; SAS Institute, Cary, NC) with an α -level of 0.05.

Results

Tree canopy condition, *A. planipennis* infestation level, and the number of *A. planipennis* beetles captured per trap differed among sites in both 2010 and 2011. In 2010, the infestation level of trees in the vicinity of traps varied considerably among the field sites (Table 1). At the Burchfield, Bear Lake, Kellogg, and Sleepy Hollow sites, trees in the vicinity of traps had higher levels of infestation than at the Jasper and Ionia sites. We intentionally selected relatively healthy

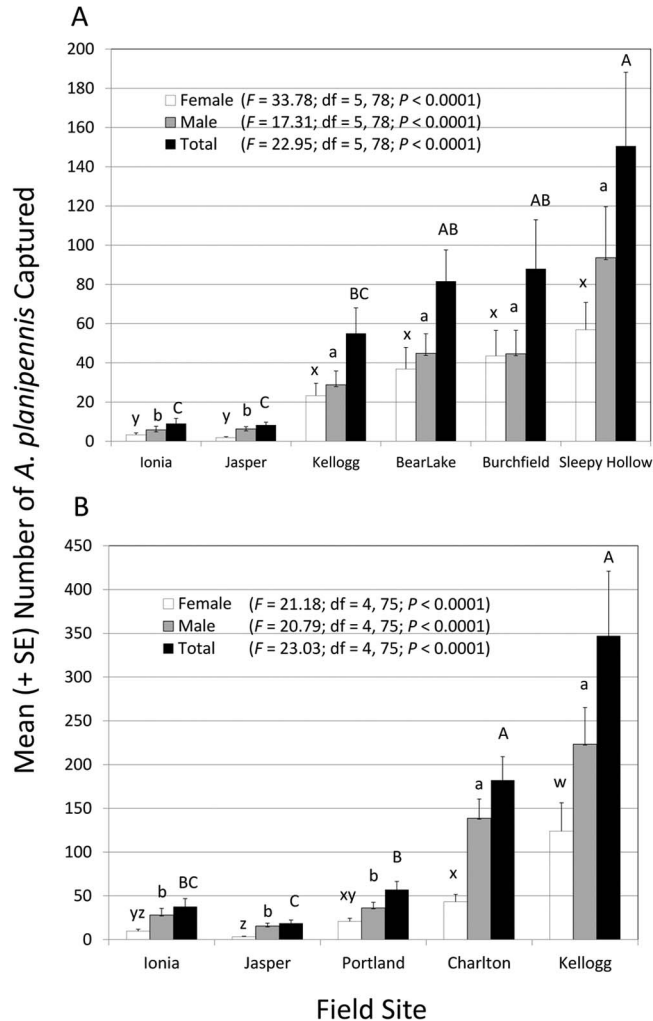


Fig. 1. Mean (\pm SE) number of *A. planipennis* females, males, and adults of both sexes captured by field site in southern Michigan in (A) 2010 and (B) 2011; $N = 8$ –24 traps per site. Within each year and sex, bars topped by the same letter are not significantly different, Tukey HSD test on data transformed by $\ln(y + 1)$, $P > 0.05$.

trees for hanging the canopy traps, and the trap-bearing trees had consistently lower infestation levels than the surrounding trees. Infestation levels of the canopy trap trees did not differ among the sites. Canopy transparency and dieback of the trees bearing canopy traps were similar among sites, but more woodpecker attacks and epicormic shoots were observed on canopy trap trees at the Bear Lake site than at the Kellogg or Ionia sites. Canopy trap trees at Sleepy Hollow, Burchfield, and Jasper had intermediate numbers of woodpecker attacks and epicormic shoots (Table 1).

In 2011, infestation levels increased at all sites, presumably reflecting *A. planipennis* population growth (Table 2). The Bear Lake, Burchfield, and Sleepy Hollow sites were dropped because many trees were dead or dying in spring 2011. Two new sites, Portland and Charlton, were added. Infestation levels increased noticeably at the Kellogg and Jasper sites, but remained fairly low at the Ionia site.

Infestation levels of trees in the vicinity of the traps were higher at Kellogg and Jasper than at Portland and Charlton and were lowest at Ionia (Table 2). Similar to 2010, we selected relatively healthy trees of the canopy trap trees were higher at the Kellogg site than at the Portland or Ionia sites and were intermediate at the Jasper and Charlton sites. Canopy transparency and dieback, woodpecker attacks, and epicormic shoots were all higher on canopy trap trees at the Kellogg site than at the Portland and Ionia sites, and were intermediate at the Charlton and Jasper sites (Table 2).

In 2010, the mean number of beetles captured per trap was higher at the Sleepy Hollow site than at the Kellogg, Ionia, and Jasper sites, whereas captures at the Burchfield and Bear Lake sites were intermediate (Fig. 1A). Similarly, more male and female *A. planipennis* were captured at Sleepy Hollow, Burchfield, Bear Lake, and Kellogg than at Ionia and Jasper. In

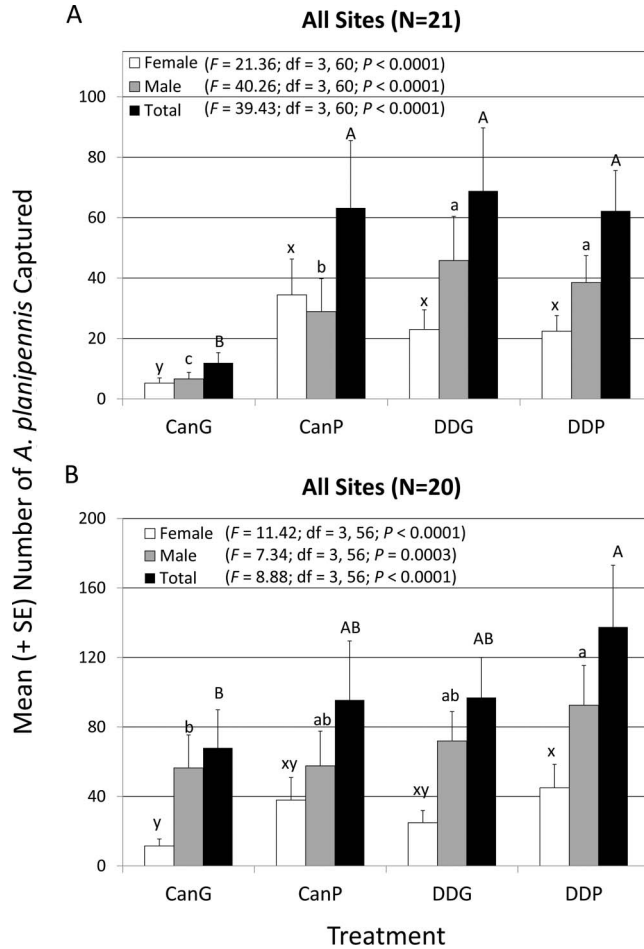


Fig. 2. Mean (\pm SE) number of *A. planipennis* females, males, and adults of both sexes captured on green canopy (Can G), purple canopy (Can P), green double-decker (DDG), or purple double-decker (DDP) traps at field sites in southern Michigan in (A) 2010 and (B) 2011. Traps in 2010 were baited with *cis*-3-hexenol released at 7.4 mg/d and an 80:20 blend of Manuka and Phoebe oil released at 50 mg/d. Green traps were a light shade of green; $N = 21$ replicates. Traps in 2011 were baited with *cis*-3-hexenol released at 7.4 mg/d and Manuka oil released at 50 mg/d. Green traps were a darker shade of green; $N = 20$ replicates. Within each year and sex, bars topped by the same letter are not significantly different, Tukey–Kramer test on data transformed by $\ln(y + 1)$, $P > 0.05$.

2011, more beetles were captured at Kellogg and Charlton than at Portland, Jasper, and Ionia (Fig. 1B). More males were captured at Kellogg and Charlton than at Portland, Jasper, and Ionia, and more females were captured at Kellogg than at Charlton, where more females were captured than at Ionia or Jasper. The number of females captured at Portland was intermediate between female captures at Charlton and Ionia (Fig. 1B).

In 2010, for all sites combined, more *A. planipennis* females and beetles of both sexes were captured on purple or green double-decker traps and on purple canopy traps than on green canopy traps (Fig. 2A). More males were captured on purple or green double-decker traps than on purple or green canopy traps. For all sites combined, 100% of the purple and green double-decker traps, 90% of purple canopy traps, and 80% of green canopy traps captured at least one beetle.

There was a significant effect of the trap type and trap color, but not their interaction, on the number of *A. planipennis* captured. Overall, double-decker traps captured more females ($F = 8.24$; $df = 1, 80$; $P = 0.005$), males ($F = 26.16$; $df = 1, 80$; $P < 0.0001$), and beetles of both sexes ($F = 19.81$; $df = 1, 80$; $P < 0.0001$) than canopy traps, and purple traps captured more females ($F = 8.52$; $df = 1, 80$; $P = 0.004$), males ($F = 3.51$; $df = 1, 80$; $P = 0.05$), and beetles of both sexes ($F = 3.98$; $df = 1, 80$; $P = 0.05$) than green traps.

In 2011, for all sites combined, more *A. planipennis* females, males, and beetles of both sexes were captured on purple double-decker traps than on green canopy traps, while captures on the green double-decker traps and the purple canopy traps were intermediate (Fig. 2B). Infestation levels increased at all sites by 2011, and there were no differences in detection rates among trap types. The number of *A. plani-*

pennis captured was significantly affected by trap color and trap type, but not their interaction. Overall, double-decker traps captured more females ($F = 5.53$; $df = 1, 76$; $P = 0.02$), males ($F = 8.67$; $df = 1, 76$; $P = 0.004$), and beetles of both sexes ($F = 8.20$; $df = 1, 76$; $P = 0.005$) than canopy traps. Purple traps captured more females ($F = 6.87$; $df = 1, 76$; $P = 0.01$) and beetles of both sexes ($F = 5.03$; $df = 1, 76$; $P = 0.03$) than green traps. Captures of males ($F = 0.86$; $df = 1, 76$; $P = 0.3$) did not differ between trap colors.

For each year, differences among treatments in *A. planipennis* captures were greater at sites with lower infestation levels than at sites with heavier infestations. In 2010, at Ionia and Jasper where infestation levels were relatively low, more *A. planipennis* were captured on purple double-decker traps than on green canopy traps. At Ionia, more *A. planipennis* males and beetles of both sexes were captured on purple double-decker traps than on green canopy traps and captures on green double-decker or purple canopy traps were intermediate. More females were captured on purple or green double-decker traps than on purple or green canopy traps (Fig. 3A). At Jasper, more *A. planipennis* males, females, and beetles of both sexes were captured on purple or green double-decker traps than on purple or green canopy traps (Fig. 3B). At the Ionia and Jasper sites, detection rates were 100% for purple or green double-decker traps, 82% for purple canopy traps, and 64% for green canopy traps.

At Kellogg and Bear Lake where *A. planipennis* infestation levels were higher, differences in beetle captures among treatments were not as pronounced. There were no differences among treatments at the Kellogg site in captures of females, males, or both sexes (Fig. 3C). At the Bear Lake site, more males and beetles of both sexes were captured on purple or green double-decker traps or purple canopy traps than on green canopy traps. More females were captured on purple canopy traps than on green canopy traps, while captures on purple or green double-decker traps were intermediate (Fig. 3D). All traps captured at least one beetle at the Kellogg and Bear Lake sites.

Similarly, at Burchfield and Sleepy Hollow, which were both heavily infested, differences among treatments were also less pronounced and all traps captured at least one beetle. There were no differences in captures of females, males, or both sexes among treatments at the Burchfield site (Fig. 3E). At Sleepy Hollow, more females and beetles of both sexes were captured on purple or green double-decker traps or purple canopy traps than on green canopy traps. More males were captured on green double-decker traps than on purple or green canopy traps. The number of males captured on purple double-decker traps was intermediate between those captured on green double-decker traps and purple canopy traps (Fig. 3F).

In 2011, differences in trap captures among treatments were again more pronounced at sites with rel-

atively low *A. planipennis* infestations. At Ionia, where the *A. planipennis* infestation remained low, more beetles of both sexes were captured on purple double-decker traps than on green canopy traps, while captures on green double-decker traps or purple canopy traps were intermediate (Fig. 4A). More males were captured on purple or green double-decker traps and on purple canopy traps than on green canopy traps. The trend was similar for females, but differences were not significant.

At the Portland site, where the *A. planipennis* infestation level was higher, more males and beetles of both sexes were captured on purple or green double-decker traps than on purple or green canopy traps. More females were captured on purple double-decker traps than on purple or green canopy traps, and the number of females captured on green double-decker traps was intermediate between purple double-decker traps and purple canopy traps (Fig. 4B). At Jasper (Fig. 4C), Charlton (Fig. 4D), and Kellogg (Fig. 4E) where *A. planipennis* infestations were heavy, there were no differences in *A. planipennis* captures among treatments.

In 2010, the number of *A. planipennis* captured per trap varied, depending on the infestation level of trees in the vicinity of the traps (≤ 10 m from each trap). As expected, more females, males, and beetles of both sexes were captured on traps when trees in the vicinity were heavily infested compared with traps surrounded by trees with very light, light, or moderate infestation levels (Fig. 5A). In 2011, the *A. planipennis* infestations were generally higher than in 2010, and none of the trees within 10 m of traps were classified as very lightly infested. More females, males, and beetles of both sexes were captured on traps when trees in the vicinity were heavily or moderately infested compared with traps surrounded by trees with light infestation levels (Fig. 5B).

The number of *A. planipennis* captured on canopy traps increased as the infestation level of canopy trap trees increased in both years. The linear relationship between number of *A. planipennis* captured ($\ln(y + 1)$ transformed) and the mean infestation level of canopy trap tree (continuous variable) was significant for females ($n = 42$, $R^2 = 0.32$, $P = 0.04$), males ($n = 42$, $R^2 = 0.35$, $P = 0.02$), and beetles of both sexes ($n = 42$, $R^2 = 0.33$, $P = 0.03$) in 2010 and for females ($n = 40$, $R^2 = 0.41$, $P = 0.008$), males ($n = 40$, $R^2 = 0.50$, $P = 0.001$), and beetles of both sexes ($n = 40$, $R^2 = 0.49$, $P = 0.002$) in 2011. In 2010, more beetles of both sexes were captured on the canopy traps hung in trees with heavy canopy trap tree condition scores than on traps hung in trees with light or very light canopy trap tree condition scores (nominal variable); captures on traps in trees with moderate canopy trap tree condition scores were intermediate. More males were captured on canopy traps hung in trees with heavy canopy trap tree condition scores than traps in trees with very light canopy trap tree condition scores; captures of males in traps hung in trees with moderate or light canopy trap tree condition scores were intermediate. Differences

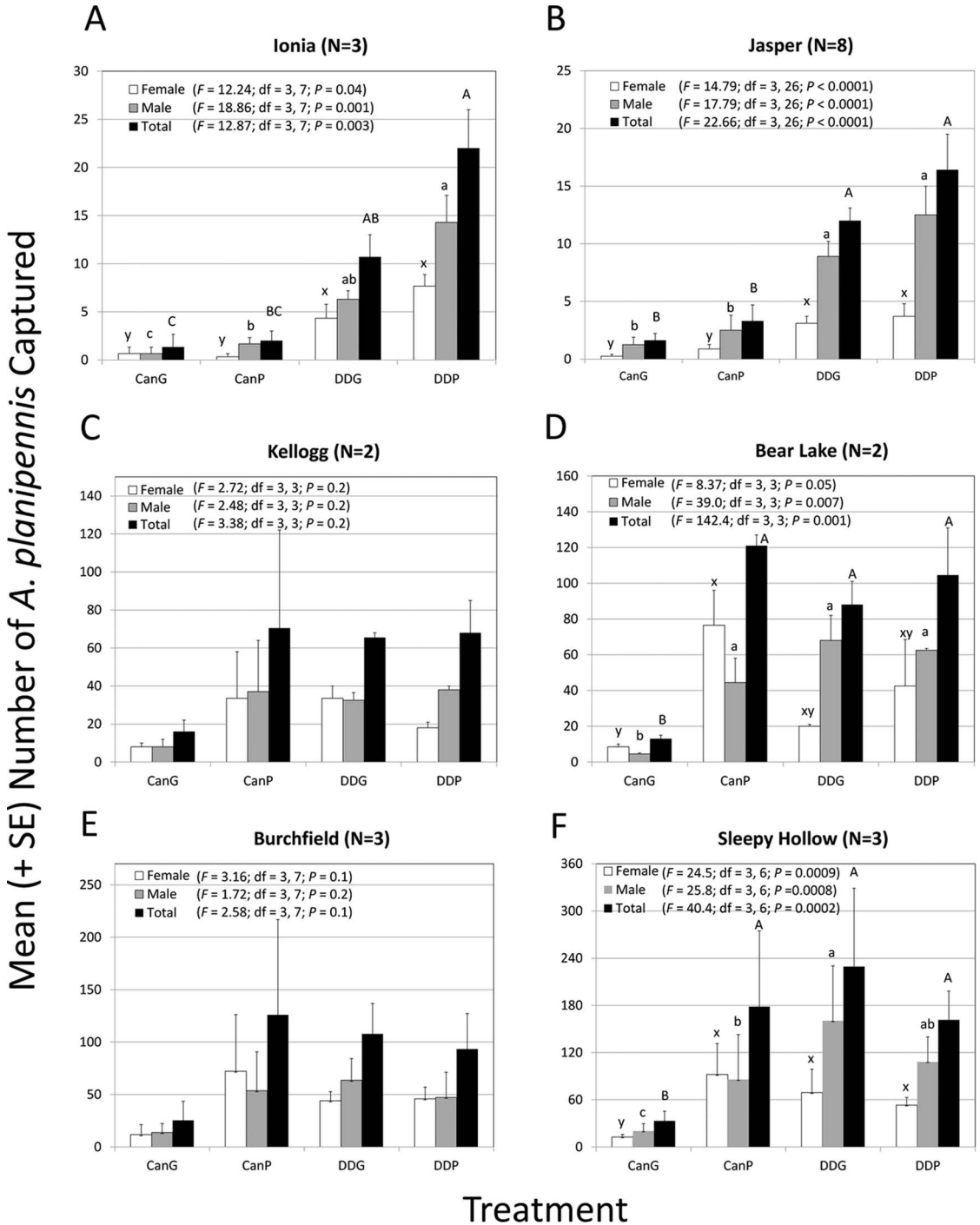
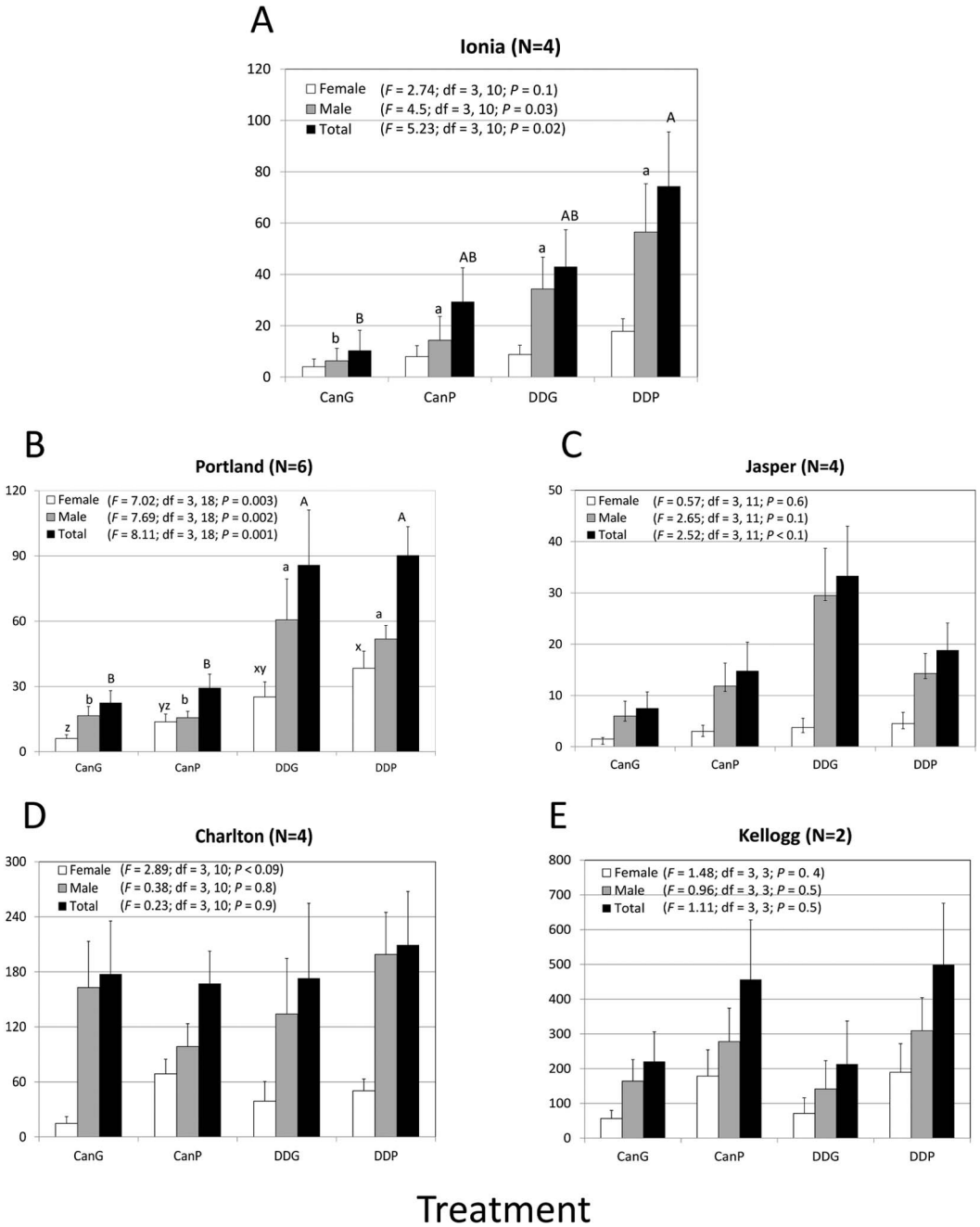


Fig. 3. Mean (\pm SE) number of *A. planipennis* females, males, and adults of both sexes captured on light green canopy (Can G), purple canopy (Can P), light green double-decker (DDG), or purple double-decker (DDP) traps at field sites in southern Michigan in 2010. Traps were baited with *cis*-3-hexenol released at 7.4 mg/d and an 80:20 blend of Manuka and Phoebe oil released at 50 mg/d. Within each field site and sex, bars topped by the same letter are not significantly different, Tukey-Kramer test on data transformed by $\ln(y + 1)$, $P > 0.05$.

in female captures on canopy traps were not affected by the condition of the trap-bearing trees (Fig. 6A). In 2011, there were no differences in the number of

females, males, or beetles of both sexes captured on canopy traps among trees with different canopy trap tree condition scores (Fig. 6B).

Mean (+ SE) Number of *A. planipennis* Captured



Treatment

Fig. 4. Mean (\pm SE) number of *A. planipennis* females, males, and adults of both sexes captured on dark green canopy (Can G), purple canopy (Can P), dark green double-decker (DDG), or purple double-decker (DDP) traps at field sites in southern Michigan in 2011. Traps were baited with *cis*-3-hexenol released at 7.4 mg/d and Manuka oil released at 50 mg/d. Within each field site and sex, bars topped by the same letter are not significantly different, Tukey-Kramer test on data transformed by $\ln(y + 1)$, $P > 0.05$.

Discussion

Our results illustrate the influence of local infestation levels on *A. planipennis* attraction to different traps, and have implications for maximizing effectiveness of artificial traps and lures used in operational

detection programs. At sites with heavy *A. planipennis* infestations, high numbers of adults were captured on all traps, indicating beetles may be less discriminatory, traps may be more likely to passively intercept beetles, and altered light conditions and abundant volatiles

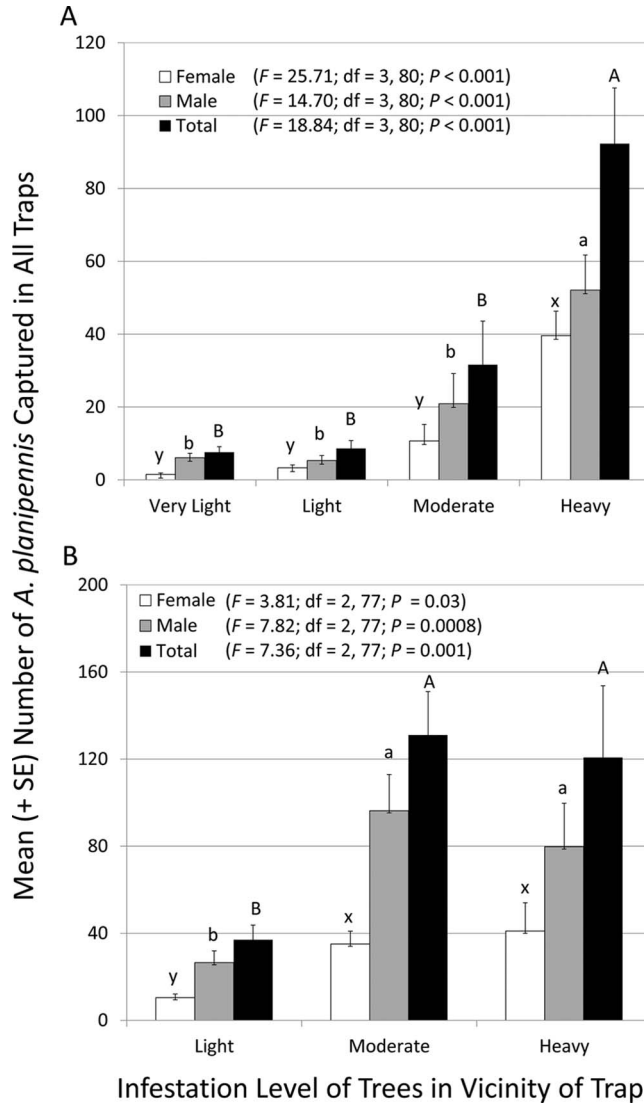


Fig. 5. Mean (\pm SE) number of *A. planipennis* females, males, and adults of both sexes captured on traps by infestation level of trees within 10 m of each trap in (A) 2010 and (B) 2011. Infestation level was ranked as follows: 1) very light, no to few symptoms; 2) light, few symptoms; 3) moderate, symptoms very evident; 4) heavy, high degree to extreme level of symptoms. All trap types (purple or green double-decker and purple or green canopy traps) and field sites were combined. Within each year and sex, bars topped by the same letter are not significantly different, Tukey HSD test on data transformed by $\ln(y + 1)$, $P > 0.05$.

from declining hosts may obscure differences in *A. planipennis* preferences for different traps and lures. In contrast, at sites with low *A. planipennis* population levels where trees appeared relatively healthy and exhibited few symptoms of infestation, more beetles were consistently captured on purple double-decker traps than on green canopy traps. When compared with purple double-decker traps, captures on green double-decker traps or purple canopy traps were either lower or intermediate. Overall, more beetles were captured on double-decker traps than on canopy traps, and more captures on purple traps than on green traps. Detection rates were also higher for purple

(100%) and green double-decker traps (100%) than for purple (82%) or green canopy traps (64%) at sites with very low to low *A. planipennis* infestation levels. These detection rates were similar to those reported for purple double-decker (95%) purple canopy (81%) and green canopy trap (67%) by Marshall et al. (2010) at sites with low *A. planipennis* population densities.

The two methods we used to categorize *A. planipennis* infestation levels at each site included a visual assessment of the condition of trees in the vicinity of each trap and an estimate of the condition of the individual tree bearing a canopy trap. We used both

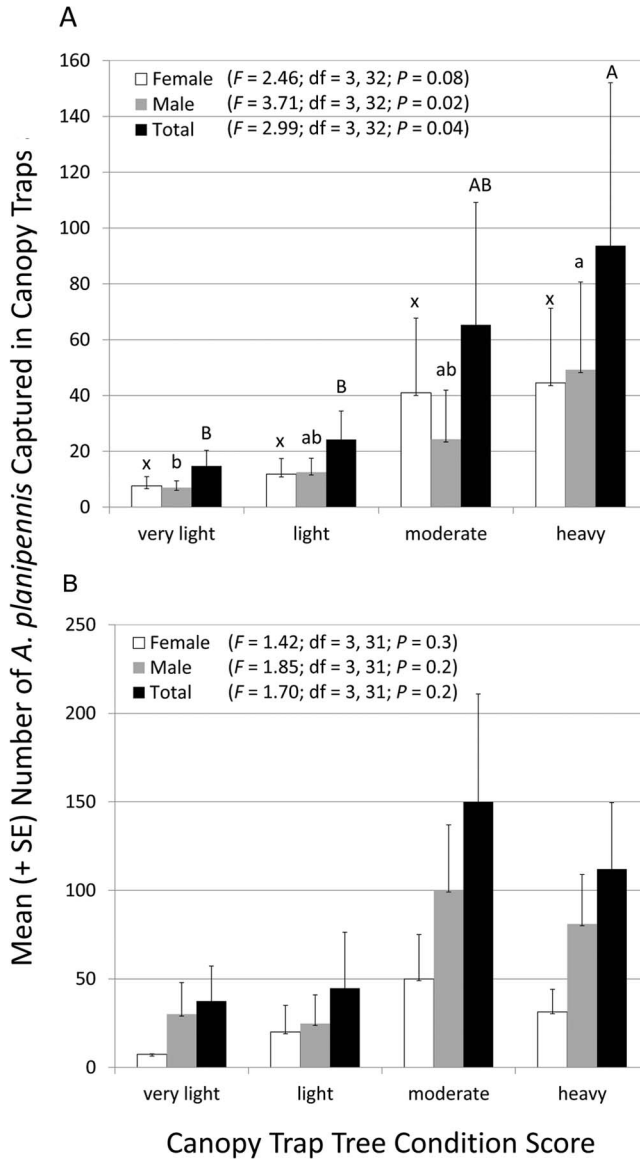


Fig. 6. Mean (\pm SE) number of *A. planipennis* females, males, and adults of both sexes captured on canopy traps by overall condition of the tree in which the canopy trap was hung in (A) 2010 and (B) 2011. Overall tree condition was ranked as follows: 1) very light, no to few symptoms; 2) light, few symptoms; 3) moderate, symptoms very evident; 4) heavy, high degree to extreme level of symptoms. Trap colors (purple or green) and field sites were combined. Within each year and sex, bars topped by the same letter are not significantly different, Tukey HSD test on data transformed by $\ln(y + 1)$, $P > 0.05$.

methods because we intentionally selected relatively healthy trees for the canopy traps to avoid passively capturing beetles as they emerged from the trees. We have previously observed beetles emerging from heavily infested trees and flying directly into adjacent canopy traps (T.M.P. and D.G.M., unpublished data). Therefore, ratings of infestation level based on canopy trap tree symptoms likely underestimated the overall infestation level of the site. The positive relationship between the number of beetles captured on canopy traps and the infestation level of the canopy trap tree may at least partially explain the variation in results

from other studies that compared different types of traps and lures hung in infested ash trees (Crook et al. 2008; de Groot et al. 2008; Grant et al. 2010, 2011; Marshall et al. 2010; Crook et al. 2012).

Federal and state agencies in the United States have substantially invested in annual detection surveys to identify *A. planipennis* infestations. Grids of girdled ash trees were used from 2005 to 2008 (Hunt 2007, Rauscher 2006); however, since 2008, most surveys have relied on networks of baited purple canopy traps (Crook et al. 2009, USDA–APHIS 2013). However, our results show that the efficacy of canopy traps was

consistently influenced by the infestation level of the tree bearing the traps. Green canopy traps were consistently the least effective trap, even in sites where infestation levels were moderate or high. Crook et al. (2008) suspended purple canopy traps at a height of 13 m along the edge of moderately to heavily infested ash stands and reported more beetles were captured on the 13-m in height traps than in similar traps suspended at a height of 1.5 m. However, even when ash trees this tall are available for surveys, attempting to hang baited sticky traps at a height of 13 m above-ground is difficult and unlikely to be practical for field crews. In operational detection surveys, canopy traps are typically hung at a height of 4–8 m on a lower limb (USDA-APHIS 2013).

When traps are placed in the canopy of ash trees, lures likely compete with volatiles emitted by the trap-bearing tree and surrounding ash trees. Foliage may obscure the trap, reducing visual attraction of beetles and leaves often become stuck on the traps, decreasing the sticky surface area available to trap beetles. Canopy traps can be difficult to hang, and it is not uncommon for traps to blow out of trees. In a recent large scale project, girdled trees and baited purple canopy traps were used to track *A. planipennis* distribution and density from 2008 to 2010 over an area of >750 km² (Mercader et al. 2013). Only 3.5% of the canopy traps that were hung in girdled ash trees that were infested with *A. planipennis* larvae captured an adult beetle. The probability of detection with girdled trees was >50%, even in areas with low *A. planipennis* densities, where an average of only five larvae per tree were present when the girdled trees were debarked. In contrast, even where average larval counts exceeded 25 larvae per tree, the probability of detection with canopy traps was <35% (Mercader et al. 2013).

Our results, along with a growing body of evidence from other studies (Marshall et al. 2010, McCullough et al. 2011, Poland et al. 2011), show that in sites with low densities of *A. planipennis*, double-decker traps are more likely to capture beetles than canopy traps. The surface area of double-decker traps is twice that of canopy traps, but more than twice as many beetles were captured on double-decker traps compared with green canopy traps in 2010 at Ionia and Jasper, and in 2011 at Ionia where infestation levels were relatively low. Lures on the free-standing double-decker traps present beetles with a distinct point source of volatiles that is not obscured by ash trees emitting competing host volatiles. Double-decker traps are placed in full sun to ensure they are visually apparent to beetles (McCullough and Poland 2009, McCullough et al. 2011, Poland et al. 2011). In addition, adult *A. planipennis* activity is greatest in full sun (Yu 1992). Field studies showed more *A. planipennis* are captured on trap trees that are exposed to sunlight than those growing in a closed canopy (McCullough et al. 2009a,b), and *A. planipennis* adults preferentially feed on ash leaves grown in sun over leaves grown in shade (Chen and Poland 2009). In a 16-ha site with a mix of open and forested areas and a very low *A. planipennis* density, McCullough et al. (2011) found evidence that

beetles actively flew to double-decker traps and were not merely intercepted. Beetles captured on double-decker traps in open areas and on the edge of stands bypassed many ash trees, and the spatially explicit data showed captures were not related to the abundance of ash phloem in the vicinity nor to the distance between traps and infested logs at the center of the site where most beetles emerged. Double-decker traps are relatively simple to set up, and in our experience, require roughly the same amount of time needed to hang a canopy trap. Double-decker traps are more expensive than individual canopy traps because they require two panels, as well as a PVC pipe and T-posts. The PVC pipe and T-posts can be reused annually, however, and in some locations, T-posts can remain in place for use in subsequent years.

Opportunity costs associated with “false negative” trap data, that is, the failure to detect an established *A. planipennis* infestation, can be substantial. Failure to detect an *A. planipennis* infestation delays implementation of quarantines designed to decrease risks that potentially infested ash trees, logs, or firewood will be transported from the area. It also reduces the time available for municipalities and private landowners to develop plans to protect, replace, or harvest ash in landscapes and forested settings. Given the consistently higher detection or captures rates reported for girdled trees (McCullough et al. 2011, Mercader et al. 2013) and double-decker traps (Marshall et al. 2010, McCullough et al. 2011, Poland et al. 2011), modifying *A. planipennis* detection survey protocols warrants consideration. Detection methods are not mutually exclusive, and incorporating girdled trees and double-decker traps, in particular those at high risk sites, along with canopy traps and visual surveys to check declining ash trees could increase the likelihood of an early detection.

Differential attraction of male and female *A. planipennis* to traps of different colors may not affect the detection efficacy, but does provide information on host-selection behavior by these beetles. In electroretinogram assays, male and female *A. planipennis* were sensitive to violet and green light (Crook et al. 2009), but only females were sensitive to red ranges of the spectrum. In field experiments with unbaited traps, more females were attracted to purple traps than males, presumably because of reflectance in the red range of the spectrum (Francese et al. 2008, Crook et al. 2009). In contrast, unbaited light green and dark green canopy traps captured more beetles and a higher proportion of males than purple traps when traps were hung at a height of 13 m in the canopy (Crook et al. 2009). Similarly, Grant et al. (2010) found that light green canopy traps baited with *cis*-3-hexenol captured more males than females. Males tend to hover around the canopies of ash trees, where they feed on foliage and opportunistically approach females feeding on foliage in the canopy (Lelito et al. 2008, Rodriguez-Saona et al. 2007). Grant et al. (2010) reported that purple canopy traps captured more males than females when baited with the leaf volatile, *cis*-3-hexenol, but equal numbers of females and males when baited with

Manuka oil, which contains sesquiterpenes present in ash bark. Females are presumably more attracted to colors in the red range of the spectrum and to sesquiterpenes present in bark because they oviposit on the trunk and branches of ash trees. Overall, more males than females were captured in our study; however, a relatively higher proportion of females were captured on purple canopy traps than on green canopy traps, and on purple double-decker traps than on green double-decker traps. Detection of female *A. planipennis* may be of particular interest, given that mature, mated females are capable of longer dispersal flights than males or younger females (Taylor et al. 2010) and can initiate new, localized infestations.

Detection and delimitation of newly established, low density *A. planipennis* infestations is likely to remain challenging. Recent evidence suggests that close range attraction of *A. planipennis* to mates may be mediated by contact or close range pheromones may eventually lead to more effective lures (Lelito et al. 2009; Pureswaran and Poland 2009; Silk et al. 2009, 2011; Ryall et al. 2012). A better understanding of *A. planipennis* dispersal and host selection behavior, in particular long range dispersal by mated females (Taylor et al. 2010, Mercader et al. 2012), would help guide operational surveys. Given the economic and ecological impacts of *A. planipennis* in North America (Gandhi and Herms 2010, Kovacs et al. 2010, Aukema et al. 2011), Moscow, and Russia (Baranchikov et al. 2008), continued research to improve detection and survey methods should remain a priority.

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