Effects of Pheromone and Plant Volatile Release Rates and Ratios on Trapping *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in China

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ABSTRACT Native to China and Korea, the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is a polyphagous wood-boring pest for which a trapping system would greatly benefit eradication and management programs in both the introduced and native ranges. Over two field seasons, a total of 160 flight intercept panel traps were deployed in Harbin, China, which trapped a total of 65 beetles. In 2012, traps using lures with a 1:1 ratio of the male-produced pheromone components (4-(n-heptyloxy)butanal and 4-(n-heptyloxy)butan-1-ol) designed to release at a rate of 1 or 4 milligram per day per component in conjunction with the plant volatiles (-)-linalool, *trans*-caryophyllene, and (Z)-3-hexen-1-ol caught significantly more *A. glabripennis* females than other pheromone release rates, other pheromone ratios, plant volatiles only, and no lure controls. Males were caught primarily in traps baited with plant volatiles only. In 2013, 10× higher release rates of these plant volatiles were tested, and linalool oxide was evaluated as a fourth plant volatile in combination with a 1:1 ratio of the male-produced pheromone components emitted at a rate of 2 milligram per day per component. Significantly more females were trapped using the pheromone with the 10-fold higher three or four plant volatile release rates compared with the plant volatiles only, low four plant volatile + pheromone, and control. Our findings show that the male-produced pheromone in combination with plant volatiles can be used to detect *A. glabripennis*. Results also indicate that emitters should be monitored during the field season, as release rates fluctuate with environmental conditions and can be strongly influenced by formulation additives.

KEY WORDS *Anoplophora glabripennis*, male-produced pheromone, plant volatile, kairomone, monitoring trap

Introduced invasive species pose a serious threat to ecosystem diversity and stability (Simberloff 1997). More than 450 species of exotic, invasive forest insects can be found across the United States, and they cost municipal governments US$1.7 billion to control annually (Aukema et al. 2011). On average, from 1860 to 2006, 2.5 new exotic, invasive species became established in U.S. forests every year (Aukema et al. 2010). Of these, the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), a polyphagous wood-borer first discovered in North America in 1996 (Haack et al. 1997), is capable of destroying 30.3% of the urban hardwood trees in the United States. This level of destruction would result in an economic loss of US$669 billion should it establish in areas that contain suitable hosts (Nowak et al. 2001). Pheromone-baited traps offer a species-specific tool to detect economically devastating pests such as *A. glabripennis* at low population densities.

Since its initial discovery in Brooklyn, NY, this beetle has been found in the greater New York City area, New Jersey, Massachusetts, Illinois, and Ohio (Dodds and Orwig 2011, USDA-APHIS 2013a), with the most recent infestation discovered in 2013 in Babylon Township, NY (USDA-APHIS 2013b). In the United States and Europe, *A. glabripennis* attacks >48 species of apparently healthy trees especially those in the genera *Acer*, *Fraxinus*, *Populus*, *Salix*, and *Ulmus* (Hu et al. 2009). In its native range in China, it is a serious pest in *Populus* plantations, while in the United States, it primarily attacks maples, including *Acer negundo* L. (boxelder), *Acer platanoides* L. (Norway maple), *Acer saccharinum* L. (silver maple), *Acer saccharum* Marsh.

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(sugar maple), and *Acer rubrum* L. (red maple; Haack et al. 2010). Failure to control *A. glabripennis* could cause the maple syrup, timber, and fall tourism industries to suffer substantial losses (USDA-NASS 2014).

To find *A. glabripennis*-infested trees, specialized ground surveyors use binoculars to locate signs of damage including exit holes, frass, and oviposition pits. However, ground surveys only locate ≈30% of infested trees (USDA-APHIS 2013a). Tree climbers can maneuver through the canopies and detect *A. glabripennis* with a higher rate of accuracy, ranging from 60 to 75%, but this method is much more expensive and time consuming (USDA-APHIS 2013a). The only effective method to eradicate this insect is to completely remove and chip infested trees (Wang et al. 2000). From 1998–2006, the costs in the United States alone to survey, remove infested trees, and prophylactically treat host trees with imidacloprid in quarantine zones as part of an eradication effort were US$249 million (U.S. Government Accountability Office [GAO] 2006). A cost-effective detection method is therefore needed to monitor for *A. glabripennis* populations.

Male *A. glabripennis* produce a volatile two-component pheromone composed of an aldehyde, 4-(n-heptyloxy)butanal, and an alcohol, 4-(n-heptyloxy)-butan-1-ol, in a 1:1 ratio (Zhang et al. 2002). Previous laboratory and field trapping studies have shown that the male-produced pheromone can act synergistically with plant volatiles and attract significantly more *A. glabripennis* than the male-produced pheromone or plant volatiles alone (Nehme et al. 2009, 2010). During the summers of 2012 and 2013, we evaluated the efficacy of multiple ratios of the male-produced pheromone components, pheromone release rates, plant volatile combinations, and plant volatile release rates on *A. glabripennis* trap catches in China.

Materials and Methods

Field Site. Field experiments were conducted from mid-July until the end of August in 2012 and 2013 at the Northeast Forestry University in Harbin, Heilongjiang Province, China (45.723263 °N, 126.639538 °E). Traps were hung in host trees whenever possible, primarily in *Betula platyphylla* (Suk), on the university campus and nearby forest research area. In 2012 and 2013, 66.7 and 45.7% of traps were hung in host trees, respectively (Table 1). Traps not hung in host trees were always adjacent to host tree stands.

Traps. We used flight intercept panel traps equipped with twist-off collection cups (ChemTica USA, Durant, OK). Traps were coated with 10% Fluon to decrease traction for trapped insects (Graham et al. 2010). Collection cups were filled with tap water and a few drops of laundry detergent (Liby Enterprise Group Co. Ltd., Guangzhou, China) to reduce surface tension. Traps were hung in the lower canopy and attached with paracord so they could easily be raised and lowered for servicing as described in Nehme et al. (2014). Distances between traps, which were limited by the size of the area available for trapping and distances between trees, varied from 5 to 15 m. Trap height, tree species, and diameter at breast height (DBH) for each trap tree were recorded.

Table 1. Percentage of traps that were hung in host trees and percentage of trees with signs of *A. glabripennis* on the trap tree and the nearest four host trees to the trap in 2012 and 2013

Characteristic[a]	2012	2013
Traps in host trees (%)	66.7	45.7
Uninfested trees (%)	69.7	79.6
Trees with oviposition pits only (%)	22.8	3.86
Trees with 1–10 exit holes (%)	7.22	13.4
Trees with 11–100 exit holes (%)	0.28	0.00
Trees with 101+ exit holes (%)	0.00	0.00
Avg trap height (m)	3.47 ± 0.06	3.77 ± 0.13
Avg tree DBH (cm)	18.8 ± 0.38	21.7 ± 1.10

Mean ± SEM are shown for trap height and DBH in meters and centimeters, respectively. Infestation ranking system corresponds to thresholds used by USDA-APHIS Cooperative *A. glabripennis* Eradication program (Nehme et al. 2014). Number of beetle signs likely overestimated due to difficulty distinguishing old from new exit holes and oviposition sites.

[a] Characteristics and signs of beetle infestation with respect to trap placement.

Experimental Design. A number of confounding factors at our field site in China needed to be addressed within the experimental design including potential microsite differences, fluctuations in the timing of beetle emergence during the field season, and variation in the spatial distribution of populations. To balance or remove these factors, traps were organized spatially into trap sets. Each trap set contained one replicate of each lure type being evaluated. Traps were checked every 3 d, and at the time they were checked, the traps were rotated within the trap set so that each lure type occupied each location within the trap set. Additionally, to balance any potential proximity-based interactions among lure types, the placement of each lure type was such that each lure type neighbored each of the other lure types an equal number of times. To avoid potential cross-contamination among lure types in the trap, the entire panel-intercept trap was moved with lure intact.

During each trap check, collection cups were emptied and filled with fresh water and detergent. Trapped females were dissected to determine mating status as described in Nehme et al. (2010). Because of limited field site space and low *A. glabripennis* populations resulting from pesticide treatments applied by local grounds managers, sampling was focused on evaluating different objectives in the two years of field study to ensure sufficient replication.

Effects of Pheromone Component Ratios and Release Rates on Number of Trapped Beetles. In 2012, our goal was to determine the optimal ratio and release rates of the two male-produced pheromone components before evaluating different kariomone release rates and components in the subsequent field season. Aerations from male *A. glabripennis* indicate the two pheromone components are emitted in a 1:1 ratio, although it was not known if this finding was the result of a time-averaged collection or chemical conversion of the aldehyde into a carboxylic acid (Zhang et al. 2002). Each of the two pheromone components was

Table 2. Lure sets used in field experiments in China in 2012 and 2013

Lure type	Release rate (mg/d)					
	Alc	Ald	L	CA	Z3	LO
2012 treatment						
1:1 MP PV	1	1	9	8	1	0
4:4 MP PV	4	4	9	8	1	0
8:8 MP PV	8	8	9	8	1	0
1:4 MP PV	1	4	9	8	1	0
1:8 MP PV	1	8	9	8	1	0
4:1 MP PV	4	1	9	8	1	0
8:1 MP PV	8	1	9	8	1	0
PV	0	0	9	8	1	0
Control	0	0	0	0	0	0
2013 treatment						
3 PV	0	0	9	8	1	0
3 PV 1× MP	2	2	9	8	1	0
3 PV 10× MP	2	2	90	80	10	0
4 PV	0	0	9	8	1	1
4 PV 1× MP	2	2	9	8	1	1
4 PV 10× MP	2	2	90	80	10	10

MP, male-produced pheromone; PV, plant volatiles; Alc, 4-(n-heptyloxy)butan-1-ol; Ald, 4-(n-heptyloxy)butanal; L, (-)-linalool; CA, *trans*-caryophyllene; Z3, (Z)-3-hexen-1-ol; LO, linalool oxide.

placed in a separate emitter designed to release at 1 mg/d; ratios and release rates were modified by altering the numbers of emitters hung on the trap. Ratios and release rates in mg/d (alcohol:aldehyde) were 1:1, 4:4, 8:8, 1:4, 1:8, 4:1, and 8:1.

All treatments except the control included a three-component plant volatile blend composed of (-)-linalool, *trans*-caryophyllene, and (Z)-3-hexen-1-ol released at 9, 8, and 1 mg/d, respectively, from separate emitters (Tables 2 and 3). Selection of kairomones and their release rates was based on experiments conducted in previous years and reported in Nehme et al. (2014). Each trap set also contained a three-plant-volatile-only treatment and an unbaited control (Table 2). Thus in 2012, there were 8 lure treatments and an unbaited control for a total of 9 treatments in each of 10 trap sets yielding a total of 90 traps (Table 2). All emitters were manufactured by ChemTica Internacional S.A. (Heredia, Costa Rica). Traps were rotated until each lure treatment had occupied each position in the trap set once. This allowed us to consolidate trap catches by trap while removing variables such as trap location from the analyses.

Table 3. Lures used in field experiments with desired and actual release rates mean (± SEM) measured gravimetrically in mg/d

Lure type	Manufacturer	Desired (mg/d)	Actual (mg/d)
2012 lures			
ALB alcohol	ChemTica	1	0.52 ± 0.03
ALB aldehyde	ChemTica	1	1.48 ± 2.00
trans-caryophyllene	ChemTica	8	10.22 ± 4.15
(Z)-3-hexen-1-ol	ChemTica	1	1.30 ± 0.10
(-)-Linalool	ChemTica	9	5.70 ± 0.10
2013 lures			
ALB alcohol	ChemTica	1[a]	0.64 ± 0.04
ALB alcohol	Synergy	2	1.80 ± 0.24
ALB aldehyde	ChemTica	2	Not weighed
ALB aldehyde—bag	Synergy	2	0.70 ± 0.09
ALB aldehyde—no bag	Synergy	2	1.60 ± 0.11
trans-caryophyllene	ChemTica	8	14.2 ± 0.54
trans-caryophyllene	Synergy	8	11.2 ± 0.26
(Z)-3-hexen-1-ol	ChemTica	1	1.78 ± 0.25
(Z)-3-hexen-1-ol	Synergy	1	2.51 ± 0.18
(-)-Linalool	ChemTica	9	14.4 ± 0.38
(-)-Linalool	Synergy	9	14.9 ± 0.88
Linalool oxide	ChemTica	1	2.07 ± 0.13
Linalool oxide	Synergy	1	2.26 ± 0.08

No extra ChemTica aldehyde lures were available to be weighed in 2013 in China.

[a] In 2013, two ChemTica alcohol lures were used in each trap to achieve a desired release rate of 2 mg/d.

Effect of Plant Volatiles on Number of Trapped Beetles. Our experimental design in 2013 focused on the plant volatile blend and release rates, particularly with respect to potential sex differences in response to lure components. Nehme et al. (2010, 2014) reported that trap catches by the male-produced pheromone of *A. glabripennis* are enhanced in the presence of plant volatiles, and preliminary data suggested that higher release rates of these kaimones, and the addition of another plant volatile linalool oxide, might increase trap catches. Thus, in 2013, in combination with the male-produced pheromone, we compared trap catches among emitters with low (1×) and high (10×) release rates of both the three-component plant volatile blend used in 2012, and a blend that had linalool oxide added as fourth component (Table 2). To create the high plant volatile release rates, we used 10 low release rate emitters per chemical component (Table 2). To determine if the plant volatiles alone could trap *A. glabripennis*, two lure treatment groups were tested using the three-plant-volatile or four-plant-volatile emitters (without the pheromone) at low release rates. The combination of six lure treatments and an unbaited control (7 treatments) were used in each of 10 replicate trap sets (Table 2). All treatments that included the male-produced pheromone were designed to release each pheromone component at 2 mg/d. High release rate plant volatiles alone were not tested because the field site was too small to accommodate additional treatments. This experiment was conducted in the Northeast Forestry University Experimental forest (the same forest site used in 2012).

The additional goal of comparing lures formulated by two different pheromone companies (ChemTica Internacional and Synergy Semiochemicals Corp., Burnaby, B.C.) was added before the deployment of traps in the 2013 trapping season to assist a regulatory agency with selecting the most effective emitters. In the 2013 trapping season, a paired replicate design was used in which a trap set containing all lure treatments made by ChemTica as followed in a line with a trap set of all lure treatments made by Synergy. This pattern was replicated five times for each manufacturer. Traps were rotated within sets, and each trap set from one manufacturer was moved down to the spot where the other manufacturer's trap set was previously. There were 15 collection periods; thus, traps and trap sets were rotated through the cycle of 10 positions 1.5 times.

The male-produced pheromone alone was not used as a treatment in either 2012 or 2013 because space was

limited and previous field studies had shown that the male-produced pheromone alone trapped very few beetles compared with treatments using the male-produced pheromone in combination with plant volatiles (Nehme et al. 2010).

Lure Release Rate Measurements. To estimate field lure release rates, three of each lure type were placed in a parallel field trial in a flight intercept panel trap and weighed every 3 d on a precision balance. In 2012, weather data for Harbin was obtained from the National Climatic Data Center (www.ncdc.noaa.gov). In 2013, weather data were collected in 5-min intervals with a HOBO H8 Pro Series Data Logger (Onset Computer Corporation, Bourne, MA).

GC Quantification of Lure Release Rates. *Chemical Analysis.* Because new formulations of the pheromone components were used in 2013 in an attempt to stabilize their release rates, laboratory lure release rates of the two male-produced pheromone components (4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal) were analyzed by volatile collection followed by gas chromatography. Pheromone lures purchased from Synergy and ChemTica were hermetically sealed by the manufacturer, and stored at −20°C before testing.

Lure release rates were measured in a laboratory fume hood at 23°C for a 10-wk period. Lures were hung freely in the middle of the hood and the sash was kept closed. Individual lures were removed weekly and placed inside 471-ml Mason jars fitted with screw-on metal canning lids with filter ports as the air entrainment jars for collection. Volatile chemicals were collected from lures using air entrainment filters packed with 30 mg HayeSep Q Adsorbent. Air was sampled from within the jars at a rate of 0.5 liters/min for 10–20 min, depending on the release rates of individual lures, on a weekly basis. Filters were eluted with 120 μl of a 1:1 mixture of n-hexanes (95% purity) and dichloromethane followed by the addition of octane and nonyl acetate as internal standards. The amount of internal standard introduced into the vial for quantification varied depending on the release rates of individual lures on a weekly basis to accommodate the initial burst and decreasing release rates over time from the emitters.

All sample analytes were quantified using a Hewlett–Packard Agilent 6890 GC equipped with a flame ionization detector (FID) and fitted with an HP-5MS bonded phase capillary column (0.25 mm × 0.25 μm × 30 m, Agilent Technologies, Santa Clara, CA). The injector was operated in splitless mode with a split delay of 0.75 min and helium carrier gas flow maintained at 1 ml/min. The oven was kept at an initial temperature of 40°C for 1 min then increased to 240°C at a rate of 10°C/min. Both the inlet and FID detector were held at 250°C. Identification of analytes was accomplished by coinjection with authentic standards as well as by GC-MS analysis using an Agilent 6890 chromatograph coupled to an Agilent 5975 C mass selective analyzer. Chromatograph parameters and columns were equivalent to those used in FID analysis with the addition of an interface temperature of 280°C. The mass selective analyzer was operated in electron ionization mode (70eV) with source and quadrupole temperatures of 230°C and 150°C, respectively. Matching of representative spectra to reference spectra in the NIST 08 library and spectra of synthetic standards was used to confirm analyte identities.

Quantification of Pheromone Components from Emitters. Quantification was based on GC-FID peak areas of analytes relative to that of the internal standard nonyl acetate. Analyte peak areas were adjusted using response factors calculated from the slopes of their respective calibration curves. Release rates were calculated as the total mass of analyte collected divided by the duration of the volatile organic compound collection. Mean release rates and standard errors were calculated for each set of two lures of each compound on each sampled date.

Estimation of Beetle Populations. To estimate beetle populations in the field sites, exit holes and oviposition pits were quantified at the end of the trapping season by examining the trap trees and the nearest four host trees to each trap. The main trunk, main branches, and lower crown were visually inspected for signs of beetle damage by walking around the tree for 1 min. In the forest, larger trees were inspected with binoculars. Because distinguishing old from new oviposition sites or exit holes was difficult, all signs were counted and summed; thus, this approach likely overestimated the current year's beetle populations. Also, trees had been sprayed for the past two seasons with deltamethrin and several beetles were observed on the ground twitching, which may explain why there were fewer exit holes observed in 2013 than in 2012. Infestation levels were approximated using the log scale rating system established by the Cooperative *A. glabripennis* Eradication Program as: 0 = no beetle damage; A = oviposition pits only; B = 1–10 exit holes; C = 11–100 exit holes; and D = 101+ exit holes (Nehme et al. 2014).

Statistics. Statistics were performed using R version 3.0.2 (R Core Team, 2012) and JMP 10 (SAS Institute Inc., 2012). The sciplot package in R was used to calculate standard errors. As is often the case with trapping studies, the data were composed of primarily zeros and ones. Thus, the data were fitted to a general linear model (GLM with link: log), using a Poisson distribution and the Firth-bias estimation method. This method uses a χ^2 analysis to determine which lure treatments were significant predictors of the number of beetles trapped. Trap height, tree DBH, and treatment were also tested as predictors of trap catches. Predictors that did not have a statistically significant effect were removed from the final model. Spearman tests were used to test for correlations.

Results

Effects of Pheromone Component Ratios and Release Rates on Number of Trapped Beetles. In total, 42 beetles were trapped between 23 July and 19 August 2012. Lure treatment had a significant effect on the number of female beetles caught ($\chi^2 = 19.6$, df = 8, $P = 0.012$; Fig. 1), of which 87% were virgins. The

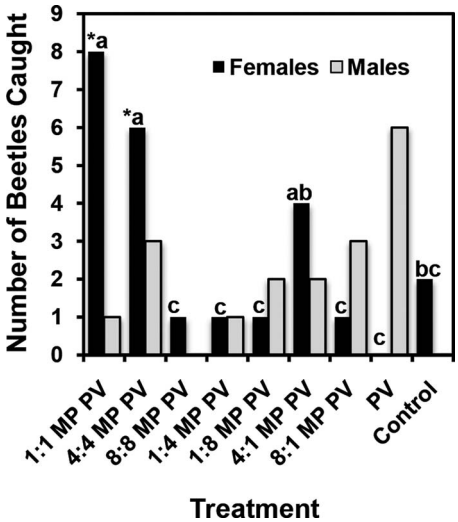


Fig. 1. Cumulative number of beetles trapped by each lure treatment by sex in 2012. Ten of each lure treatment ($N = 10$) were hung in 10 trap sets for 27 d. An asterisk over the bar indicates treatments that were significant linear predictors of the number of female beetles trapped from the GLM analysis. MP, male-produced pheromone; PV, three plant volatiles: (-)- linalool, *trans*-caryophyllene, and (Z)-3-hexen-1-ol.

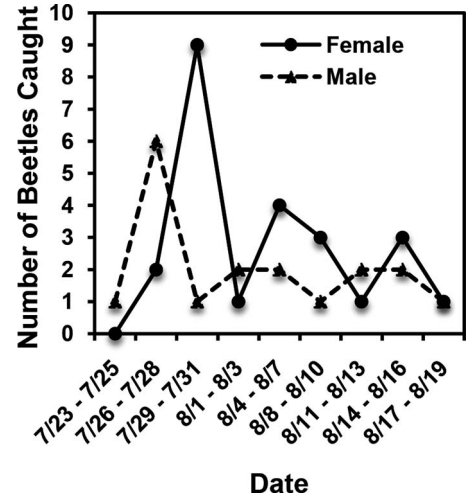


Fig. 2. Number of beetles caught for all lure treatments combined by sex during each trap check in 2012. Dates correspond to the duration between trap checks. $N = 10$ replicates per treatment.

1:1 and 4:4 male-produced pheromone + plant volatile treatments were significant linear predictors of the number of female beetles caught (Fig. 1). Two female beetles were caught in control traps.

Treatment did not have a significant effect on the number of male beetles caught ($\chi^2 = 12.5$, df = 8, $P = 0.130$; Fig. 1) nor of the total number of beetles caught, i.e., males plus females ($\chi^2 = 15.3$, df = 8, $P = 0.054$).

Peak male catches occurred during the second trap check from 26 July to 28 July (Fig. 2), while peak female catches occurred during the third trap check from 29 July to 31 July. After the end of July, the number of beetles caught in traps during each sampling period varied but generally decreased until the end of the experiment.

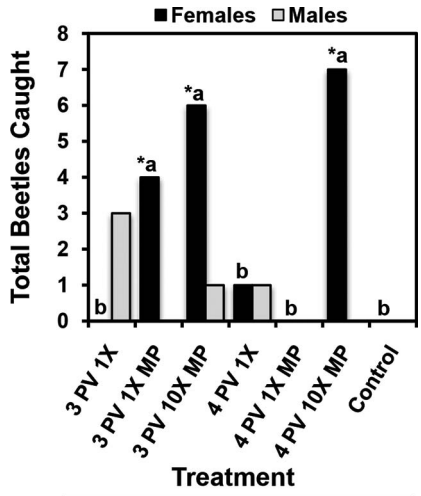
Effect of Plant Volatiles on Number of Trapped Beetles. In total, 23 beetles were trapped between 21 July and 25 August 2013. Treatment had a significant effect on the number of female beetles caught ($\chi^2 = 22.5$, df = 6, $P = 0.001$; Fig. 3); 83% of these females were virgin. The high release rate of three plant volatiles + male-produced pheromone ($\chi^2 = 8.9$, $P = 0.003$), high release rate of four plant volatiles + male-produced pheromone ($\chi^2 = 11.7$, $P = 0.001$), and low release rate of three plant volatiles + male-produced pheromone treatments ($\chi^2 = 3.96$, $P = 0.047$) were significant linear predictors of the number of females trapped (Fig. 3). There was no clear pattern in the timing of when beetles were trapped in 2013 for either sex, but trap catches generally declined during the last three sampling dates (Fig. 4).

Effect of Trap Height and DBH on Beetle Catches. In the 2012 analysis, traps were hung at a mean height of 3.47 ± 0.06 m (Table 1); neither height nor DBH were significant predictors of the number of beetles caught. In the 2013 analysis, traps were hung at a mean height of 3.77 ± 0.13 m (Table 1); trap height was a significant positive linear predictor of the number of female beetles caught ($\chi^2 = 16.9$, df = 1, $P < 0.001$) and total beetles trapped ($\chi^2 = 16.6$, df = 1, $P < 0.001$), though it was not a significant linear predictor of male trap catches ($\chi^2 = 0.063$, df = 1, $P = 0.427$). The mean height (±SEM) of traps that caught beetles was 4.71 ± 0.28 m, whereas traps that did not catch beetles were hung from a mean height of 3.75 ± 0.04 m irrespective of treatment.

Effect of Different Lure Manufacturers on Beetles Caught. In 2013 when lures from different companies were separated out, a significant treatment effect was observed for female and total beetles trapped in trap sets with ChemTica lures ($\chi^2 = 18.5$, df = 6, $P = 0.005$; $\chi^2 = 20.6$, df = 6, $P = 0.002$, respectively; Fig. 4) but not Synergy lures ($\chi^2 = 2.51$, df = 6, $P = 0.867$; $\chi^2 = 4.97$, df = 6, $P = 0.547$, respectively).

Empirical Measurements of Lure Release Rates in the Field. Average lure release rates for the 2012 and 2013 trapping season as measured gravimetrically in the field differed from the desired release rates in most cases (Table 3). Lure release rates generally peaked during the first week of the experiment (the initial burst period) and then gradually decreased for the remainder of the season (Supp Figs. 1–3 [online only]). Gas chromatography measurements indicated that the true pheromone release rates were far lower than the desired release rates (Table 4). Fluctuating temperatures paralleled fluctuating lure release rates (Supp Fig. 4 [online only]).

Estimation of Beetle Population in Field Sites. Fewer trees with oviposition pits were observed in 2013 compared with 2012, while a higher percentage of trees had exit holes. *A. glabripennis* is known to have a 2-yr life cycle in its northern range, such as in Harbin,



ChemTica	♀	0	3	*5	0	0	*5	0
	♂	0	0	1	1	0	0	0
Synergy	♀	0	1	1	1	0	2	0
	♂	*3	0	0	0	0	0	0

Fig. 3. Cumulative number of beetles trapped by each lure treatment and lure manufacturer by sex in 2013. Ten of each lure treatment (5 from ChemTica and 5 from Synergy) were hung in 10 trap sets for 35 d ($N = 10$ per lure type with $N = 5$ lure sets per manufacturer). Asterisks indicate treatments that were a significant linear predictor of beetles trapped from the GLM analysis. Table indicates how many beetles of each sex were caught using lures produced by each manufacturer. 3 PV—three plant volatile mix: (-)-linalool, *trans*-caryophyllene, and (Z)-3-hexen-1-ol; 4 PV—four plant volatile mix: (-)-linalool, *trans*-caryophyllene, (Z)-3-hexen-1-ol, and linalool oxide. MP, male produced pheromone; 1×, low plant volatile release rates; 10×, high plant volatile release rates. Synergy—all emitters were manufactured by Synergy; ChemTica—all emitters were manufactured by ChemTica.

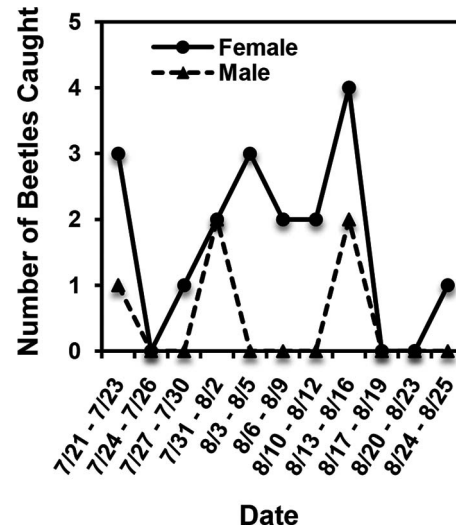


Fig. 4. Number of beetles caught for all lure treatments combined during each trap check in 2013. Dates correspond to the duration between trap checks. $N = 10$ replicates per lure type.

China (Hu et al. 2009). Exact numbers of adults present each year could not be determined because the beetle signs were difficult to date visually, and some individuals were also killed by pesticide applications.

Discussion

Emitters designed to release at a 1:1 ratio of the two male-produced pheromone components at rates of 1 or 4 mg/d, but <8 mg/d, in conjunction with a higher release rate of plant volatiles, trapped the most beetles, and this effect was most pronounced for females. Higher trap catches of females were expected for lures that contained a pheromone component because this is a male-produced sex pheromone (Nehme et al. 2010). Adding the plant volatile linalool oxide to the three-component blend ((-)-linalool, *trans*-caryophyllene, and (Z)-3-hexen-1-ol) did not significantly increase trap catches, indicating the addition of this compound may not add to the trap's efficacy. Given the relative high cost of linalool oxide, leaving this component out would reduce the cost of the lure sets. Males, but not females, were slightly more attracted to traps baited with plant volatiles without the pheromone in both years, suggesting the potential for male-produced sex-pheromones to act as a deterrent to males.

Male-produced sex and aggregation pheromones have been discovered in several cerambycid species, such as *Hedypathes betulinus* Klug, *Neoclytus acuminatus* F., *Tetropium fuscum* F., and *Monochamus galloprovincialis* Megerle (Aldrich et al. 1984, Pajares et al. 2004, Hanks et al. 2007, Lacey et al. 2007, Fonseca and Zarbin 2009, Silk et al. 2010). In contrast to most moth sex pheromones, which are mostly female-emitted, the described volatile sex pheromones in the

Table 4. Mean ± SEM (mg/d) pheromone lure release rates at 23°C produced by ChemTica and Synergy as determined by volatile collection and analysis by gas chromatography using pure chemical standards

Day	Alcohol, 4-(n-heptyloxy)butan-1-ol		
	ChemTica 2012 (mg/d)		Synergy 2013 (mg/d)
7	0.66 ± 0.02		1.69 ± 0.15
14	0.09 ± 0.01		0.13 ± 0.01
21	0.08 ± 0.01		0.13 ± 0.01
28	0.06 ± 0.00		0.08 ± 0.00
35	0.05 ± 0.01		0.10 ± 0.00
	Aldehyde, 4-(n-heptyloxy)butanal		
	ChemTica 2012 (mg/d)	ChemTica 2013 (mg/d)	Synergy 2013 (mg/d)
7	3.42 ± 0.50	2.35 ± 2.07	3.85 ± 0.94
14	0.21 ± 0.04	1.45 ± 0.86	0.20 ± 0.04
21	0.08 ± 0.02	0.49 ± 0.11	0.10 ± 0.00
28	0.04 ± 0.00	0.29 ± 0.06	0.10 ± 0.02
35	0.03 ± 0.00	0.26 ± 0.02	0.12 ± 0.02

Lamiinae subfamily are all male-produced. However, in this group of beetles, the pheromones alone are often not sufficient for optimal trapping, and these data suggest *A. glabripennis* shares this characteristic. Improved attraction to pheromones by combining them with plant volatiles has been observed in several insect taxa (Nakamuta et al. 1997, Reddy and Guerrero 2004, Sweeney et al. 2010). Our results show that use of the male-produced pheromone in combination with the plant volatiles significantly improved the capture of female *A. glabripennis*, while plant volatiles alone were attractive only to male beetles.

Pheromone component ratios are often critical for attracting conspecifics; for example, male tortrix moths, *Adoxophyes orana* (Lepidoptera: Tortricidae, Fischer von Roslerstamm, 1834), and elm bark beetles, *Scolytus mutistraitus* (Coleoptera: Curculionidae, Marsham, 1802), have been shown to be sensitive to variations in pheromone component ratios (Minks and Voerman 1973, Cuthbert and Peacock 1978). In the pheromone ratio experiment reported herein, *A. glabripennis* were caught in the treatments with desired pheromone component ratios that deviated from 1:1 ratio (Fig. 1); however, the nominal release rate for the 4:1 treatment that caught the most beetles was actually close to 1:1 for at least part of the time, indicating *A. glabriopennis* is likely sensitive to variation in the ratios of the pheromone components.

In trapping programs, knowledge of the range of effective pheromone release rates is important for cost considerations. Development of an optimal lure emitter depends on finding an ideal emission rate. Rates that are too high may carry an increased cost with additional chemical use, and there is a potential for high concentrations to repel insects. Conversely, rates that are too low may result in ineffective draw ranges. Results from the 2012 study suggest that a pheromone release rate between 1 and 4 mg/d was more effective for trapping *A. glabripennis* than higher rates. However, GC volatile collections indicate that the actual pheromone release rate was far lower than the design specifications after the initial burst period (Table 4). A previous field trapping study for this insect in China used 0.010 mg of male-produced pheromone applied to rubber septa, but at these low septum loadings, trap catches were low (Nehme et al. 2010), suggesting the optimal release rate is perhaps slightly <1 mg/d.

In 2013, traps baited with 10× higher release rates of three or four plant volatiles in combination with the pheromone caught significantly more beetles than the low release rate of four plant volatiles in combination with the pheromone, plant volatiles alone, and the control. The higher plant volatile release rates may allow traps to successfully compete against background plant volatiles. Interestingly, the low release rate of four plant volatiles with male-produced pheromone (but not the low release rate of three plant volatiles with male pheromone) caught fewer beetles than the high release rate of four plant volatiles + pheromone. This suggests the addition of the fourth plant volatile, linalool oxide, at low release rates may be ineffective or even counter-productive for capturing *A. glabripennis.*

Although our study showed that moderately high release rates of plant volatiles synergized responses to the pheromone for females, it is also possible that extremely high release rates may be inhibitory. For *Ips pini* Say, increasing the release rates of α-pinene increased trap catches up to a point, but the response was not linear and trap catches declined when α-pinene release rates exceeded 110.5 mg/d (Erbilgin et al. 2003). Similarly, for the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), optimal beetle trap catches were seen in traps baited with its pheromone ferrugineol and the plant volatile ethyl acetate released at 57–350 mg/d (Vacas et al. 2013). However, when the same amount of pheromone was released with 2,200 mg/d of ethyl acetate, female trap catches decreased.

The low release rate, three-plant-volatile treatment captured males in both years, which is consistent with a previous field study in China showing that males are attracted to plant volatiles alone (Nehme et al. 2010). In both study years, treatments with three or four plant volatiles alone caught only males. Limited sample size may explain the lack of significance, although in our 2013 experiment, the three plant volatiles without pheromone treatment was a significant linear predictor of male captures in trap sets using Synergy lures.

Trap height has been shown to have a significant effect on the diversity and types of insects sampled (Graham et al. 2012, Rodriguez-Saona et al. 2012). In our study, trap height was a significant linear predictor of the number of *A. glabripennis* caught in 2013, but not in 2012. Variation in the apparent role of trap height between the years may be related to differences in the trapping locations. In 2013, traps were located only in large forest trees, while in 2012 about half the traps were in trees distributed primarily within an urban site (campus arboretum) where trees were smaller and the other half were hung in the large forest trees at the same location as the 2013 traps. The lower range of trap heights in 2012 may have reduced the sensitivity of our ability to detect height effects. Yet, the 2013 results suggest that hanging traps higher in the canopy may increase the number of *A. glabripennis* caught, perhaps by reducing the distance adults have to travel to a trap. Beetle damage is seldom found on the lower trunk unless a tree is heavily infested (Haack et al. 2006).

During the 2012 field experiment in which different ratios and release rates of the male-produced pheromone were tested, there was a clear peak in the number of beetles caught at the end of July. Male catches peaked 3 d before female catches. Male insects typically develop faster than females, although there is considerable variation among insect orders (Fairbairn et al. 2007). This may be partially true for *A. glabripennis* males, which tend to be smaller than females. The sooner males develop, emerge, and are able to fly, the more likely they are to be the first individual to mate with a female (Wiklund and Fagerstrom 1977). This phenomenon highlights the importance

of hanging traps early in the season before beetles are active to maximize catches. In both experiments, traps captured primarily virgin females, which is consistent with results from previous field studies (Nehme et al. 2010, 2014). Fewer virgin females may be present at the end of the season, which could explain the decrease in beetles caught. In areas with denser *A. glabripennis* infestations, females may have more mating opportunities and there may be more competition from natural sources of male-produced pheromones, so fewer females may be detected if traps are not deployed before the initial emergence peak.

Pheromone release rates determined by volatile collection and GC analysis differed considerably from gravimetric measurements (Table 4). About 80–90% of the measured lure weight loss could not be attributed to loss of pheromone components. The summer of 2013 was the first time the lure manufacturers used a different formulation to try to improve the release rates of the pheromones and to stabilize the aldehyde pheromone component. Many aldehydes oxidize readily and require the addition of antioxidants or other adjuvants to prevent formation of carboxylic acids and prevent trimerization (Ishihara et al. 1986). Although just before the field season the lure formulations were evaluated by the manufacturers and by our group using gravimetric methods at a constant 25°C to obtain specified release rates, that approach was only an approximation. Temperatures in the field vary on an hourly basis, making it difficult to predict the overall temperature-dependent release rates before field deployment. Lures often release components very rapidly during the first week or so after deployment in the field while the vapor pressures of components equilibrate with the external environment. This phenomenon, known as "blow-off," was observed and expected in our experiments.

Although not as accurate, gravimetric monitoring of lure release rates is an easy, convenient method to determine whether or not lures are depleted. This method allows researchers to replace lures as necessary throughout the season, but may be inaccurate when there is heavy precipitation (some emitters will absorb water) or when lures contain adjuvants that volatilize at a different rate from the pure pheromone or volatile component.

This study demonstrated that traps baited with 1:1 ratios of *A. glabripennis* pheromone components released at <8 mg/d together with high release rates of the three or four plant volatiles tested trapped significantly more beetles than any other lure type tested, and can be used to detect *A. glabripennis*, even when beetle populations are fairly low. This assertion is supported by a recent report of a four-year trapping study conducted in the 110 m^2 regulated area in Worcester, MA, in which *A. glabripennis* were caught only in traps with lures (Nehme et al. 2014); no beetles were ever trapped in unbaited control traps, which were spaced ≈100 m apart. There was a reduction in the number of beetles collected by traps through time, which was consistent with the reduction in the overall number of beetles found in the regulated area over the course of the study due to an aggressive eradication program using survey and removal of infested trees (Nehme et al. 2014). Importantly, although the total number of beetles found each year in Worcester decreased, the proportion of beetles found using traps increased in concert with lure improvements based on the findings in this study in China. Moreover, in several cases trap catches of *A. glabripennis* led to more rapid discovery and management of previously unknown areas of infestation in the Worcester county regulated area (Nehme et al. 2014).

These findings demonstrate the potential for traps baited with both male-produced pheromones and plant volatiles to assist in the rapid detection and delineation of *A. glabripennis* infestations. This in turn may place eradication programs in a better position to rapidly eliminate established populations in invaded regions.

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