Attraction of *Anoplophora glabripennis* to Male-Produced Pheromone and Plant Volatiles

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ABSTRACT The male-produced pheromone of *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), which is an equal blend of 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal, was used in laboratory bioassays and in the greenhouse to determine its potential for attracting *A. glabripennis* adults. In modified "walking wind tunnels," virgin females were most attracted to the alcohol component, and virgin males were repelled by the pheromone blend at the lowest and highest amounts offered. Y-tube olfactometer bioassays also showed that females were significantly more attracted to the pheromone and its components than males were. However, males were more attracted to plant volatiles than females. Of 12 plant volatiles tested, δ-3-carene and (*E*)-caryophyllene were highly attractive to males, whereas (Z)-3-hexenyl acetate was repellent to males. Combining the male pheromone blend with (−)-linalool alone or with (Z)-3-hexen-1-ol attracted significantly more males than did the pheromone alone. We tested four trap designs in our quarantine greenhouse with eight different lures. The Intercept Panel traps and the hand-made screen sleeve traps caught more beetles than the Plum Curculio traps and Lindgren funnel traps. Intercept traps worked best when baited with male blend and (Z)-3-hexen-1-ol, whereas screen sleeve traps were most attractive when baited with (−)-linalool. Our findings provide evidence of the attractiveness of the *A. glabripennis* male-produced pheromone and suggest that it has a role in mate-finding. It is also a first step toward the development of an efficient trap design and lure combination to monitor *A. glabripennis* infestations in the field.

KEY WORDS *Anoplophora glabripennis*, male-produced pheromone, mate-finding, trap design, Cerambycidae

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae, subfamily Lamiinae), commonly known as the Asian longhorned beetle, is a high-risk invasive species (USDA–APHIS 1998, EPPO 2004). It was introduced into the United States in solid wood packing materials from Asia and was first discovered in New York in 1996 (USDA–APHIS 2008). *Anoplophora glabripennis* larvae feed in the wood of deciduous trees. Adults emerge in early summer and feed on small twigs and leaves. They spend most of their time in shaded areas on the trunk of the same tree they emerged from (Williams et al. 2004). After resource depletion, flight to a healthy host is believed to be guided by olfactory cues from the host trees (Lance et al. 2000).

In the United States, monitoring of *A. glabripennis* infestations is restricted to scouting by climbing individual trees and searching for oviposition scars, sap flow, larval frass, or dime-sized adult emergence holes. Large bucket trucks or tree climbers are sometimes used to aid in detection (USDA–APHIS 2005a). This approach is expensive and time consuming. Rapid detection of this serious, invasive species is necessary to intercept new introductions before they establish and verify success (or failure) of eradication efforts. Development of an attractive lure to be used in monitoring traps is a first major step toward the improvement of detection techniques for *A. glabripennis*.

Recent studies showed taxonomic patterns in pheromone production within subfamilies of the Cerambycidae (Hanks 1999, Allison et al. 2004, Ray et al. 2006). In the Cerambycinae, volatile pheromones were found in most of the species studied thus far (Sakai et al. 1984, Kuwahara et al. 1987, Lacey et al. 2004, Hall et al. 2006, Ray et al. 2006). These pheromones are produced by males to attract females and share a similar structural motif. Some of these compounds also attract males and thus likely play a role in aggregation (Lacey et al. 2004, Hanks et al. 2007, Lacey et al. 2007). Pheromone production was correlated positively with the presence of pores on the prothorax of male cerambycids (Ray et al. 2006). However, prothoracic pores are not as common in the lamiines (Ray et al. 2006). In fact, electron microscopy of *A. glabripennis* (Coleoptera: Cerambycidae: Lamiinae) adults showed no difference in prothoracic pores between males and females (L. Hanks, personal communica-

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tion); but, consistent with the cerambycines, male-produced pheromones were identified in two Aseminae, *Tetropium fuscum* L. and *Tetropium cinnamopterum* (Kirby) (Silk et al. 2007), and the two lamiines *Hedypathes betulinus* (Klug) (Vidal et al. 2008) and *A. glabripennis* (Zhang et al. 2002).

Putative pheromone components produced by *A. glabripennis* males consist of two functionalized dialkyl ethers, one alcohol [4-(n-heptyloxy)butan-1-ol] and one aldehyde [4-(n-heptyloxy)butanal] (Zhang et al. 2002). Antennae of both males and females responded to the blend (1:1; vol:vol) of these two compounds using gas chromatography–electroantennographic detection (GC-EAD). Preliminary olfactometer bioassays showed that these compounds were equally "stimulatory" to both sexes, but only moderately so (Zhang et al. 2002).

Several species of cerambycids use plant compounds rather than long-range pheromones as mediators for mate location. Such is the case for most flower-visiting cerambycids that depend largely on floral scents (Hanks 1999). Attraction to plant volatiles has been exploited for pest management and in monitoring traps, alone or in combination with insect pheromones (Linsley 1959; Byers et al. 1988; Hanks 1999; Allison et al. 2004; Sweeney et al. 2004, 2007; Ibeas et al. 2006; Miller 2006).

Anoplophora glabripennis feeds primarily on poplars in China and maples in the United States. Studies have been conducted in both countries on host location, including detection of plant volatiles, and the possibility of using host volatiles in baited traps for monitoring. Volatiles of ashleaf maple, *Acer negundo*, were studied extensively. Li et al. (2003) found that, in ashleaf maple, (Z)-3-hexen-1-ol, 1-butanol, 1-pentanol, and 2-pentanol were significantly attractive to adults. Wickham and Teale (2009) extracted volatiles from a range of *A. glabripennis* host trees in the United States and found several volatiles that are common across the host range (and absent in nonhosts), including (Z)-3-hexen-1-ol, (*E*)-caryophyllene, delta-3-carene, (−)-linalool, linalool oxide, and camphene. Our knowledge about *A. glabripennis* attraction to plant volatiles, however, is still in its infancy.

The purpose of this study was to develop an attractive lure to be used for monitoring *A. glabripennis* populations in the field. This involved testing a series of hypotheses followed by empirical testing of various trap designs and lure combinations. First, we tested the hypothesis, suggested by preliminary GC-EAD results, that the two male-produced compounds, 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal, either together or alone, were attractive to females and play a role in sexual communication in this species. A corollary to this hypothesis included determining whether there is a difference in attractiveness of the two putative pheromone components.

The second hypothesis was that host plant volatiles enhance performance of the putative pheromone components, and we chose a list of volatiles and essential oils to test based on previous studies with *A. glabripennis* (Chenier et al. 1989, Allison et al. 2001, Ping et al. 2001, Francese 2005, Wickham and Teale 2009). These plant volatiles were evaluated alone, in mixtures, and/or in combination with the male-produced pheromone to identify the most effective combination and to detect any possible synergism or antagonism between different chemicals.

Our third objective was to determine the best trap design in combination with the best lure. Francese (2005) tested five different trap designs without lures and found the screen sleeve trap to be the most effective by far. However, semiochemical-based trapping for *A. glabripennis* has been done almost exclusively with Intercept panel traps (Lund et al. 2004, Wickham and Teale 2009). In this study, we tested four different trap designs with eight different lures in a quarantine greenhouse to determine the most attractive combination of trap design and lures to be further tested as a monitoring tool for *A. glabripennis* in the field.

Materials and Methods

Insects

Most *A. glabripennis* adults were reared in the USDA–Forest Service Northern Research Station Quarantine laboratory in Ansonia, CT. These beetles came from two colonies: one established from adults that emerged from infested wood obtained from the Ravenswood area, Chicago, IL, infestation (UIC; reared for nine generations) and the other from large larvae imported from Inner Mongolia, China (IMC; reared for six generations). Insects used in the greenhouse trapping trials were from the *A. glabripennis* colony at Penn State University, which is of mixed origins (field collected from New York and laboratory reared). All *A. glabripennis* were transported and reared under USDA permits at each quarantine facility. Voucher specimens of the Ansonia *A. glabripennis* colonies were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT, and those of the Penn State colony were deposited at the Frost Museum, The Pennsylvania State University, University Park Campus, PA. All insects were reared on artificial diet (Keena 2005) until pupation. All life stages were kept at 25°C, 60% humidity, and L:D 16:8 h with constant ventilation. Males and females were kept individually in 750-ml glass jars after emergence and supplied with Norway maple twigs (*Acer platanoides* L.) for feeding. For mating, one male and one female *A. glabripennis* were placed in a 3.75-liter glass jar and provided with an ≈5-cm-diameter by 20-cm-long Norway maple bolt and twigs for oviposition and feeding, respectively. For all experiments, only healthy 15- to 30-d-old insects were used. For experiments using mated insects, beetles were used after the first oviposition event to ensure mating compatibility and sexual maturity (Keena 2002). Beetles used in the pheromone experiments were well fed, whereas those used in plant volatiles experiments were starved 5–7 h to enhance attraction.

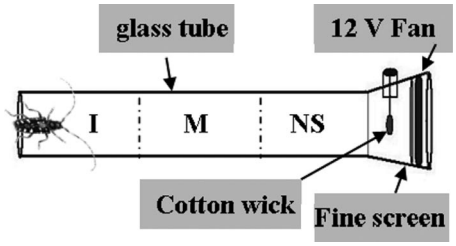

Fig. 1. The modified walking wind tunnel, composed of a 1.5-m-long glass tube connected to a plastic beaker closed by a 12-V fan at the end. Chemicals were applied on the cotton wick and beetles introduced at the free end of the tube and allowed to walk inside for 20 min. Tubes were virtually divided into three compartments: I (initial), M (middle), and NS (near source).

Response of *A. glabripennis* Adults to the Two Putative Male-Produced Pheromone Components

Wind Tunnel Bioassays. To test the hypothesis that the two male-produced compounds are attractive to females and play a role in sexual communication, we offered *A. glabripennis* males and females 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal alone or in an equal ratio blend before and after mating in modified wind tunnels. The alcohol, 4-(n-heptyloxy)-butan-1-ol, and aldehyde, 4-(n-heptyloxy)butanal, components of the putative pheromone, were synthesized following the protocol described by Zhang et al. (2002). One microgram per microliter solutions of each were prepared in hexane (mixture of isomers, 98.5%, high-performance liquid chromatography grade; EMD Chemicals, Gibbstown, NJ). Blends were created by mixing equal amounts of pure chemicals and diluting in hexane.

The modified "walking" wind tunnels consisted of 1.5-m-long by 1-mm-thick by 10-cm-diameter glass tubes (Chemglass, Vineland, NJ). For each tube, one plastic beaker (VWR International, West Chester, PA) was connected tightly to one end. Part of the base of the beaker was removed to allow the insertion of a 12-V computer fan. The fan was placed face out and manually adjusted with the aid of a flow meter such that it provided unidirectional air flow at 0.5 liters/min through the beaker to the inside of the tube. Chemicals were applied to a sterile cotton wick (TIDI Brand; Banta Healthcare Group, Boston, MA) and inserted in front of the fan through a small covered opening in the beaker (Fig. 1). The advantage of this no-choice test is that, unlike commonly used olfactometers where treatments offered mix in the central zone rather than staying separate and where air turbulence is likely to affect the directionality of the odor plume (Saïd et al. 2006), the modified wind tunnels limit air turbulence and provide a homogeneous unidirectional flow, ensuring purity of the chemical offered.

Four identical tunnels were used for each replicate, placed parallel to each other, ≈30 cm apart, on a flat surface directly under the room's ambient fluorescent lighting. The four treatments, 4-(n-heptyloxy)butan-1-ol alone; 4-(n-heptyloxy)butanal alone; blend of the two; and hexane control, were randomly distributed in the four tunnels. Four insects of the same sex/mating status were introduced simultaneously to the four tunnels to correct for possible temporal variation in insect response to the different treatments. All experiments were conducted in the late morning–early afternoon period (1100–1200 and 1400–1600 hours), when beetles were observed to be active in their feeding jars. Cotton wicks were replaced, and tunnels were cleaned with hexane between replicates. Relative positions of the test compounds in the four parallel tunnels were alternated every other replicate to randomize potential environmental factors. Tunnels were virtually divided lengthwise into three equal compartments: "initial," "middle," and "near source" (Fig. 1). One insect was introduced at the free end of each tunnel at the same time (opposite end to the fan). Beetles were allowed to walk inside the tunnel for 20 min. The time spent by each insect in each compartment was recorded, as well as the number of times each insect touched the cotton wick. Four different amounts of chemicals were tested: 0.05, 0.5, 1, and 2 μg. Ten each of virgin males, virgin females, mated males, and mated females were tested separately for each treatment and each amount. All tests were conducted in the quarantine facility in Ansonia, CT, under conditions suitable for *A. glabripennis* (25°C, 60% humidity) (Keena 2006).

Y-Tube Olfactometer Bioassays. After determining optimal amounts of the putative pheromone to be used in the wind tunnels, we conducted choice tests in a Y-tube olfactometer for verification purposes. Bioassays were conducted to test attractiveness of the male-produced compounds, each alone or in a 1:1 blend, at the 1-μg level, when offered against a hexane control. The Y-tube olfactometer used was built following the description by Ginzel and Hanks (2005) (6 cm diameter, main tube 26 cm long, arm length 22 cm, angle between arms 70°; Penn State University Glass Shop). Clean air was pushed into the two arms using two air pumps (Push-Pull pump; Bryan Banks, Penn State University) at ≈0.5 liters/min using 90 cm of Teflon tubing (6.35 by 9.52 mm, VWR brand; VWR Scientific Products, San Francisco, CA), and air was pulled from the main tube using another Push-Pull pump calibrated at ≈1 liter/min. Chemicals were applied on 1 by 1-cm^2 pieces of filter paper (Filter paper 413; 9.0 cm OD; VWR Brand; VWR International), and the solvent was allowed to dry before initiating the trial. The olfactometer was washed with hexane and allowed to dry completely between replicates to avoid residual odors. Treatment and control arms were alternated every other replicate. Fifteen virgin males and 15 virgin females were tested for each of the male-produced blend and its components. Choice and time to choice were recorded when beetles had moved forward at least 10 cm within the arm. All tests were conducted in the quarantine facilities in Ansonia, CT, or Penn State University, PA, under *A. glabripennis* rearing conditions.

Response of *A. glabripennis* Adults to Plant Volatiles

Based on previous studies with *A. glabripennis* (Chenier et al. 1989, Allison et al. 2001, Francese 2005,

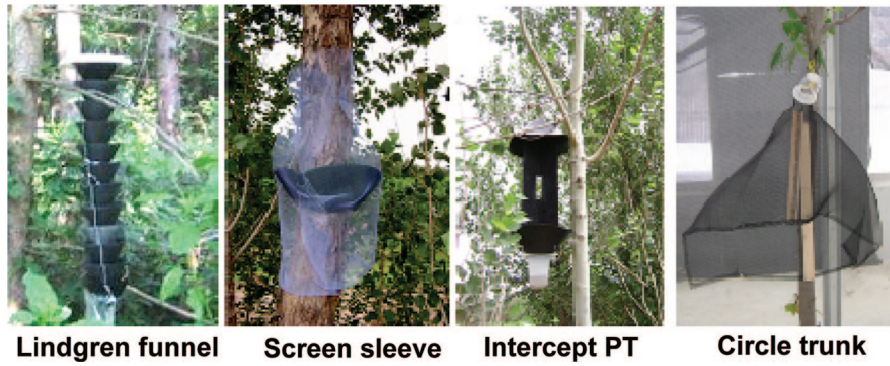

Fig. 2. Trap designs used in the greenhouse trapping experiment. From left to right: the Lindgren funnel trap, the hand-made screen sleeve trap, the Intercept Panel trap, and the circle trunk curculio trap.

Wickham and Teale 2009), we chose a list of plant volatiles and essential oils to test to determine the most attractive plant volatiles that could be used as lures in monitoring traps for this species. Two essential oils were tested: the commonly used eucalyptus oil and manuka oil (Silky Scents, Riverside, CA), which has been recently shown to be moderately attractive to the emerald ash borer (Crook et al. 2008) and other Coleoptera (Hanula and Sullivan 2008). Twelve plant volatiles were also purchased from VWR International and tested. These were (−)-verbenone (Fluka, Buchs, Switzerland; purity 99%), and (*E*)-pinocarveol (Fluka; purity 96%), which are attractants used to trap bark beetles and other wood-boring species (Reddy et al. 2005, Ibeas et al. 2006); (Z)-3-hexen-1-ol (Fluka; purity 98%), (−)-linalool (Fluka; purity 98.5%), linalool oxide (Fluka; purity >97%; GC grade), camphene (Chem Service, West Chester, PA; purity >96%), delta-3-carene (Fluka; purity 98.5%), (*E*)-caryophyllene (MP Biomedicals, Solon, OH; purity 98%), myrcene (Alfa Aesar, Ward Hill, MA; purity 95%), (±)-2-pentanol (Alfa Aesar; purity 99%), (Z)-3-hexenyl acetate (Alfa Aesar; purity 99%), and benzenyl acetate (Fluka; purity 99.7%), which are produced either by healthy or stressed *Acer mono* Maxim. and *A. negundo* and/or have been shown in different studies to attract *A. glabripennis* and other cerambycid adults (Chenier et al. 1989, Allison et al. 2001, Ping et al. 2001, Francese 2005, Wickham and Teale 2009).

All plant volatiles and essential oils were tested in the Y-tube olfactometer against a hexane control as described previously. In all cases, 50 μg of chemicals was used for each replicate. Depending on availability of beetles, 15–20 replicates each of virgin males and virgin females were tested for each chemical.

Response of *A. glabripennis* Adults to a Combination of the Male-Produced Compounds and Plant Volatiles in the Y-Tube Olfactometer

Previous studies showed that combining plant volatiles with insect pheromones enhances beetle trap catches significantly (Byers et al. 1988). To verify whether this would apply to *A. glabripennis*, we tested combinations of the putative male-produced pheromone blend with each of (−)-linalool alone, (−)-linalool + (Z)-3-hexen-1-ol, and delta-3-carene + (Z)-3-hexenyl acetate, offered against the putative pheromone blend in the Y-tube olfactometer as previously described. The choice of plant volatiles to be used in these combinations was based on previous Y-tube olfactometer and greenhouse trapping results. Ten to 20 virgin males and virgin females were tested for each combination (depending on availability of beetles). Choice and time to choice were recorded and analyzed for comparisons.

Comparison of Trap Design and Lures in the Greenhouse

Baited trap catches were compared among four trap designs in the quarantine greenhouse at Penn State University. The four traps tested were the Intercept Panel Trap (or Intercept PT; APTIV, Portland, OR), which was designed for bark beetles and other wood borers, and was used in research work with *A. glabripennis* (Francese 2005); a circle trunk trap (or curculio trap; Great Lakes Integrated Pest Management, Vestaburg, MI) used for plum curculio, pecan, and acorn weevils; the Lindgren funnel trap (12-funnel size; Contech, Delta, Canada), a widely used trap for bark beetles and other wood-boring beetles; and a screen sleeve trap, designed by Victor Mastro and Dave Lance (USDA–APHIS–PPQ, Otis, MA) for *A. glabripennis* after the limited success of the commonly available trap types (V. Mastro, personal communication) and manually made from metal screen (Fig. 2).

The quarantine greenhouse contains two screen cages of equal dimensions (≈3 by 3 by 3.9 m). In each cage, four different traps were placed in the four corners. The two walk-in trap designs (circle trunk and screen sleeve traps) were placed around the trunk of (≈3 m high, 10–15 cm DBH) sugar maple trees (*Acer saccharum* Marsch.), whereas the flight traps (Intercept Panel and Lindgren funnel) were attached to the screen wall of the cage. Because of limited time and limited number of beetles, we combined trap design and lure testing in one experiment. The pur-

Table 1. Lure treatments used in the greenhouse trapping experiments

Abbreviation	Lure treatment	Dosage (μg)	Replicates
MP	Male pheromone[a]	1	3
L	(−)-Linalool (98.5%; Fluka)	50	3
LO	Linalool oxide (97%; Fluka)	50	3
Hex	(Z)-3-hexen-1-ol (98%; Fluka)	50	3
HA	(Z)-3-hexenyl acetate (99%; Alfa Aesar)	50	3
MPL	(−)-Linalool (98.5%; Fluka) + male pheromone	1 MP + 50 L	3
MPHex	(Z)-3-hexen-1-ol (98%; Fluka) + male pheromone	1 MP + 50 Hex	3
MPHA	(Z)-3-hexenyl acetate (99%; Alfa Aesar) + male pheromone	1 MP + 50 HA	3

[a] Male pheromone = blend 1:1 [4-(n-heptyloxy) butan-1-ol: 4-(n-heptyloxy) butanal], purity 100%; synthesized by Aijun Zhang, USDA–ARS.

pose was to acquire preliminary information about what lures and trap designs to use for field testing in China the subsequent summer. For this, eight lure treatments were tested and compared with empty control traps (see Table 1 for lure information). All lures were dispensed on rubber septa (stopper sleeve 5 by 11; VWR Scientific, West Chester, PA), widely used dispensers for insect pheromones (Butler and McDonough 1981, Weatherston 1989). Each week, all four traps were tested using the same lure treatment (or empty control) making trap design the only variable. Three temporal replicates (each 1 wk long) were completed for each lure and the control. Relative placement of traps and greenhouse cages were rerandomized for each replicate. To correct for time and potential microclimate influences, lure treatments and empty controls were completely randomized over the weeks. Five *A. glabripennis* males and five females were released in the middle of each of the two greenhouse cages and allowed to move freely within each cage. Traps were checked daily. Trapped beetles were recorded, marked, and released back in the middle of the same cage. Dead beetles were continuously replaced. Greenhouse temperature was maintained between 24 and 28°C with 50–70% humidity.

Statistical Analyses

In the wind tunnel, two responses of *A. glabripennis* adults were recorded: time spent near source and number of visits to source. Time spent near source varied between 0 and 1,200 s. Generalized linear models (GLMs) for both data sets were performed by chemical amount, insect sex/mating status, and pheromone treatment for the three highest chemical amounts, because mated adults were not tested at the lowest amount. Separate GLMs were performed for each of the four chemical amounts tested by pheromone treatments and insect categories (combinations of sex and mating status) because of significant interactions among these variables. Orthogonal contrasts (OCs) were performed after each GLM for both pairwise and multiple mean comparisons.

For all insects used in the wind tunnel, age was divided into classes 1–6, with each class consisting of 3 consecutive days, starting at 15 d old, with class 6 grouping those individuals 30 d old or more. Spearman's correlation test was used to determine the effect of age class and insect weight on the time spent near source by *A. glabripennis* adults and the number of visits to source in the wind tunnel.

All Y-tube olfactometer results followed a binomial distribution and were analyzed using frequency analysis followed by a probability test, which allowed comparison of the real means to hypothetical means of 50%, (i.e., no choice was made) at the 95% confidence level using Pearson's test of significance. Comparisons between males and females were performed using the Fisher exact test. Spearman's correlations were used to determine the relationship between choice and time to choice.

Greenhouse trapping data were compared by trap design and lure using a GLM followed by OCs. All data were analyzed using JMP 7.0 (SAS Institute 2007).

Results

Response of *A. glabripennis* Adults to the Two Putative Male-Produced Pheromone Components

Wind Tunnel Bioassays. The response of *A. glabripennis* adults to the putative male-produced pheromone and its two components differed significantly by sex and mating status (Table 2). Overall, mated males and mated females showed no significant difference in the time spent near source or the number of visits to source across chemical treatments and amounts (data not shown).

Because of significant interactions of chemical amount with chemical treatment and sex/mating status (Table 2), we analyzed the time spent near source and number of visits to source data for each chemical amount separately to determine the effect of pheromone treatment and sex on insect response.

Overall, time spent near source was highest at the 1-μg level (OC: $\chi^2 = 18.2$; df = 2; $P = 0.0002$). At this level, virgin females spent more time near the source of the alcohol treatment compared with the remaining three treatments (OC: $\chi^2 = 5.26$; df = 3; $P = 0.021$) and visited the source of the alcohol treatment significantly more often than the blend (OC: $\chi^2 = 5.54$; df = 1; $P = 0.018$; Fig. 3). Virgin males visited the source of the alcohol/aldehyde blend and the aldehyde alone

Table 2. Generalized linear model results: effects of tests for time spent near source and no. of visits to source over the three highest amounts (0.5, 1, and 2 μg) of the male-produced blend or its components

GLM effects	Time spent near source			Number of visits to source		
	χ^2	df	*P*	χ^2	df	*P*
Whole model	329	20	<0.0001[a]	78.8	47	0.002[a]
Treatment	39.3	3	0.024[a]	4.31	3	0.229
Amount	25.2	2	<0.0001[a]	0.0001	2	0.999
Sex/mating status	35.2	3	0.002[a]	9.89	3	0.019[a]
Treatment × amount	55.6	6	0.003[a]	2.92	6	0.819
Treatment × sex/mating status	39.6	9	0.121	3.24	6	0.840
Amount × sex/mating status	115	6	<0.0001[a]	4.93	9	0.778
Treatment × amount × sex/mating status	129	18	<0.0001[a]	29.9	18	0.0383[a]

[a] Significant *P* values at the 95% confidence level.

more than the control and alcohol sources (OC: $\chi^2 = 4.74$; df = 3; $P = 0.029$; Fig. 3).

At the lowest chemical amount (0.05 μg), the number of visits to the source was different by sex/mating status (GLM: $\chi^2 = 19.78$; df = 3; $P = 0.0195$). Virgin males visited the source in control tunnels significantly more times than the alcohol (OC: $\chi^2 = 4.15$; df = 1; $P = 0.04$; Fig. 3) and spent more time near the source in control wind tunnels compared with the three chemical treatments (OC: $\chi^2 = 16.13$; df = 3; $P < 0.0001$; data not shown). Virgin females did not show any significant response at this level.

Anoplophora glabripennis adults showed no significant difference in their response to the alcohol, aldehyde, or blend treatments at the 0.5- and 2-μg levels (Fig. 3). At the 2-μg level, the number of visits to source was only significantly different by sex (GLM: $\chi^2 = 32.9$; df = 3; $P = 0.0098$), and virgin males visited the source more frequently (OC: $\chi^2 = 7.95$; df = 3; $P = 0.0047$) in the control tunnels than any of the putative pheromone component treatments (Fig. 3).

Y-Tube Olfactometer Bioassays. There was no significant difference in attraction to the alcohol or aldehyde or the blend of the two between males and females as a main effect (Fisher exact test: df = 1; $P > 0.5$; Table 3). However, females preferred the blend (Pearson: $\chi^2 = 5.4$; df = 14; $P = 0.02$) to the control, whereas males did not make a significant choice in any of the three tests. In no case did time to choice correlate significantly with quality of choice, suggesting that response times did not differ among any of the treatments.

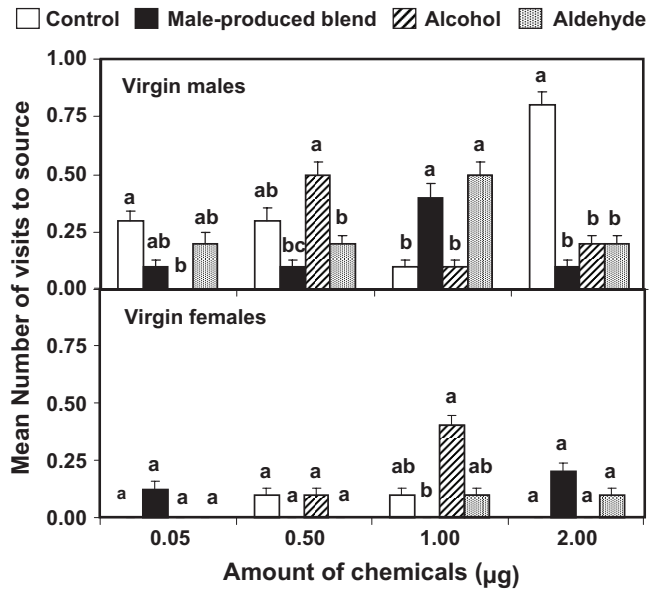

Fig. 3. Mean number of visits of *A. glabripennis* adults to the source in the walking wind tunnel at four different chemical amounts (0.05, 0.5, 1, and 2 μg) during 20 min. Treatments include the male pheromone (*A. glabripennis* male-produced pheromone blend), alcohol [4-(n-heptyloxy) butan-1-ol], and aldehyde [4-(n-heptyloxy) butanal] offered against a hexane control. Each pheromone treatment and chemical amount combination was tested separately for virgin males and virgin females ($n = 10$ for each). Error bars represent SEM. Letters over bars represent significantly different means within chemical amount and sex/mating status at the 95% confidence level.

Table 3. Percentage of *A. glabripennis* virgin males and virgin females that chose the male-produced blend, the alcohol alone, or the aldehyde alone when offered 1 μg of each against a hexane control in a Y-tube olfactometer (mean percentage ± SEM)

Sex	Male-produced blend	Alcohol	Aldehyde
Virgin males	46.7 ± 13.3%	33.0 ± 12.5%	60.0 ± 13.1%
Virgin females	80.0 ± 10.7%[a]	46.7 ± 13.3%	73.3 ± 11.8%

[a] Significant choice compared with the control at the 95% confidence level.

Effect of Age and Weight on *A. glabripennis* Response to Pheromones

Insect age and weight did not affect the capacity of *A. glabripennis* adults to walk toward the end of the wind tunnel. No correlation was found between insect weight or age and any of the response data (Spearman: $P > 0.5$), even when analyzed separately for each insect sex/mating status group.

Response of *A. glabripennis* Adults to Plant Volatiles in the Y-Tube Olfactometer

Of the 12 plant volatiles tested, 7 elicited more than a 50% response in virgin males, whereas only 4 of them attracted >50% of virgin females (Fig. 4). The difference between male and female attraction was significant for both δ-3-carene (Pearson: $\chi^2 = 7.5$; $n = 20$; $P = 0.0062$) and (*E*)-caryophyllene (Pearson: $\chi^2 = 4.46$; $n = 14$; $P = 0.0346$). δ-3-Carene was highly attractive for males, with 90.0% of virgin males choosing the treatment arm in the Y-tube olfactometer (Pearson: $\chi^2 = 6.4$; df = 1; $P = 0.0114$) compared with 22.2% of females.

When (Z)-3-hexenyl acetate or myrcene were offered against a hexane control, males chose the control arm of the olfactometer significantly more often (Pearson: $\chi^2 = 3.8$; df = 1; $P = 0.029$ and $\chi^2 = 3.85$; df = 1; $P = 0.04$, respectively). Females preferred the control significantly more frequently when offered against camphene (Pearson: $\chi^2 = 3.85$; df = 1; $P = 0.04$; Fig. 4).

Response of *A. glabripennis* Adults to a Combination of the Male-Produced Compounds and Plant Volatiles in the Y-Tube Olfactometer

When linalool was combined with the blend of these two compounds, twice as many adults of both sexes chose the combination compared with the male-produced blend alone (Fig. 5). This difference was especially significant for males (Pearson: $\chi^2 = 4$; df = 1; $P = 0.04$). When (Z)-3-hexen-1-ol was added to the mixture above, 90% of males chose the combination (Pearson: $\chi^2 = 4.5$; df = 1; $P = 0.034$), whereas only 10% chose the male-produced blend alone; in contrast, females did not make a choice.

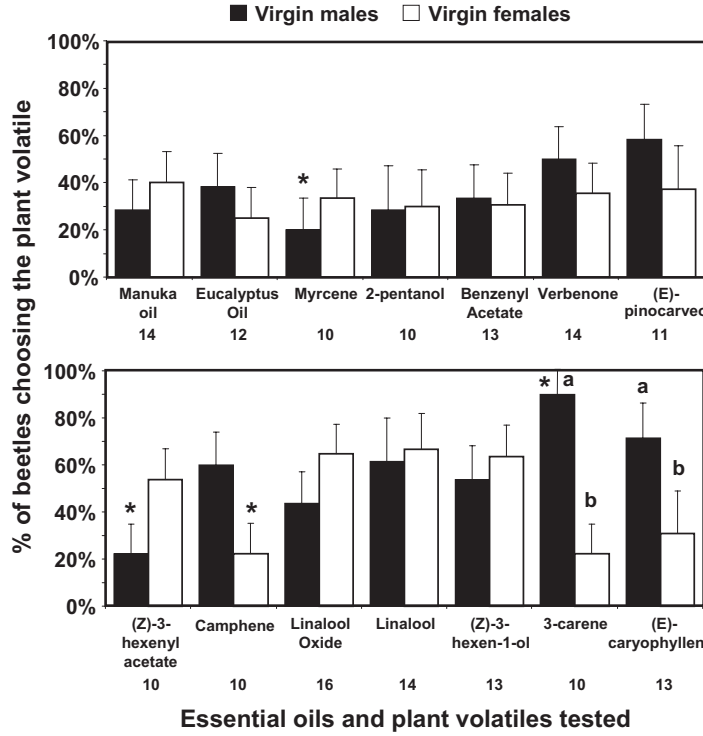

Fig. 4. Percentage of virgin males and virgin females that chose plant volatiles when offered against a hexane control in a Y-tube olfactometer. Error bars represent SEM. Letters over bars represent significantly different responses between males and females at the 95% confidence level. Percentages that are significantly higher or lower than 50% are labeled with an asterisk. Numbers under plant volatiles names represent the number of beetles of each sex tested.

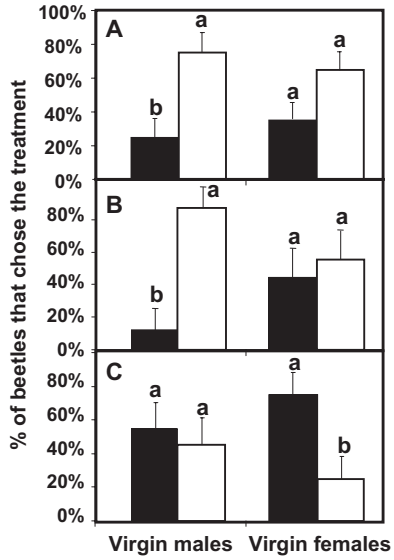

Fig. 5. Response of *A. glabripennis* virgin males and virgin females to combinations of the male-produced pheromone blend with plant volatiles offered against the pheromone alone (closed bars) in a Y-tube olfactometer. Open bars represent the response to (A) the male blend plus (−)-linalool; (B) the male blend plus (−)-linalool and (Z)-3-hexen-1-ol; and (C) the male blend plus (Z)-3-hexenyl acetate and δ-3-carene. Error bars represent SEM. Letters over bars represent significantly different means between treatments at the 95% confidence level.

We then chose δ-3-carene as one host volatile that is only attractive to males and (Z)-3-hexenyl acetate that elicited more response from females than males to test whether the combination of both with the male-produced blend would attract both sexes of *A. glabripennis.* In this case, 75% of females preferred the male blend alone, whereas males were equally attracted to both treatments (Fig. 5).

Comparison of Trap Designs and Lures in the Greenhouse

When the four trap designs were tested in the greenhouse, the Intercept panel traps and the screen sleeve traps caught equal numbers of beetles per week on average across all lures. Mean trap catches per week were significantly lower for the circle trunk and Lindgren funnel traps compared with the Intercept panel and screen sleeve traps (GLM: $\chi^2 = 17.5$; df = 3; $P = 0.0006$; Fig. 6). Although overall mean trap catches were equal between the intercept panel and screen sleeve traps, mean trap catches per week differed by lure (GLM: $\chi^2 = 27.2$; df = 8; $P = 0.0007$) and by the interaction of trap design and lure (GLM: $\chi^2 = 20.5$; df = 8; $P = 0.0087$). Screen sleeve traps baited with linalool caught the highest number of beetles per week, whereas Intercept panel traps baited with the same plant volatile did not catch any beetles. Intercept traps baited with (Z)-3-hexen-1-ol caught twice as many beetles on average than screen sleeve traps. The

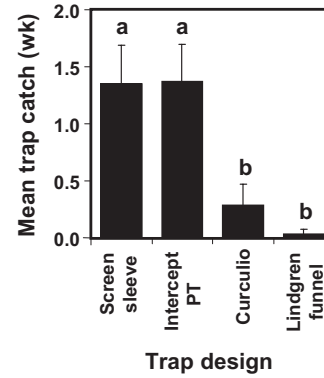

Fig. 6. Mean trap catches per week for the four trap designs tested in the greenhouse: the screen sleeve trap, Intercept Panel trap (or Intercept PT), Circle trunk trap (or Curculio trap), and Lindgren funnel trap. Error bars represent SEM. Letters over bars represent significantly different means at the 95% confidence level.

combination of the male-produced blend with (Z)-3-hexen-1-ol attracted higher numbers of beetles in both trap designs. This combination caught significantly more beetles than (Z)-3-hexen-1-ol in screen sleeve traps (OC: $\chi^2 = 4.15$; df = 1; $P = 0.04$), the (Z)-3-hexenyl acetate alone (OC: $\chi^2 = 10.4$; df = 1; $P = 0.0012$), or in combination with the male blend (OC: $\chi^2 = 10.4$; df = 1; $P = 0.0012$; Fig. 7). (Z)-3-hexenyl acetate had the lowest overall trap catches when offered alone or in combination with the male-produced blend. Although some trap designs × lure treatments were significantly

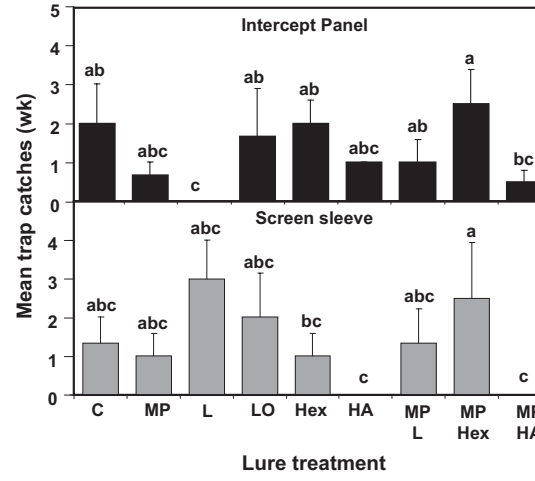

Fig. 7. Mean trap catches per week per lure for the Intercept panel (top) and screen sleeve (bottom) traps. Error bars represent SEM. Letters over bars represent significantly different means within lure treatments at the 95% confidence level. [C, control; MP, *A. glabripennis* male pheromone blend; L, (−)-linalool; LO, linalool oxide; Hex, (Z)-3-hexen-1-ol; HA, (Z)-3-hexenyl acetate; MPL, male pheromone blend + (−)-linalool; MPHex, male pheromone blend + (Z)-3-hexen-1-ol; MPHA, male pheromone blend + (Z)-3-hexenyl acetate; ref. Table 1 for amounts].

more attractive than others, none was significantly different from empty control traps in the greenhouse.

Discussion

The *A. glabripennis* male-produced compounds 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal showed potential as attractive lures for monitoring this species. Based on trends observed in both the wind tunnel and Y-tube olfactometer bioassays, females responded behaviorally to the compounds alone or in combination, which suggests they are both required components for attraction. This result is consistent with findings for several members of the Cerambycidae where males produce volatile pheromones to attract females (Sakai et al. 1984, Kuwahara et al. 1987, Schröder et al. 1994, Fettköther et al. 1995, Lacey et al. 2004, Hall et al. 2006, Ray et al. 2006). More recently, the male-produced pheromone of another member of the subfamily Lamiinae (to which *A. glabripennis* belongs), *Hedypathes betulinus*, was also identified (Vidal et al. 2008).

Attraction of males to male-produced pheromones has been reported for other species in the family (Lacey et al. 2004, 2007; Hanks et al. 2007) and may explain the response of *A. glabripennis* males to the blend at the 1-μg level, as well as the EAD response of both sexes to the pheromone found by Zhang et al. (2002). Cases of males responding to pheromones produced by other males of the same species are abundant in the literature and are not limited to cerambycids but seem to be common for wood-boring beetles (e.g., bark beetles, see Byers et al. 1988). In bark beetles, the ability of males to cue in on other males' pheromones helps them overcome tree defenses through aggregation (Raffa and Berryman 1983). In the case of *A. glabripennis*, there is no evidence of plant defenses limiting the beetles' development in preferred hosts; this species is known to recolonize the natal host or adjacent trees. *A. glabripennis* males' ability to respond behaviorally to other males' pheromone might help such "opportunistic" males increase their chances of finding females through competition and interception, as males are known to do in many other insect groups.

The response of *A. glabripennis* males to the male-produced blend varied with the amount of pheromone offered, as observed when comparing males' attraction to the blend with the control in the wind tunnel bioassays. In fact, males showed no significant response to the blend at the 0.05- and 0.5-μg levels, but they were attracted to the blend at the 1-μg level. At the 2-μg level, the response of the males to the blend was significantly lower than at the 1-μg level. This suggests that, at high beetle densities, a certain proportion of *A. glabripennis* males might prefer to avoid competition by staying far from pheromone sources and that they may use the concentration of this pheromone as a spacing/dispersal signal. Reported observations of *A. glabripennis* dispersal propose a higher flight propensity when the host is exhausted (Williams et al. 2004). In mark-release-recapture studies, beetle dispersal from release trees was positively associated with the abundance of beetles at the release tree (Bancroft and Smith 2005). We suggest that higher flight propensities could also be correlated with a higher number of males on the host; males have been observed to fly more than females (Keena and Major 2001). This can be translated into males moving to new hosts to avoid competition and to draw females to new healthy trees where resources are abundant.

The large difference between the response of males to the treatments, and especially the males' response to the control at the 2-μg level compared with controls at the three other treatment levels, may be an artifact of the bioassay itself. Because all four treatments were done at the same time for each chemical level, and the treatments were separated by only 1 m of space during the experiment, it is possible that some of the pheromones or its components from the other tunnels at the highest (2 μg) level were pulled into the fan and subsequently into the control wind tunnel, eliciting a response from the males in the control. Also, insects, and more particularly *A. glabripennis*, are generally less active on artificial substrates than on natural ones. We believe that this might have contributed to the overall low response of beetles in the laboratory bioassays, particularly in the walking wind tunnels, and may have contributed to the observed difference in beetle responses between the wind tunnel and the Y-tube olfactometer experiments. This difference might be an artifact of the differential speed of oxidation of alcohols versus aldehydes and caused by differences in distance traveled by the chemical and exposure time to ambient oxygen in both settings. In the wind tunnels, beetles had to walk for a much longer distance inside a glass tube than in the Y-tube; however, the fact that a significant number of them walked >1 m in the walking wind tunnel toward the odor source suggests that they are indeed attracted to it. Most of the beetles that did not show a response remained at their introduction point without moving for 20 min or circled around the inside of the first 0.5 m of the tunnel.

In addition to their smaller size and higher flight propensity, the greater attraction of males to plant volatiles compared with females provides additional support for their role in host location and later mate attraction to new resources. The higher attraction of males toward delta-3-carene and (*E*)-caryophyllene suggests that these two terpenes might play a role in males' host choice. Li et al. (2003) and Wickham and Teale (2009) found linalool and (*Z*)-3-hexen-1-ol to be attractive to both sexes of *A. glabripennis*. We have seen a similar trend in our Y-tube olfactometer results, although the attraction was not significant. Greenhouse results further suggest that these two plant volatiles might be used in lures for field trapping, especially with screen sleeve traps. (*Z*)-3-hexenyl acetate, a stress-induced volatile in *A. negundo* (Li et al. 2003), repelled males in the Y-tube olfactometer. Traps baited with this plant volatile did not catch any beetles. This correlates well with the known preference of *A. glabripennis* for slightly weakened but not highly stressed hosts (Hanks 1999).

Some of the volatiles we tested are commonly used lures for cerambycids and other wood-boring insects. For example, (−)-verbenone was found in earlier

studies to enhance attraction of *Monochamus galloprovincialis* Olivier (Coleoptera: Cerambycidae) to a blend of alpha-pinene, ipsenol, and methyl butanal (Ibeas et al. 2006). The monoterpenes (+)-alpha-pinene, (−)-verbenone, (−)-(E)-pinocarveol, and (+)-terpenen-4-ol were significantly attractive to *Hylotrupes bajulus* L. (Reddy et al. 2005). *A. glabripennis* adults, however, were not attracted to any of these commonly available lures, which excludes the possibility of their use for monitoring. We also tested alpha-pinene and ethanol, which were found to be attractive to eight species of Cerambycidae and some Buprestidae, Elateridae, and Curculionidae (Miller 2006) but were not attractive to *A. glabripennis* (unpublished data).

The greenhouse study provided needed information for further field studies. The equal trap catches in both Intercept panel traps and screen sleeve traps suggests that both trap types could be used for monitoring purposes. However, the significant interaction between lures and trap design showed that the quality of the lure used will affect success in trapping. Based on these results, we plan to test screen sleeve traps baited with (−)-linalool and Intercept traps baited with a combination of the male-produced pheromone blend and (Z)-3-hexen-1-ol in the field for confirmation. This is especially important given that the greenhouse studies contained inherent limitations; the traps were touching the screened walls of the greenhouse cages and the number of beetles released per cage was relatively low, both of which may be responsible for the small variation found between treatments.

This study was a first step toward developing an attractive lure for use in traps to monitor *A. glabripennis* populations and detect new introductions in the field. Results of these laboratory and greenhouse experiments will be used as a basis for further field trapping experiments in China.

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