Improving Detection Tools for Emerald Ash Borer (Coleoptera: Buprestidae): Comparison of Multifunnel Traps, Prism Traps, and Lure Types at Varying Population Densities

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ABSTRACT The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest of North American ash (*Fraxinus* spp.) that has caused devastating mortality since it was first identified in North America in 2002. In 2012, we conducted field trapping assays that tested the efficacy of purple prism and fluon-coated green multifunnel (Lindgren funnel) traps. Traps were baited with combinations of several lures that were previously shown to be attractive to *A. planipennis*: manuka oil—a sesquiterpene-rich oil, (3Z)-hexenol—a green leaf volatile, or (3Z)-dodecen-12-olide [= (3Z)-lactone], a sex pheromone. Eighty-nine blocks (trap lines) were tested throughout nine states along the outer edges of the currently known *A. planipennis* infestation in North America. Trap catch was highest on fluon-coated green multifunnel traps, and trap detections at sites with low *A. planipennis* population density ranged from 72 to 76% for all trap and lure types tested. (3Z)-hexenol and (3Z)-lactone baited traps functioned as well as (3Z)-hexenol and manuka oil-baited traps. Independent of the lure used, detection rates on green fluon-coated multifunnel traps were comparable with glued purple prism traps in areas with low *A. planipennis* population densities.

KEY WORDS *Agrilus planipennis*, sex pheromone, kairomone, multifunnel trap, fluon

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive pest of North American ash (*Fraxinus* spp.) introduced from Asia (Haack et al. 2002) in the mid-1990s (Siegert et al. 2007). Since its discovery near Detroit, MI, and Windsor, Ontario, Canada, in 2002, the beetle has been detected in 21 additional states (Colorado, Connecticut, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Massachusetts, Maryland, Minnesota, Missouri, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, and Wisconsin) and one additional province (Quebec; Haack et al. 2002, Emerald Ash Borer Info 2014). To date, tens of millions of ash trees have been infested and have subsequently died (Poland and McCullough, 2006; Emerald Ash Borer Info 2014). Symptoms of infestation, such as D-shape exit holes, epicormic branching, crown dieback, and bark deformities, are not noticeable until populations have become well established (Cappaert et al. 2005; Poland and McCullough, 2006). As a result, discovering new, localized outlier populations following establishment may take several years (Francese et al. 2013b). One of the primary goals of the U.S. Department of Agriculture–Animal and Plant Health Inspection Service–Plant Protection and Quarantine (USDA–APHIS–PPQ) Emerald Ash Borer Cooperative Project has been to develop an effective and sensitive monitoring system that is able to detect low-density populations of *A. planipennis* when no visible symptoms of attack are apparent on trees. Purple prism traps suspended in the canopy of ash trees are currently used for large-scale detection surveys (Emerald Ash Borer Info 2014). These traps are currently baited with manuka oil and (3Z)-hexenol.

Early detection is critical if effective management and control measures are to be implemented (Silk et al. 2011). Chemical, visual, and behavioral studies that describe how *A. planipennis* selects mates and hosts could lead to improved lures and trap designs (Crook and Mastro 2010, Crook et al. 2012).

Several insect vision and color studies have led to the development of effective colors for trapping for *A. planipennis* (Oliver et al. 2002; Francese et al. 2005,

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2008, 2010a,b, 2011, 2013b; Crook et al. 2009, 2012; Grant et al. 2010). Trapping studies have shown that green (530–540 nm wavelengths) traps (Crook et al. 2009, Francese et al. 2010a) painted in the mid-range (22–67%) of reflectance (brightness) and purple traps painted with a color originally shown to be attractive to buprestids are highly attractive to *A. planipennis.* In an unbaited field trap study (Francese et al. 2010a), significantly more adults were caught on green prism traps by decreasing the reflectance of traps from 67 to 49% (a darker green). Electro-retinogram assays found that mated females were sensitive to red wavelengths of light (640–650 and 670 nm) while males were not (Crook et al. 2009). This sensitivity then translated into trapping assays where purple traps were found to be more attractive to females than males (Crook et al. 2009; Francese et al. 2010a, 2013b). Based on electro-retinogram and color trapping assays, green and purple pigments were incorporated into multifunnel traps as paints and plastics (Francese et al. 2011). These traps eliminate the need for an adhesive trap coating and provide a reusable, user-friendly tool for surveyors. In subsequent studies, green multifunnel traps (530 nm, 49% reflectance) were shown to be a promising tool for emerald ash borer survey.

In addition to color, green multifunnel traps were modified to increase effectiveness. A coating to increase slipperiness (i.e., reduce insect adherence) was found to be essential, with Rain-X-coated traps catching significantly greater numbers of beetles than untreated traps (Francese et al. 2011). Graham et al. (2010) demonstrated that cerambycid trap catch could be significantly increased on intercept panel traps by applying a coating of fluon, a fluoropolymer. Fluon has also been shown to be an effective trap coating for emerald ash borer (Lyons et al. 2012, Francese et al. 2013a). Green traps coated with untinted (white) fluon caught almost four times as many adult *A. planipennis* as Rain-X-coated traps, and almost 33 times more beetles than untreated control traps (Francese et al. 2013a).

Trap placement is also an important factor in capturing adult *A. planipennis.* Survey traps used by the USDA–APHIS–PPQ Emerald Ash Borer Cooperative Project are placed in the lower canopy (usually on the lowest live branch) of host ash trees (≈4–8 m above the ground). However, traps placed in the mid to upper canopy (≈13 m) have been shown to catch two to three times more beetles than those placed in the lower canopy (Francese et al. 2008, 2010b; Crook et al. 2009). Although traps placed in the mid to upper canopy of ash trees catch more beetles, this can be logistically difficult in wide-scale monitoring programs (Crook and Mastro 2010, USDA–APHIS–PPQ 2013). Recent emerald ash borer trapping studies have been conducted in the lower canopy range (and/or on smaller trees) to match the 4–8 m operational height (Marshall et al. 2010; Francese et al. 2011, 2013a, 2013b; Grant et al. 2011; Silk et al. 2011; Crook et al. 2012; Lyons et al. 2012; Ryall et al. 2013; Poland and McCullough 2014).

Two types of ash volatiles have been shown to be attractive to *A. planipennis:* bark and foliage volatiles (Crook and Mastro 2010). Six antennally active compounds (for male and female beetles) were identified in aerated bark material removed from girdled green ash trees (*Fraxinus pennsylvanica* Marshall). These compounds were identified as α-cubebene, α-copaene, 7-*epi*-sesquithujene, *E*-β-caryophyllene, α-humulene (=α-caryophyllene), and eremophilene (Cossé et al. 2008, Crook et al. 2008). Due to the difficulty and expense of synthesizing these six compounds, two natural tree oil distillates have been used in field studies: manuka oil [from New Zealand manuka tea tree, *Leptospermum scoparium* J.R. Forst & G. Forst (Myrtaceae)] and phoebe oil [from Brazilian walnut, *Phoebe porosa* Nees & Mart (Lauraceae)]. Both sexes of *A. planipennis* are attracted to these oils in field tests (Crook et al. 2008). Phoebe oil contains all six of the antennally active bark compounds compared with manuka oil which contains five (it lacks 7-*epi*-sesquithujene). This absence of 7-*epi*-sesquithujene has been suggested as a reason why phoebe oil lures attract significantly more adult *A. planipennis* than manuka oil lures in field trapping experiments (Crook et al. 2008). Unfortunately, phoebe oil is not currently commercially available.

Several ash green leaf volatiles have also been identified as potential host attractants (Rodriguez-Saona et al. 2006). Of those studied, (3Z)-hexenol has been shown to increase trap catch, especially of males (Grant et al. 2010, 2011; Poland et al. 2011; Crook et al. 2012). Grant et al. (2010) further tested (3Z)-hexenol by using a "light green" plastic prism traps that was matched to the wavelength (540 nm) and reflectance (64%) of green paint tested by Crook et al. (2009). Crook et al. (2012) tested (3Z)-hexenol on dark green "Sabic" prism traps (green plastic, 540 nm, 49% reflectance) but found male trap catch to be significantly better in only one of three field studies. They hypothesized that the previously reported kairomonal attractancy of (3Z)-hexenol (for males) on light green prism traps was not as obvious because of the improved attractancy to the darker (lower reflectance) green trap.

Bartelt et al. (2007) identified a macrocyclic lactone, (3Z)-dodecen-12-olide [(3Z)-lactone] that was hypothesized to act as a sex pheromone. Several studies have since shown that the (3Z)-lactone can significantly increase male trap catch when combined with (3Z)-hexenol on green prism traps hung high in the mid- to upper-canopy of ash trees (Silk et al. 2011; Ryall et al. 2012, 2013).

Previous studies have shown that in areas with low *A. planipennis* population density, purple traps catch more adult *A. planipennis* per trap and have higher detection rates than their green counterparts (Marshall et al. 2010, McCullough et al. 2011, Poland et al. 2011, Francese et al. 2013b). In a large scale, nine-state comparative study (n = 77), Francese et al. (2013a) tested four trap designs all baited with a manuka (50 mg/d) and (3Z)-hexenol (50 mg/d) lure. The four trap designs tested included three prism traps (standard "Program used" purple, Sabic purple, and Sabic green) and a green multifunnel trap (coated with Rain-X). Detection rates (recording at least one catch on a trap over the course of the trapping season, or not) were highest on the Sabic purple prism (86%) compared with 73, 66, and 58% for the standard purple prism, Sabic green prism, and green multifunnel traps, respectively. Catches on green traps have been shown to vary greatly from trap to trap when compared with purple traps of the same design (Francese et al. 2010a, 2011, 2013b).

The main goals of this study were to compare *A. planipennis* trap catch and detection rates on varying trap types. Fluon-coated, multifunnel traps and glue-coated, Sabic purple prism traps were baited with either manuka oil and (3Z)-hexenol or (3Z)-lactone and (3Z)-hexenol lures at a height specified by current large scale monitoring protocols (USDA–APHIS–PPQ 2013).

Materials and Methods

Traps and Lures. Two trap designs were used for field testing in 2012: Sabic purple (420 nm, 21.7% reflectance and 670 nm, 13.6% reflectance; Great Lakes IPM, Vestaburg, MI) prism traps and green multifunnel (12 U) traps (530 nm, 57% reflectance; Chemtica Internacional, San Jose, Costa Rica). Four trap and lure combinations were tested, as each of the two trap types were baited with one of two lures, either 1) manuka oil (50 mg/d) and (3Z)-hexenol (50 mg/d) or 2) a (3Z)-hexenol (50 mg/d) and (3Z)-lactone (2 μg/d).

The outer surfaces of prism traps were coated with Tanglefoot insect trapping glue (brushable formulation; Contech, Grand Rapids, MI). Beetles were removed from the glued trap surface by using soft, wide tip forceps (Bioquip Products, Rancho Dominguez, CA). All of the beetles collected from a trap on a given day were placed in a single, labeled plastic zippered bag.

Green multifunnel traps were coated with fluon (Insect-A-Slip Insect barrier; Bioquip products, Rancho Dominguez, CA) by using dish sponges. Fluon was applied to the inside and the outside of each funnel. Traps were allowed to dry in the laboratory for 24 h before being deployed in the field. Trap collection cups were filled with 150–200 ml of propylene glycol (Camco Easygoing-50, Camco, Greensboro, NC, USA), acting as a surfactant and preservative for captured beetles. During periodic checks, the contents of each trap cup were strained with a medium mesh paper paint filter (Trimaco, Mooresville, NC). Paint filters were then placed in individual, labeled Whirl-Pak sampling bags (Nasco, Fort Atkinson, WI) with ≈2 ml of ethanol added for preservation until sorting and identification could be conducted. It was not necessary to clean insects from trap catch samples to identify them.

Evaluation of Detection Tools in Outlying Infestation Sites. This study was conducted on public and private land in nine states along or near the outer edges of the current emerald ash borer infestation. Traps were hung in host trees with ropes and hoisted to the desired height in the lower canopy (5–8m). Eighty-nine blocks (trap lines) of each of the four trap and lure combinations were placed in a randomized complete block design in the following states: Illinois (n = 10), Kentucky (n = 10), Maryland (n = 10), Minnesota (n = 10), New York (n = 9), Pennsylvania (n = 10), Tennessee (n = 10), West Virginia (n = 10), and Wisconsin (n = 10). Traps were placed at least 30 m apart within blocks. Blocks were placed at least 800 m from each other. Depending on the states being surveyed, traps were placed in the field from late April to early June. Traps were first placed in southern states in anticipation of earlier flight by emerald ash borer. Traps were checked periodically (once every two weeks to monthly) from mid-May to mid-August, depending on the states surveyed.

Statistical Analyses. For analyses, 78 blocks that recorded at least one beetle capture (within a block) were used. We defined blocks that had low- and high-density beetle populations in accordance with Marshall et al. (2009). Low-density populations were defined as having ≤87 beetles caught per block; whereas high-density populations were defined as having ≥274 beetles per block. When trap catches in a block were between 88 and 273, the population density was classified as medium. Collected beetles were summed for each individual trap over the entire field season. Summed catch was log-transformed (y + 0.5) before statistical analysis to normalize the data, which was confirmed by testing residuals after ANOVA. A two-way analysis of variance (ANOVA) was performed on the total number of *A. planipennis* adults captured per trap with trap type and lure type as the main effects (JMP version 8.0.2, SAS Institute 2003). The interaction of trap and lure type was also included in the model. Differences in catch were compared using Tukey's honestly significant difference (HSD) test (α <0.05). Confidence intervals (95%) were calculated from the standard error of the transformed trap catch. Means and confidence intervals were then back-transformed for presentation of the data.

χ^2 tests were used to identify independence of *A. planipennis* detection from trap and lure types for the overall catch and catch at low *A. planipennis* population density sites. For purposes of the analysis, detection was defined as finding at least a single beetle on a trap, and it was recorded as a binary response (yes/no) over the entire field season.

Results

Of the 89 replicates, 11 had no beetle catches (5 in Maryland and 6 in Minnesota). We classified 50 of the 78 replicates as low-density populations, 16 as high density and 12 as medium density (Marshall et al. 2009).

There was a significant effect of trap type on trap catch (F = 13.6446; df = 1, 308; P = 0.0004; Table 1), with green multifunnel traps catching more beetles than purple prism traps (Table 2). Lure type had no significant effect on trap catch. There were no signif-

Table 1. Effect of trap type and lure type on total trap catch of *A. planipennis* (log transformed), tested by ANOVA

Source of variation	df	Sum of squares	*F*	*P*
Trap type	1	8.828321	13.6446	0.0004
Lure type	1	0.0001844	0.0003	0.9868
Trap type × lure type	1	0.0070579	0.0104	0.9187
Error	308	208.448		

icant interactions between trap and lure type on the trap catch (Table 1).

Detection of *A. planipennis* was independent of the trap and lure type used on all traps at all sites with detections (χ^2 = 0.977; df = 3; P = 0.807; Table 2). While detection rate was 100% on all traps within high-density sites and at or near 100% at medium-density sites, detection rates were lower, but independent of trap and lure type, at low-density sites (χ^2 = 0.2895; df = 3; P = 0.9619). Trap detections at low-density sites ranged from 72–76% for all trap and lure types tested (Table 2).

Discussion

Our tests indicate that fluon-coated, baited, green multifunnel traps provide equal beetle detection rates when compared with glued, baited, purple prism traps in low beetle density areas. Fluon-coated multifunnel traps have been shown to be effective for several years (Graham and Poland 2012) whereas Rain-X coatings may need to be reapplied season to season (Francese et al. 2011). Fluon concentration on funnel traps could be reduced by 50% and still maintain *A. planipennis* capture rates equal to undiluted fluon, thus reducing trapping costs (Francese et al. 2013a).

The two lure combinations tested in this study captured similar numbers of *A. planipennis* and provided similar rates of detection. In this study, traps were placed on the lowest branch and suspended below the crown, based on operational set-up requirements. Previous studies have shown, however, that addition of the (3Z)-lactone to a green trap baited with (3Z)-hexenol significantly synergized trap captures only when traps were placed well within the canopy of the ash tree (Silk et al. 2011, Ryall et al. 2012). In Silk et al. (2011), a significant increase in male captures occurred when (3Z)-hexenol was combined with either the (3Z)-lactone or (3*E*)-lactone in field trials conducted in Ontario. In Michigan, mean male captures on traps baited with (3Z)-lactone and (3Z)-hexenol were ≈50% greater than traps baited with (3Z)-hexenol alone; however, capture rate variation was particularly high in Michigan and therefore no statistical differences were detected. Silk et al. (2011) suggested the inconsistent results were possibly due to differences in tree size and trap placement with respect to the canopy at the Ontario and Michigan field sites. In Michigan, trees ranged from 10 to 30 m in height, with traps hanging at ≈6 m from the first available branch under the canopy. In Ontario, the green prism traps were placed in the mid-canopy of trees that were 4–6 m tall. The fact that the female-produced (3Z)-lactone lures are more effective when placed in the mid to upper canopy of trees is not surprising, as mating activity of *A. planipennis* has been shown to occur in the canopy of ash trees in bright sunshine (Lance et al. 2007, Lelito et al. 2007, Rodriguez-Saona et al. 2007). Further prism trap studies demonstrated that (3Z)-lactone alone is attractive to male *A. planipennis* in some field trials and that this male attraction decreased when release rates were too high (Ryall et al. 2012). Ryall et al. (2013) also evaluated whether the addition of (3Z)-lactone to dark green prism traps baited with (3Z)-hexenol would increase capture (and detection) rates when placed in low-density infestations. Using a branch sampling method to determine the lowest measurable population density (i.e., 0.5–2.0 larval galleries per m^2), they reported a detection rate of 88% for green prism (mid-canopy) traps baited with (3Z)-lactone and (3Z)-hexenol compared with 60% detection on traps without the (3Z)-lactone. This combination of a green trap baited with (3Z)-hexenol and the (3Z)-lactone is an effective monitoring tool for *A. planipennis*, when placed within the mid canopy of ash trees (Silk et al. 2011; Ryall et al. 2012, 2013).

For large-scale surveys (in low-density population areas) where traps are mainly hung just below the canopy of tall ash trees, our results indicate that green or purple fluon-coated traps offer equally effective detection rates, irrespective of what lure combination is used. With regards to trap design, this offers a (non-glue) option with regards to the growing number of methods for surveying *A. planipennis.* Poland and Mc-

Table 2. Mean (95% confidence interval) trap catch and trap detections of *A. planipennis* adults captured in comparative field assay conducted April–August 2012 in nine U.S. states (Illinois, Kentucky, Maryland, Minnesota, New York, Pennsylvania, Tennessee, West Virginia, and Wisconsin; *n* = 78)

Trap type lure	Mean catch (95% CI)	Total no. of traps with detections (proportion of detections)	Proportion of trap detections		
			High density (n = 16)	Medium density (n = 12)	Low density (n = 50)
Green multifunnel (3Z)-lactone + (3Z)-hexenol	10.58 (6.61–16.96)	66 (0.85)	1.00	1.00	0.74
Green multifunnel Manuka oil + (3Z)-hexenol	10.32 (6.44–16.52)	65 (0.83)	1.00	1.00	0.76
Purple prism (3Z)-lactone + (3Z)-hexenol	4.77 (1.46–6.88)	65 (0.83)	1.00	0.92	0.76
Purple prism Manuka oil + (3Z)-hexenol	4.86 (3.26–7.24)	65 (0.83)	1.00	0.83	0.72

Cullough (2014) recently showed that "double-decker" traps (3-m-tall PVC pipe with either two purple or green glued prism traps attached), baited with green leaf volatiles and manuka oil, gave good detection of *A. planipennis* in low population areas. These traps offer a good free standing trap option but still obviously involve the use of glue. Trapping methods for *A. planipennis* are clearly not mutually exclusive, and survey protocols should incorporate a mix of trap types and visual surveys to be most effective in identifying established infestations (Poland and McCullough, 2014).

As a result of this study, a new county detection was added for *A. planipennis* in Pennsylvania in May 2012 at two sites in Millersburg, Perry County.

Ongoing studies will test if the use of volatile lures is still warranted for large-scale survey programs in which traps are hung under the canopy. We also aim to determine the effectiveness of fluon-coated green and purple multifunnel traps that have been used for several trapping seasons.

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