Evaluating the Use of Male-Produced Pheromone Components and Plant Volatiles in Two Trap Designs to Monitor *Anoplophora glabripennis*

M. E. NEHME,[1] M. A. KEENA,[2] A. ZHANG,[3] T. C. BAKER,[1] Z. XU,[4] AND K. HOOVER[1,5]

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ABSTRACT *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), commonly known as the Asian longhorned beetle, is a wood-boring invasive species introduced from Asia to North America and Europe in solid wood packing material. Efficient monitoring traps are needed to assess population density and dispersal in the field and to detect new introductions at ports of entry. For this purpose, we conducted field trapping experiments in China in the summers of 2007 and 2008. In 2007, we tested Intercept panel traps hung on poplar trees. In 2008, we used Intercept panel traps hung on poplar trees, screen sleeve traps wrapped around poplar trunks, and Intercept panel traps hung on bamboo poles 20 m away from host trees. Traps were baited with *A. glabripennis* male-produced pheromone alone or in different combinations with plant volatiles. Traps baited with the male-produced pheromone alone caught significantly more females than control traps in both years. The addition of a mixture of (−)-linalool, (Z)-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene, and *trans*-pinocarveol to the pheromone significantly increased trap catches of females, 85% of which were virgin. Screen sleeve traps baited with a combination of (−)-linalool and the pheromone caught the highest number of beetles overall in 2008, whereas traps placed on bamboo polls caught the lowest number. Although the logistics for the most effective implementation of a trapping program using a mixture of the pheromone and plant volatiles require additional studies, these results indicate that this pheromone has considerable promise as a monitoring tool for *A. glabripennis* in the field.

KEY WORDS *Anoplophora glabripennis*, male-produced pheromone, plant volatiles, monitoring traps

Recent globalization trends and international trade movements have greatly strengthened the links between continents and countries, which have tremendously accelerated introduction of non-native pests (Liebhold et al. 1995). The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae: Lamiinae), is a wood-boring invasive species introduced from Asia to North America and Europe in solid wood packing material. In the United States, *A. glabripennis* was first discovered in New York City, NY, in 1996. More field infestations have since been found in New York, New Jersey, and Illinois. Most recently, a new infestation was reported in Worcester County, MA (USDA–APHIS 2008). As of 18 April 2009, in the Worcester area, MA, >20,000 trees have been removed, including >8,000 infested trees and >12,000 host trees found near the infested trees. More than 635,000 trees are awaiting survey (MA Introduced Pests Outreach Project 2009).

The host range of *A. glabripennis* includes >24 deciduous tree species. Tree infestations result in canopy dieback. Trees weakened by insect tunneling pose a serious threat to pedestrians and vehicles from falling limbs and trees. Insect tunneling thus can cause serious economic damage to the lumber, nursery, and tourism industries (USDA–APHIS 2005b). Preference of *A. glabripennis* for maple species (Haack et al. 1996, Li et al. 1999) can have a serious impact on maple forests managed for lumber and maple-dependent industries, such as maple syrup production. The estimated potential urban impact of *A. glabripennis* in the United States is a loss of nearly 35% of total canopy cover, 30.3% tree mortality, and a compensatory value loss of $669 billion (Nowak et al. 2001).

The USDA–APHIS is currently attempting to eradicate *A. glabripennis*, as well as to detect potential outlier populations. Monitoring of *A. glabripennis* populations is restricted to scouting by climbing individual trees and searching for oviposition scars, sap flow, larval frass, and dime-sized adult emergence holes (USDA–APHIS 2005a). This approach is highly labor intensive and time consuming. Efficient monitoring

[1] Department of Entomology, The Pennsylvania State University, University Park, PA 16802.
[2] U.S. Forest Service Northern Research Station, Hamden, CT 06514.
[3] USDA–ARS, Invasive Insect Biocontrol and Behavior Laboratory, BARC-West, Beltsville, MD 20705.
[4] Beijing Forestry University, Beijing 100083, China.
[5] Corresponding author, e-mail: kxh25@psu.edu.

traps are needed to assess population density and dispersal in the field and to detect new introductions at ports of entry before establishment.

Anoplophora glabripennis males produce a pheromone that consists of a blend of two dialkylethers [4-(*n*-heptyloxy)butan-1-ol and 4-(*n*-heptyloxy)butanal] (Zhang et al. 2002), which was shown to attract females, especially virgins, in laboratory assays. Attractiveness of the male-produced pheromone to *A. glabripennis* adults in a Y-olfactometer was further enhanced when coupled with plant volatiles such as (−)-linalool and (Z)-3-hexen-1-ol (Nehme et al. 2009). Greenhouse trapping studies also provided additional evidence for the attractiveness of this pheromone and its potential use for monitoring. In the greenhouse, both Intercept panel traps and screen sleeve traps were found to effectively trap *A. glabripennis* adults. However, efficacy of each trap design depended largely on the lure used (Nehme et al. 2009).

Using the male-produced pheromone described in the preceding paragraph, we conducted field trapping studies in China for 2 consecutive years 2007 and 2008, to determine the most effective combination of lure and trap design for catching *A. glabripennis* adults in the field. We also studied the effect of adding different combinations of plant volatiles to the male-produce pheromone on trap catches of males and females.

Materials and Methods

Comparison of Lure Treatments for Intercept Panel Traps in 2007. In July 2007, trapping studies were conducted for 3 wk in Qing Tong Xia, Ningxia Province, China (hereafter referred to as site A; 38°06′82.40″ N; 105°91′60.60″ E), to determine the attractiveness of the male-produced pheromone to *A. glabripennis* adults in the field and its potential for use in monitoring. The site consisted of a vineyard with poplars planted as wind breaks between the 20-m-wide grapevine plots. The poplar trees included in the study were ≈10 and 20 cm in diameter (measured ≈1 m above ground). Fourty-nine black Intercept Panel Traps (or Intercept PT; cross-vein panels; height: 122 cm; cross panels, 81 by 30 by 30 cm; APTIV, Portland, OR) were hung on host trees so that the bottoms were 1.5–2 m aboveground. Seven traps were placed, 7–10 m apart, in each of seven rows of poplar trees. The distance between traps was necessitated by the total number of traps needed for replication of all treatments and the size of the field. Treatments consisted of traps baited with five live males in cages; five live females in cages; 10 μg of 4-(*n*-heptyloxy)butan-1-ol; 10 μg of 4-(*n*-heptyloxy)butanal; and 10 μg of the pheromone blend (equal ratio of the two components). An additional preliminary treatment was added to test the potential effect of combining plant volatiles with the male-produced pheromone on trap catches. This treatment consisted of 10 μg of the pheromone blend with 100 μg of each of (−)-linalool (Fluka; purity 98.5%) and *trans*-pinocarveol (Fluka; purity 96%); these plant volatiles were chosen based on preliminary studies in the laboratory (data not shown). The amount of chemicals used as lures in the field was based on previous studies conducted in the laboratory and in the greenhouse (Nehme et al. 2009). The alcohol [4-(*n*-heptyloxy)butan-1-ol] and aldehyde [4-(*n*-heptyloxy)butanal] components of the male-produced pheromone were synthesized by Zhang et al. (2002). Solutions of both chemicals were prepared in hexane [mixture of isomers, >98.5%, high-performance liquid chromatography (HPLC) grade; EMD Chemicals, Gibbstown, NJ). The blend was created by mixing equal volumes (1:1) of each pure pheromone component in hexanes.

Treatments were compared with control (unbaited) traps and randomized within rows. Live insect baits were replenished as needed. All chemicals were applied on rubber septa (Stopper sleeve 5 by 11; VWR Scientific, West Chester, PA), which are commonly used dispensers for insect pheromones (Butler and McDonough 1981, Weatherston 1989). Septa were hung in the empty space in the middle of the trap and changed every 4–5 d. Because septa were not touching the trap itself, we were able to rerandomize lure treatments in traps with every change of lure. Trap cups were filled with a solution of ≈5% propylene glycol (or antifreeze; purchased locally). Traps were checked daily, and beetles caught were preserved individually in alcohol.

Comparison of Lure Treatments, Trap Design, and Placement in 2008. In July and August 2008, trapping studies were conducted again at site A and in a second field site in the same town (hereafter referred to as site B; 38°08′50.80″ N; 105°91′82.10″ E); site B was planted in corn and alfalfa. Poplars were also planted as windbreaks in site B; in rows around ≈20-m-wide plots. The purpose of this study was to determine: (1) the most attractive lure combination, (2) the best trap design, and (3) the best trap placement, for monitoring *A. glabripennis* adults in the field. Trap design and placement treatments included (1) Intercept panel traps hung on host trees, (2) Intercept panel traps hung on a row of bamboo poles 20 m away from host trees, and (3) screen sleeve traps (handmade from metal screen, designed by V. Mastro and D. Lance; USDA–APHIS–PPQ, Buzzards Bay, MA). Screen sleeve traps are "walk-in" traps, wrapped around host tree trunks, 1.5–2 m aboveground. Beetles walking up the tree trunk would walk between the outside perimeter of the cup and the screen and get caught in the upper section of the trap, unable to escape from the tightly closed top or to backtrack because of the funnel shape of the cup (Fig. 1). All Intercept panel traps were hung so that the bottoms were 1.5–2 m aboveground. The three trap design/placement treatments were randomized within both of the field sites. Six to seven traps were placed, 7–10 m apart, in each of 12 rows of poplar trees in site A and seven rows in site B. Rows of poplar were ≈20 m apart in both sites. Five lure treatments were tested against control (unbaited) traps and randomized within each trap design/placement (Table 1). Traps were checked daily and beetles caught in traps were preserved in alcohol. All females

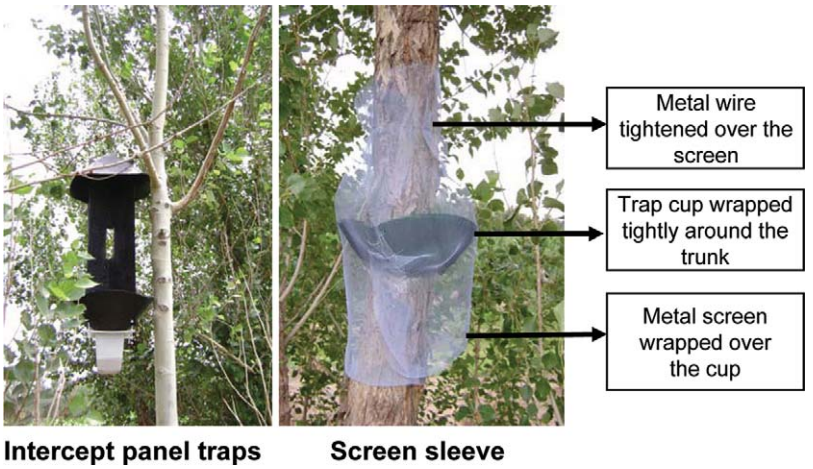

Fig. 1. Intercept PT traps (left) and screen sleeve traps (right). (Online figure in color.)

caught were dissected and the spermatheca removed to determine mating status. We did not have access to a microscope or light source at the rural field station, so only females with a completely flatted spermatheca, as observed with a hand lens, were considered virgin, whereas any swelling of the spermatheca was attributed to the presence of sperm, and such females were classified as mated.

The alcohol [4-(*n*-heptyloxy)butan-1-ol] and aldehyde [4-(*n*-heptyloxy)butanal] were prepared as described above for the 2007 field study. In addition to (−)-linalool and *trans*-pinocarveol, (Z)-3-hexen-1-ol (Fluka; purity 98%), linalool oxide (Fluka; purity >97%), and *trans*-caryophyllene (MP Biomedicals; purity 98%) were included in this study. Our choice of plant volatiles was based on previous Y-olfactometer bioassays in which (−)-linalool, linalool oxide, and (Z)-3-hexen-1-ol were moderately attractive to both sexes of *A. glabripennis* (Nehme et al. 2009). *Trans*-caryophyllene and *trans*-pinocarveol were mainly attractive to males and were added to the mixture to enhance attraction of the blend to both males and females. All chemicals were again applied on rubber septa. Septa carrying plant volatiles were placed in amber polyethylene bags (VWR reclosable UV-protectant amber bags 2 by 3 cm; VWR Scientific, West Chester, PA) for protection from photo-oxidation and to slow the release rate.

On 14 July, 24 July, and 2 August 2008, we walked through each of the poplar rows and counted all beetles that could be seen, carefully looking at each tree from all directions and using binoculars when needed. The numbers of beetles observed on the three dates were averaged to account for differences in time. Trees in each row were counted and first classified as "host" and "nonhost", following Sawyer (2003). Host trees at each site were further classified into four categories according to their level of infestation determined by the number of exit holes and dead branches on a tree (Table 2). The number of traps and beetles caught in traps per row were calculated at the end of the season. This information was used to estimate the proportion of the population trapped (beetles trapped per beetles observed on trees) and the relationship between level of infestation and trap catches. Temperature and precipitation information were collected from the local weather station.

Statistical Analyses. Traps catches were recorded daily and summed at each change of lure (every 4–5 d). Comparisons among treatments in 2007 and among

Table 1. Lure treatments used in field experiments in China in 2008

Abbreviation	Lure treatment components	Dose (μg)	Application
MP	Male-produced pheromone[a]	10	Rubber septa
L	(−)-Linalool	100	Rubber septa
LMP	Male-produced pheromone	10	Rubber septa
	(−)-Linalool	100	Rubber septa
Mix	(−)-Linalool	100	Rubber septa in amber
	(Z)-3-hexen-1-ol	100	Bags
	(−)-*Trans*-pinocarveol	100	
	Linalool oxide	100	
	Trans-caryophyllene	100	
MM	Male-produced pheromone	10	Rubber septa
	(−)-Linalool	100	Rubber septa in amber
	(Z)-3-hexen-1-ol	100	Bags
	(−)-*Trans*-pinocarveol	100	
	Linalool oxide	100	
	Trans-caryophyllene	100	

[a] *A. glabripennis* male-produced pheromone consists of an equal blend of 4-(*n*-heptyloxy)butan-1-ol and 4-(*n*-heptyloxy)butanal.

Table 2. Categories of host trees assigned according to infestation level

Infestation level	No. exit holes	No. dead branches	Overall appearance
1	<10	0	Healthy
2	10–20	<2; large	Weak
3	>20	>2; large	Dying but with few green branches
Dead	>20	All	Dead

treatments and trap design/placement in 2008 were conducted using generalized linear models (GLMs) followed by orthogonal contrasts (OCs) for mean comparisons. Because the data fit a poisson distribution instead of a normal distribution, the GLM produces a χ^2 statistic. Correlation analyses between treatments and tree infestation levels, and beetle and tree counts per row were conducted using Spearman's correlation analysis. All statistical analyses were performed using JMP v7.0 software (SAS Institute 2007).

Results

Comparison of Lure Treatments for Intercept Panel Traps in 2007. Total trap catches per change of lure data fit a Poisson distribution (goodness-of-fit test: $\chi^2 = 3.06$; $P = 0.22$). The pheromone-baited Intercept traps, containing both alcohol and aldehyde components, caught nine beetles over the 3-wk trapping period (five females and four males), which was the highest number over all lure treatments. Significantly more females were caught in pheromone-baited traps than the control, live male-baited traps and aldehyde-baited traps (OC: $\chi^2 = 5.54$; df = 3; $P = 0.018$; Fig. 2). Pheromone-baited traps also caught significantly more males than the alcohol and aldehyde-baited traps (OC: $\chi^2 = 11.0$; df = 2; $P = 0.0009$), but not significantly more than either the control, live insects, or the combination of the pheromone, (−)-linalool and *trans*-pinocarveol. The alcohol component of the pheromone attracted only females, while the aldehyde alone did not attract any beetles of either sex.

Comparison of Lure Treatments for Intercept Panel Traps in 2008. Temperature and precipitation averages were quite similar throughout the time of the study at the two sites, but site B was more prone to flooding. The average daily low temperature from 1 July to 7 August 2008 was 17–18°C, with an average high of 28°C and an average precipitation of 0.05–0.1 cm/d.

The data for total trap catches per change of lure in 2008 also fit a Poisson distribution (goodness-of-fit test: $\chi^2 = 4.53$; $P = 0.21$). Intercept panel traps baited with the pheromone caught more females at both sites. At site A, traps baited with the pheromone blend and the full mix of five plant volatiles (MM traps) had the highest overall female trap catches (GLM: $\chi^2 = 15.6$; df = 5; $P < 0.0001$) and caught significantly more females than males (GLM: $\chi^2 = 6.69$; df = 1; $P = 0.0097$). In fact, MM traps caught 15 of the 44 total beetles trapped in Intercept panel traps on host trees at this site (≈34%), and 12 of these 15 beetles were females. The number of males trapped did not differ among lure treatments (Fig. 3A).

Trap catches were lower at site B (17 beetles in total) compared with site A (44 beetles in total) for Intercept panel traps hung on host trees (GLM: $\chi^2 = 29.4$; df = 1; $P < 0.0001$). No significant difference was found among lure treatments at site B. However, females were caught only in traps baited with the pheromone alone or in combination with plant volatiles; no beetles were caught in control traps and more males were caught in traps baited with plant volatiles than in those baited with the pheromone alone (Fig. 4).

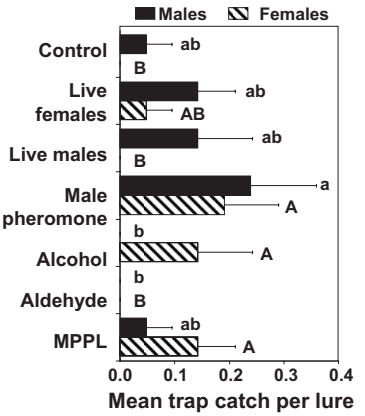

Fig. 2. Mean number of *A. glabripennis* adults caught in Intercept PT traps in China (July 2007), based on total beetles caught per change of lure. Controls consisted of unbaited traps. Live female and live male traps contained five live females or males, respectively, in small cages attached to the trap. The pheromone treatment consisted of 10 μg of the male-produced pheromone blend; the alcohol treatment of 10 μg of 4-(*n*-heptyloxy) butan-1-ol; the aldehyde treatment of 10 μg of 4-(*n*-heptyloxy) butanal; and MPPL of 10 μg of the pheromone and 100 μg of each of linalool and (−)-*trans*-pinocarveol. All chemicals were applied on rubber septa. Error bars represent SEM. Letters over bars represent significantly different means at the 95% confidence level among lure treatments for each gender (lowercase letters for males and capital letters for females). (Online figure in color.)

Comparison of Trap Designs and Placement in 2008. In 2008, relative effectiveness of trap designs and trap placement were compared at both sites. At site B, no screen sleeve traps were set up because of lack of space. Intercept panel traps on host trees caught a total of 13 beetles compared with only four beetles caught in traps hung on bamboo poles.

At site A, screen sleeve traps had the highest mean catch per trap (GLM: $\chi^2 = 41.6$; df = 2; $P < 0.0001$), followed by Intercept panel traps hung on host trees (Table 3). Intercept panel traps hung on bamboo poles caught only three beetles in total, which were found in one trap baited with the pheromone alone and two traps baited with a combination of the pheromone and the mix of five plant volatiles. Consequently, only Intercept panel traps hung on host trees were used in further comparisons with screen sleeve traps. Trap catches increased toward the end of July; peak trap catches occurred on 26 July 2008 for the screen sleeve traps and on 1 August 2008 for the Intercept panel traps (data not shown).

Female trap catches at site A differed significantly by lure treatment and by the interaction of lure treatment with trap design, whereas male trap catches were not significantly different across lure treatments or trap designs (Table 4). Significantly more females were caught in screen sleeve traps baited with the male-produced pheromone and (−)-linalool than in

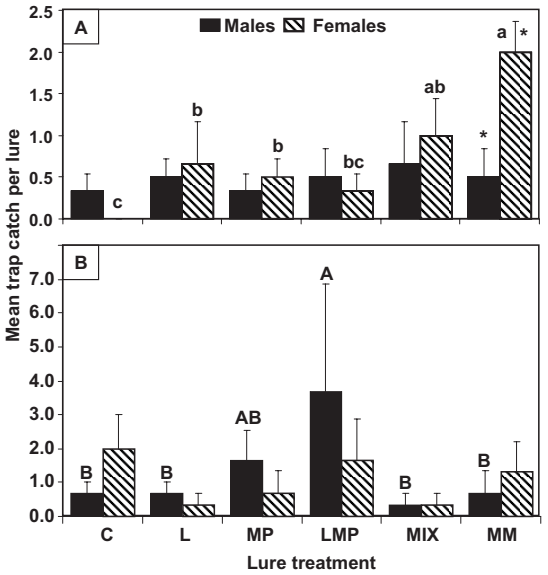

Fig. 3. Mean number of male and female *A. glabripennis* caught in Intercept PT traps hung on host trees (A) and screen sleeve traps (B) at site A in July and August 2008, based on total number of beetles caught per change of lure. C, control (unbaited); L, linalool; MP, male-produced pheromone; LMP, male-produced pheromone + linalool; Mix, linalool + (Z)-3-hexen-1-ol + (−)-*trans*-pinocarveol + linalool oxide + *trans*-caryophyllene; MM, male-produced pheromone + linalool + (Z)-3-hexen-1-ol + (−)-*trans*-pinocarveol + linalool oxide + *trans*-caryophyllene. Error bars represent SEM. Letters over bars represent significantly different means at the 95% confidence level across lure treatments for each sex. An asterisk over the bar represents significantly different means between males and females. Male trap catches were not significantly different among lures (no letters over bars).

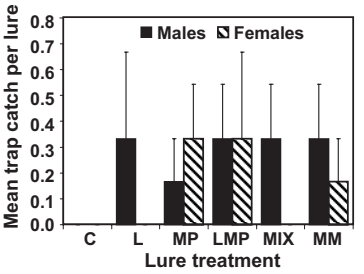

Fig. 4. Mean numbers of male and female *A. glabripennis* caught in Intercept PT traps hung on host trees (trap type 1) at site B, China, in July and August 2008, based on total caught per change of lure. C, control (unbaited); L, linalool; MP, male-produced pheromone; LMP, male-produced pheromone + linalool; Mix, linalool + (Z)-3-hexen-1-ol + (−)-*trans*-pinocarveol + linalool oxide + *trans*-caryophyllene; MM, male-produced pheromone + linalool + (Z)-3-hexen-1-ol + (−)-*trans*-pinocarveol + linalool oxide + *trans*-caryophyllene (Table 3). No significant differences were found among treatments or between sexes.

Intercept panel traps baited with the same lure (OC: $\chi^2 = 4.23$; df = 1; $P = 0.03$). More females were also caught in control screen sleeve traps than in control Intercept panel traps (OC: $\chi^2 = 13.1$; df = 1; $P = 0.0002$). Trap catches with the remaining lures did not differ significantly between the two trap designs.

Trap catches of males in screen sleeve traps were significantly different across lure treatments (GLM; $\chi^2 = 15.3$; df = 5; $P = 0.009$), whereas females were not. In screen sleeve traps, the combination of the pheromone and (−)-linalool caught significantly more males than unbaited controls (OC: $\chi^2 = 6.85$; df = 1; $P = 0.008$), (−)-linalool alone (OC: $\chi^2 = 6.85$; df = 1; $P = 0.008$), the mix of five plant volatiles (OC: $\chi^2 = 9.75$; df = 1; $P = 0.001$) or the combination of the pheromone with the mix of five plant volatiles (OC: $\chi^2 = 6.85$; df = 1; $P = 0.008$). However, the combination of the pheromone and (−)-linalool in screen sleeve traps was not significantly different from the male pheromone alone (OC: $\chi^2 = 2.3$; df = 1; $P = 0.12$; Fig. 3B).

Of the females caught in traps baited with the combination of the pheromone with the mix of five plant volatiles, 85% were virgins, whereas most females caught in the remaining treatments had mated, as evidenced by the presence of sperm in the spermatheca (Fig. 5).

Influence of Tree Infestation Level on Trap Catches. Site information on beetle abundance and infestation level of host trees in the 2008 summer study sites are given in Table 5. At site A, beetles (observed) were more abundant in rows with a higher number of host trees (Spearman: $\chi^2 = 0.67$; $P = 0.033$). Trap catches for both Intercept panel traps hung on host trees and screen sleeve traps correlated positively with the number of hosts ranked at infestation levels 1 and 3 (Spearman: $\chi^2 = 0.07$; $P = 0.0221$ and $\chi^2 = 0.68$; $P = 0.0281$; respectively), and with the number of nonhost trees (Spearman: $\chi^2 = 0.78$; $P = 0.007$). At this site, the two trap designs together caught ≈22% of the observed beetle population (Table 5).

At Site B, trap catches were not correlated with any of the parameters evaluated. Site B was characterized by a low insect population (mean number of beetles

Table 3. Mean total trap catches of *A. glabripennis* for each trap design/placement at sites A and B (Qing Tong Xia, Ningxia, China) for all lures for the entire trapping season of 11 July to 7 Aug. 2008

Trap design/placement	Site A		Site B	
	No. replicates[a]	Mean no. beetles caught ± SE[b]	No. replicates	Mean no. beetles caught ± SE[b]
Intercept PT on host tree	36	1.22 ± 0.09ab	36	0.36 ± 0.12a
Intercept PT on bamboo poles	18	0.16 ± 0.22b	12	0.33 ± 0.25a
Screen sleeve trap on poplar tree	18	2.38 ± 0.85a	0	NA

[a] No. replicates = no. lures (=6 for all) × no. traps baited with each lure (different across trap design/placement).

[b] Mean no. beetles caught per change of lure. Means with different superscript letters within a column are significantly different at the 95% confidence level.

NA, not applicable.

Table 4. Generalized linear model results from analysis of trapping data to test main effects of lure treatments (six total) and trap designs (hung on host trees) on total, female, and male trap catches at site A

	Total trap catch			Female trap catch			Male trap catch		
	χ^2	df	*P* value	χ^2	df	*P* value	χ^2	df	*P* value
Trap design	2.71	1	0.099	2.65	1	0.1029	3.17	1	0.075
Lure treatment	14.1	5	0.014	20.4	5	0.0011	1.01	5	0.962
Trap design × lure treatment	26.1	5	<0.0001	18.5	5	0.0024	7.61	5	0.178

observed per row = 4.20 ± 2.33). Intercept traps hung on poplar trees at this site caught ≈67% of the observed beetle population, with trap catches per row averaging 2.80 ± 0.66 beetles (Table 5).

Discussion

Trapping results indicate that the male-produced pheromone of *A. glabripennis*, especially when combined with specific plant volatiles, shows promise as a lure for monitoring of this exotic species. Previous laboratory and greenhouse bioassays showed significant attraction of females to the male-produced pheromone, in addition to moderate attraction of opportunistic males (Nehme et al. 2009). In the field, trapping results provided additional evidence for the attractiveness of the pheromone blend and for its role in mate-finding. More females were caught in Intercept panel traps baited with the pheromone blend compared with control traps both in 2007 and 2008 and at both field sites. Although pheromone-baited Intercept panel traps caught some males, the number of males caught was not significantly different from control traps, which suggests that, for both baited and unbaited traps, the males might be attracted more to the shape and color of the trap than to the pheromone itself. The females that were caught in the control traps may have been an artifact of the trapping design; drift from traps placed close together could have had a minor influence on trap catch.

Between the two components of the male-produced pheromone, the alcohol attracted only females to Intercept panel traps, whereas the aldehyde-baited traps did not catch any beetles. Although some attraction to the aldehyde component was observed in laboratory experiments, especially for males, the absence of beetles in aldehyde-baited traps in the field might be caused by conversion of the aldehyde into the acid under ambient oxygen conditions and high temperatures in the field. Alternatively, the discrepancy could also be caused by a difference in beetle behavior between laboratory and field environments.

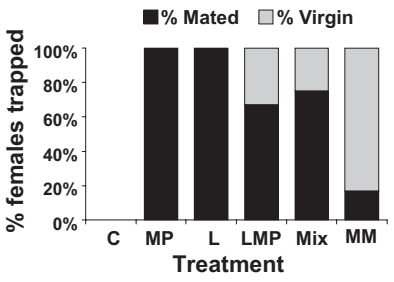

Fig. 5. Mating status of female *A. glabripennis* caught in all trap types per treatment at both sites in China (2008). C, control (unbaited); L, linalool; MP, male-produced pheromone; LMP, male-produced pheromone + linalool; Mix, linalool + (Z)-3-hexen-1-ol + (−)-*trans*-pinocarveol + linalool oxide + *trans*-caryophyllene; MM, male-produced pheromone + linalool + (Z)-3-hexen-1-ol + (−)-*trans*-pinocarveol + linalool oxide + *trans*-caryophyllene (Table 3).

Anoplophora glabripennis female trap catches were enhanced by the addition of (−)-linalool, (Z)-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene, and *trans*-pinocarveol to the pheromone. Most of the females caught in traps baited with this combination were virgins. Enhancement of trap catches of cerambycids and other wood-boring insects through the addition of plant volatiles to insect pheromones is a common practice. Since the 1970s, it has been known that bark beetles can be captured in greater numbers in traps baited with a combination of plant volatiles and bark beetle pheromones (Pitman et al. 1975, Byers et al. 1988). Combining the male sex pheromone of *Hylotrupes bajulus* L. (Coleoptera: Cerambycidae) with four monoterpenes was found to significantly improve trap catches (Reddy et al. 2005). Ginzel and Hanks (2005) also provided evidence for the role of host volatiles in mate finding for three species of longhorned beetles, *Xylotrechus colonus* (Fabricius), *Megacyllene caryae* (Gahan), and *Neoclytus mucronatus mucronatus* (Fabricius). Plant volatiles seem to play a similar role for *A. glabripennis* virgin females searching for a mate.

These trapping results, combined with previous laboratory results showing beetle preference to a combination of the male-produced pheromone with plant volatiles over the pheromone alone in a Y-olfactometer (Nehme et al. 2009), suggest that *A. glabripennis* may follow the model posed by Saint-Germain et al. (2007), where males respond to plant volatiles to find a host tree and females respond to the combination of plant volatiles and the male-produced pheromone blend to find a mate. This is also supported by our field observations where females were observed walking toward the males, but stopping short. The male walked to meet the female, which could also be guided by additional, unknown short-range cues, including vision. Contact pheromones, which occur on the elytra of females, likely provide the final cue for male recognition of con-specific females (Zhang et al. 2003).

In terms of trap design, as we observed earlier in the greenhouse (Nehme et al. 2009), Intercept

Table 5. Site details and correlation with trap catches at both sites in 2008

Mean ± SE (*P* value)	Site A		Site B	
	IPT on host trees + screen sleeve traps	IPT on bamboo poles	IPT on host trees	IPT on bamboo poles
No. host trees	58.1 ± 7.32 (0.13)	0.00 ± 0.00 NA	174 ± 36.0 (0.55)	0.00 ± 0.00 NA
No. nonhost trees	4.30 ± 0.93 (0.007)[a]	0.00 ± 0.00 NA	48.6 ± 7.47 (0.21)	121 ± 13.5 NA
No. beetles observed per row	36.8 ± 6.61 (0.44)	14.0 ± 14.0 (0.66)	4.20 ± 2.33 (1.00)	0.00 ± 0.00 NA
No. trees at infestation level 3	30.0 ± 4.26 (0.022)[a]	0.00 ± 0.00 NA	22.8 ± 6.49 (0.74)	0.00 ± 0.00 NA
No. trees at infestation level 2	22.5 ± 4.49 (0.60)	0.00 ± 0.00 NA	44.6 ± 14.7 (0.21)	0.00 ± 0.00 NA
No. trees at infestation level 1	4.20 ± 1.27 (0.028)[a]	0.00 ± 0.00 NA	107 ± 37.9 (0.55)	0.00 ± 0.00 NA
No. beetles caught per row	8.10 ± 1.77	0.75 ± 0.25	2.80 ± 0.66	2.00 ± 2.00

P values are for the results of Spearman nonparametric correlation tests of each variable with "no. beetles caught per row" (JMP7.0; SAS Institute 2007).

[a] Significant *P* values.

IPT, Intercept TM panel traps; NA, correlation analysis not applicable because of nonpositive values or small sample size.

panel traps, and screen sleeve traps were equally successful in trapping *A. glabripennis* adults in the field. The significant interaction observed between trap design and lures in the greenhouse (Nehme et al. 2009) also persisted in the field where numbers of beetles captured by the two trap types depended on the lure used. Screen sleeve traps caught the highest number of beetles when baited with a combination of the male-produced pheromone and (−)-linalool, whereas Intercept traps had the highest catches when baited with the male-produced pheromone and a mix of five plant volatiles: (−)-linalool, (Z)-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene, and *trans*-pinocarveol. Implementation of either of the trap types will depend on availability, feasibility, location, tree type and shape, accessibility, and other economic factors beyond the scope of this paper. Although we believe the combination of plant volatiles used was successful in trapping *A. glabripennis* in the field when combined with the male-produced pheromone, additional field studies using variations of the combination of the plant volatiles used in 2008 and other plant volatiles shown to be attractive in Y-olfactometer bioassays are needed. For example, 3-carene was found to be highly attractive to males (Nehme et al. 2009) and may be useful when combined with the pheromone blend.

Our trapping results from site B (2008), although not statistically significant, are promising given the low density of *A. glabripennis* observed at this site. In the United States, quarantine regulations require eradication of known infestations. Unfortunately, too often infestations are not discovered until a large number of trees are infested (e.g., New York and Worcester, MA). Thus, the need for monitoring traps to detect outlier populations or the presence of low numbers of beetles remaining in a quarantine zone is critical at places such as ports of entry, adjacent to known infestation areas, and other high-risk sites such as those released from quarantine when eradication efforts have been completed. If beetles are present at these types of locations, they will usually be at low to very low population densities, more similar to what we observed at site B than at site A in this study. The finding that at site B, Intercept panel traps caught >50% of the observed beetle population, including similar numbers of males and females, is a highly promising result given the likely similarity to *A. glabripennis* populations in the United States. Being able to trap both sexes is very important at ports of entry where there is an equal chance of introducing males and females. At the same time, the finding that the females were able to discriminate between lures that contained the male-produced pheromone and those that did not adds to the value of this pheromone for monitoring purposes. Trapping a good proportion of females at low population densities has major advantages and greatly reduces the likelihood of establishment. This can occur both by reducing the number of ovipositing females and by a diminished likelihood of mate finding, producing an Allee effect where a decrease in population density can lead to local extinction (Allee 1931).

In conclusion, the *A. glabripennis* male-produced pheromone is a promising lure for monitoring when combined with (−)-linalool, (Z)-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene, and *trans*-pinocarveol and placed in Intercept panel traps. In addition, screen sleeve traps, baited with this pheromone and (−)-linalool, seem to provide a potential alternative.

Challenges to the success of this monitoring technique in the United States include differences in host tree species composition, including height, shape, and position of host trees, compared with China. These factors are likely to affect beetle behavior and attractiveness to specific plant volatiles. Consequently, trap designs, trap placement, lure combinations, and the type of emitters used in China should be tested and adapted as needed to optimize their efficacy under field conditions in the United States.

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