WHERE’S THAT SMELL? TRAPPING AND SENSORY BIOLOGY OF THE ASIAN LONGHORNED BEETLE, Anoplophora glabripennis

A Thesis in
Entomology

by

Peter S. Meng

© 2014 Peter S. Meng

Submitted in Partial Fulfillment
of the Requirements
for the Degree of
Master of Science

May 2014
The thesis of Peter S. Meng was reviewed and approved* by the following:

Kelli Hoover  
Professor of Entomology  
Thesis Co-Advisor

Thomas C. Baker  
Distinguished Professor of Entomology  
Thesis Co-Advisor

Melody A. Keena  
Adjunct Faculty  
Research Entomologist, USDA Forest Service Northern Research Station

James H. Marden  
Professor of Biology

Gary W. Felton  
Professor of Entomology  
Head of the Department of Entomology Graduate Program

*Signatures are on file in the Graduate School
ABSTRACT

The Asian longhorned beetle, *Anoplophora glabripennis*, is an invasive, polyphagous, wood-boring pest that threatens to ravage forests across the U.S. and Europe if left uncontrolled. This thesis begins by discussing the results from a two summer field study in Harbin, China. In 2012, different release rates and ratios of the two-component male-produced pheromone released in combination with a 3-plant volatile blend were tested. In 2013, the effects of low and high release rates of 3 and 4-plant volatile blends with the male-produced pheromone on *A. glabripennis* was evaluated. Results indicated that 1 or 4 mg/day of male produced pheromone released in a 1:1 ratio with a high release rate of plant volatiles was most effective for capturing *A. glabripennis*. The 3-plant volatile mix without pheromone was most attractive to male beetles.

The second research chapter explores the sensory biology and neurophysiology of *A. glabripennis*. The scanning electron microscope was used to describe the morphological landscape of sensilla on the antennae, labial and maxillary palps. Lastly, electroantennogram responses from male and female beetles to the two-component male produced pheromone and the plant volatile, (-)-linalool are presented. Male beetles had significantly more basiconic sensilla compared to females on some antennal segments. Basiconic sensilla are typically broadly tuned to plant volatiles. This sexual dimorphism may explain why male beetles are highly attracted to traps baited with plant volatiles alone. Female beetles had significantly more chetiform-1 sensilla compared to males on some antennal segments. Chetiform sensilla are mechanoreceptive. This sexual dimorphism suggests females may have greater proprioceptive abilities compared to males. Female *A. glabripennis* are observed antennating substrates in search of nutrients and oviposition sites and males rub the female antennae while mating to make them receptive. These behaviors may explain the females’ need for greater proprioceptive senses.
Female responses to the two-component male-produced pheromone have not been published. In the latter portion of the second research chapter, male and female electroantennogram responses to both components of the male-produced pheromone are presented. Antennae from both sexes responded to both components of the male-produced pheromone in a dosage-dependent manner. Repeated stimulation with pheromone or plant volatile components caused desensitization.

Together, the results from the neurophysiological, morphological and field trapping studies can be combined to advance our understanding of *A. glabripennis* behavior. Additionally, the descriptions of the sensillar landscape on *A. glabripennis* antennae, labial and maxillary palps lay the foundation for future neurophysiological studies.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>List</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xix</td>
</tr>
</tbody>
</table>

Chapter 1  Introduction .................................................................................. 1

  Host Range and Damage ................................................................................. 2
  Life Cycle ........................................................................................................ 3
  Operational Detection and Eradication Methods for *A. glabripennis* .......... 3
  *A. glabripennis* and Cerambycid Pheromones ............................................. 4
  *A. glabripennis* Host Plant Kairomones ...................................................... 5
  *A. glabripennis* Traps .................................................................................. 6
  *A. glabripennis* Antennae and Mouthparts .................................................. 7
  Conclusions ..................................................................................................... 8
  Objectives/Null Hypotheses .......................................................................... 9
    Approaches ..................................................................................................... 9

Chapter 2  Effects of pheromone and plant volatile release rates and ratios on trapping

  *Anoplophora glabripennis* in China .............................................................. 11

  Abstract ......................................................................................................... 11
  摘要 ............................................................................................................... 12
  Introduction .................................................................................................... 13
Chapter 3  Sensory Biology and Neurophysiology of Anoplophora glabripennis .......... 45

Abstract ................................................................. 45
Introduction ............................................................... 45
Methods ................................................................. 47
Results ................................................................. 50
Discussion .............................................................. 55
Acknowledgements ................................................... 59
Tables ................................................................. 60
Figures ................................................................. 64

Chapter 4  Conclusions .................................................... 94

Appendix ...................................................................... 97

Field Site Maps of Harbin, China .................................... 97
Still Frames of Beetle Flying into Flight Intercept Trap ............ 100

References .................................................................... 104
LIST OF FIGURES

Figure 2-1: Cumulative number of beetles trapped by each lure treatment by sex in 2012.

Ten of each lure type were hung in 10 different plots for 27 days. An asterisk over the bar indicates treatments that were significant linear predictors of the number of female beetles trapped from the GLM analysis. Different letters over a bar indicate statistically significant differences in female trap catches among treatments based on orthogonal contrasts at P < 0.05. MP = male-produced pheromone, PV = 3 plant volatiles: (−)- linalool, trans-caryophyllene and (Z)-3-hexen-1-ol. ........................................... 33

Figure 2-2: Number of beetles caught by sex during each trap check in 2012. Dates correspond to the duration between trap checks .................................................... 34

Figure 2-3: Cumulative number of beetles trapped by each lure treatment and company by sex in 2013. Ten of each lure type were hung in 10 different plots for 35 days. Asterisks indicate treatments that were a significant linear predictor of beetles trapped from the GLM analysis. Different lowercase letters over the bars indicate statistically significant differences among treatments (both manufacturers combined) for females compared to the control, at P < 0.05 using orthogonal contrast tests. Table indicates how many beetles of each sex were caught using lures produced by each manufacturer. 3 PV = 3 plant volatile mix: (−)-linalool, trans-caryophyllene and (Z)-3-hexen-1-ol, 4 PV = 4 plant volatile mix: (−)- linalool, trans-caryophyllene, (Z)-3-hexen-1-ol and linalool oxide. MP = male produced pheromone, 1X = low plant volatile release rates, 10X = high plant volatile release rates. Synergy = all emitters were manufactured by Synergy. ChemTica = all emitters were manufactured by ChemTica. ........................................................................................................................................ 35
Figure 2-4: Number of beetles caught during each trap check in 2013. Dates correspond to the duration between trap checks. ................................................................. 37

Figure 2-5: Mean (± SEM) release rates of pheromone and plant volatile lure components in mg/day during the trapping study in 2012 measured gravimetrically. Some standard errors are too small to be visible. Horizontal dashed lines indicate desired plant volatile release rates of 9, 8, 1 and 1 mg/day for (-)-linalool, trans-caryophyllene, (Z)-3-hexen-1-ol and pheromone components, respectively. ...................... 38

Figure 2-6: Mean (± SEM) pheromone aldehyde and alcohol lure release rates given in mg/day during the 2013 study measured gravimetrically. Heavy rain accumulation caused erroneous weight measurements during days 24-27 of the experiment. Some standard errors are too small to be visible. Horizontal dashed line indicates desired pheromone release rates of 2 mg/day. Ald = pheromone aldehyde, Alc = pheromone alcohol. ................................................................. 40

Figure 2-7: Mean (± SEM) plant volatile lure release rates given in mg/day during the 2013 study measured gravimetrically. Horizontal lines indicate desired plant volatile release rates of 9, 8, 1 and 1 mg/day for (-)-linalool, trans-caryophyllene, linalool oxide and (Z)-3-hexen-1-ol respectively. ............................................................... 42

Figure 2-8: Daily field site temperatures in 2012 and 2013. In 2012, Temperatures were obtained from the National Climatic Data Center. In 2013, temperatures were collected in the field using a HOBO H8 data logger. Day 1 corresponds to 7/23 and 7/21 in 2012 and 2013, respectively. ................................................................. 44
Figure 3-1: SEM images of flattened white scales on various segments of male *A. glabripennis* antennae. Under natural light, these appear as decorative bands encircling the proximal 1/3 of each antennal segment on both males and females. Dense clumps of morphologically similar, if not identical, scales form decorative spots on *A. glabripennis* male and female elytra (see Fig. 3-2). **A)** Male *A. glabripennis* antenna under natural light with visible black–white banding pattern. Scale bar = 2 mm. **B)** A group of scales (arrows) on segment 2. Note their typical swollen mid-section (arrow) and narrowed proximal and distal ends. Scale bar = 100 µm. **C)** Higher magnification of a single scale on Segment 5. Note the swollen, ovoid mid-region and the pointed tip. Scale bar = 10 µm. **D)** Still higher magnification of a scale’s tip. The scale appears to be hollow (note what appears to be a hole), and this may contribute to the luminous, bright-white appearance of these scales under natural light. Scale bar = 1 µm. Panel D image taken by Kathleen Shields.

Figure 3-2: Cluster of white, broadly flattened scales on female elytra (arrows). These scales bear similarities to the scales located on the antennae. On older females, the scales are sometimes missing. Scale bar = 100 µm. Image taken by Kathleen Shields...

Figure 3-3: Böhm sensilla on the pedicel of male and female *A. glabripennis*. **A)** Böhm sensilla (arrows) on the female pedicel. As the pedicel rotates inside of the scape, sensilla come into contact with the side of the scape. Ped = Pedicel, Sca = Scape. Scale bar = 50 µm. **B)** Shorter Böhm sensilla on the male pedicel (arrows). Fine sculpting of the pedicel cuticle is visible on the surface. Scale bar = 50 µm. **C)**
Close-up of Böhm sensilla on a female pedicel. Scale bar = 10 µm. Images taken by Kathleen Shields..........................67

Figure 3-4: Distribution of Böhm sensilla on antennae ± SD There are significantly more Böhm bristles on female *A. glabripennis* compared to males. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females). ..........................................................69

Figure 3-5: Long chetiform-1 sensilla from male and female *A. glabripennis*. These sensilla were primarily found toward the distal end of antennal segments. A) Distal end of female Segment 3 showing chetiform-1 sensilla (arrows). Scale bar = 100 µm. B) Chetiform-1 sensilla on in the middle of male antennal Segment 2 (arrow). Sculpted scales can also be seen across the entire segment. Scale bar = 100 µm. C) Chetiform-1 sensilla on the male scape (arrows). Ped. = Pedicel Sca. = Scape. Scale bar = 100 µm. D) Close-up of chetiform-1 sensillium on female Segment 4 showing longitudinal striations. Scale bar = 10 µm. Panel A and C images taken by Kathleen Shields. ..........................................................71

Figure 3-6: Distribution of chetiform sensilla on antennae ± SD. There are significantly more chetiform sensilla on Segments 3 - 5 of females than males. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females). ..........................................................72

Figure 3-7: Long basiconic sensilla on male and female *A. glabripennis*. These sensilla were observed on the midsegments of flagellomeres. A) Long basiconic sensilla on the tip of male antennal segment 11 (arrows). Sculpted scales can also be seen. Scale
bar = 50 µm. B) Long basiconic on female antennal Segment 3 (arrow) between several sculpted scales. Scale bar = 50 µm. C) Long basiconic on male antennal Segment 6 projecting away from cuticle surface. Grooves are possible slit-pores on long basiconic sensillum. Scale bar = 50 µm.

Figure 3-8: Distribution of long basiconic sensilla on antennae ± SD. There are significantly more long basiconic sensilla on the scape and Segment 7 of male beetles compared to females. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females).

Figure 3-9: Trichoid and short basiconic sensilla on *A. glabripennis* antennae. These sensilla may have an olfactory function. A) Trichoid sensilla (arrows) interspersed between sculpted scales on male antennal Segment 6. A long basiconic sensilla can also be seen. Scale bar = 100 µm. B) Sharp tipped trichoid (Tr) and blunt short basiconic sensilla (Ba) interspersed between sculpted scales on female antennal Segment 9. Trichoid sensilla have raised sockets (arrows) Scale bar = 10 µm. C) Cluster of short basiconic and trichoid sensilla (dotted black line) in the middle of female antennal Segment 11. Scale bar = 100 µm. D) Hidden pocket of short basiconic sensilla (arrows) at proximal end of a male antennal segment. Scale bar = 100 µm. Panel A, C and D images taken by Kathleen Shields.

Figure 3-10: Distribution of trichoid sensilla on antennae ± SD. Trichoid sensilla are present primarily on Segments 4 – 11. No sexual dimorphism in distribution or abundance was observed.
Figure 3-11: Distribution of short basiconic sensilla on antennae ± SD. Short basiconic sensilla are present primarily on Segments 4 – 11. Male Segments 5, 8 and 11 have significantly more short basiconic sensilla compared to the corresponding female segments. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females).

Figure 3-12: Campaniform sensilla. Campaniform sensilla are circular, raised, structures recessed into Segment 11 of both sexes. These pictures are from female beetles. A) Campaniform sensilla (arrows) hidden between sculpted scales. Scale bar = 10 µm. B) Close-up of campaniform sensilla. The inner and outer diameters can be seen. Scale bar = 5 µm. Images taken by Kathleen Shields.

Figure 3-13: Distribution of campaniform sensilla on antennae ± SD. Basiconic sensilla are primarily found on the tip of antennae. No sexual dimorphism in distribution was observed.

Figure 3-14: Labial and maxillary palps of *A. glabripennis*. Arrows denote Segment 2 and 3 of labial and maxillary palps, respectively on panels A and B. A) Posterior female labial palp. Scale bar = 1 mm. B) Posterior male maxillary palp. Scale bar = 1 mm. C) Close-up of female labial palp basic sensilla (arrows) inside the cone on Segment 2. Raised sockets are visible. Scale bar = 50 µm.

Figure 3-15: Sculpted, flattened scales on *A. glabripennis* maxillary palps. These scales closely resemble the sculpted scales on the antennae. A) Segment 3 of the female maxillary palp mostly covered with sculpted scales (arrows). Small chetiform
sensilla can be seen orthogonal to the cuticle surface. Scale bar = 50 µm. B) Close-up of sculpted scales on the Segment 3 of a male maxillary palp. Scale bar = 10 µm. ... 84

Figure 3-16: Short mechanoreceptive chetiform-2 sensilla are abundant on Segments 2 and 3 of the labial and maxillary palps, respectively. A) Chetiform-2 (Ch 2), chetiform-3 (Ch 3) and coeloconic sensilla (arrows) are visible. Scale bar = 100 µm. B) Short chetiform-2 sensilla on Segment 2 of a female labial palp. Scale bar = 10 µm. C) Close-up of chetiform-2 sensilla on the male maxillary palp Segment 2. Longitudinal grooves are clearly visible. Scale bar = 1 µm. ................................. 86

Figure 3-17: Distribution chetiform-2 sensilla on A. glabripennis mouthparts ± SD. No sexual dimorphism in abundance and distribution was observed. L = Labial palp, M = Maxillary palp. Segments 2 and 3 are the most distal segments of the labial and maxillary palps, respectively................................................................. 87

Figure 3-18: Long chetiform-3 sensilla are concentrated on the proximal palp segments of A. glabripennis. A) Long chetiform-3 sensilla on Segment 2 of a female labial palp. Longitudinal grooves are clearly visible. Scale bar = 1 µm. B) Long chetiform-3 sensilla on posterior of male maxillary palp Segment 2. Scale bar = 50 µm. ................. 88

Figure 3-19: Distribution of chetiform-3 sensilla on the labial and maxillary palps ± SD. Significantly more chetiform-3 sensilla were found on the Second labial palp segment of females compared to males. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females). L = Labial palp, M = Maxillary palp. Segments 2 and 3 are the most distal segments of the labial and maxillary palps, respectively. ................................. 89
Figure 3-20: Coeloconic sensilla were observed on the most distal segments of *A. glabripennis* mouthparts. Above photos are from Segment 3 of a female maxillary palp. A) Close-up of coeloconic sensilla. Distribution can be seen in Fig. 3-16A (arrows) Scale bar = 5 µm. .................................................................................................................................................90

Figure 3-21: Distribution of coeloconic sensilla on *A. glabripennis* mouthparts ± SD. No sexual dimorphism in distribution was observed. Tip is most distal mouthpart segment. ..........................................................................................................................................................91

Figure 3-22: Distribution of basiconic-2 sensilla on *A. glabripennis* mouthparts ± SD. No sexual dimorphism in distribution was observed. The tip is the most distal mouthpart segment. ..........................................................................................................................................................92

Figure 3-23: Female EAG response after exposure to 100 µg of aldehyde pheromone. Female antennae exhibited a clear depolarization immediately after stimulation by individual male-produced pheromone components. Red bars indicate when the pheromone was puffed over the antenna........................................................................................................................................93

Figure A-1: Map of trap locations and plots in Harbin, China in 2012. Numbers denote plot number. Red dots indicate trap locations..............................................................................................................................................98

Figure A-2: Map of trap locations in Harbin, China in 2013. Different colored dots indicate lures from different manufacturers. Black (data not presented) = combination lures in which all plant volatiles and the alcohol pheromone component were manufactured by ChemTica. Aldehyde pheromone lures were manufactured by Synergy and placed in a pill bag. Pink = lures manufactured by Synergy. Yellow =
lures manufactured by ChemTica. ChemTica and Synergy trap lines were rotated in an alternating pattern through the forest every 3 days. Numbers denote plot number. ... 99

Figure A-3: Still frames of a female A. glabripennis flying into a flight intercept panel trap in Harbin, China. The beetle spends several minutes searching for a direct, walkable path to the trap at the end of the branch before conceding and flying into the trap. Blue boxes indicate the beetle’s location. ........................................................................................................ 103
LIST OF TABLES

Table 2-1: Lure sets used in field experiments in China in 2012 and 2013. 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.................................................................29

Table 2-2: Percentage of traps that were hung in host trees and percentage of trees with insect signs on the trap tree and the nearest 4 host trees to the trap in 2012 and 2013. 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).............30

Table 2-3: Lures used in field experiments with desired and actual release rates mean (± SEM) measured gravimetrically in mg/day. No extra ChemTica aldehyde lures were available to be weighed in 2013 in China. *In 2013, two ChemTica alcohol lures were used in each trap to achieve a desired release rate of 2 mg/day..........................31

Table 2-4: Mean ± SEM (mg/day) 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. Numbers following company names indicate if lures were produced in 2012 or 2013. ChemTica alcohol pheromone lures were used for the 2012 and 2013 field seasons.................................................................32
Table 3-1: Antennal segment lengths (mm) and diameters (mm) ± SD. The lengths of male antennal Segments 3 - 11 are significantly longer than the corresponding female antennal segments. The male pedicel, Segments 4, 7 and 11 are significantly wider in males compared to females.

Table 3-2: Maxillary palp segment lengths (mm) and diameters (mm) ± SD. The tip of female maxillary palp is significantly longer and wider at the center compared to males \((t = 4.85, df = 9.35, p = 0.001)\) and \((t = 3.76, df = 8.27, p = 0.005)\) respectively. The second Segment of the female labial palp is significantly shorter and narrower at both ends compared to males \((t = 32.5, df = 5, p < 0.001), (t = 5.48, df = 6.3, p = 0.001)\) and \((t = 2.3, df = 9.24, p = 0.049)\) respectively. Segment 3 is significantly longer and wider on the distal end on females compared to males \((t = -3.4, df = 9.0, p = 0.007)\) and \((t = 4.66, df = 7.00, p = 0.002)\), respectively. Segment 3 is the most distal maxillary palp segment.

Table 3-3: Maxillary palp segment lengths (mm) and diameters (mm) ± SD. The tip of the female labial palp is significantly shorter and narrower at the distal end compared to males \((t = 6.6, df = 7.87, p < 0.001)\) and \((t = 8.1, df = 9.4, p < 0.001)\) respectively. The first Segment of the female labial palp is significantly shorter and narrower at the proximal end compared to males \((t = 46.0, df = 5, p < 0.000)\) and \((t = 2.7, df = 9.8, p = 0.029)\), respectively. Segment 2 is the most distal labial palp segment.

Table 3-4: Ratio of EAG responses standardized to 10 µg of linalool ± SD. EAG responses to pheromone components appear to be dosage dependent. Female
antennal response ratios are were greater, but not statistically different compared to males.
ACKNOWLEDGEMENTS

A big thanks goes out to Penn State University and the entire Department of Entomology for the assistance and support they have provided over the last 3 years. The research herein would not have been possible without the guidance provided by the faculty, post-docs, technicians and colleagues within and outside of the department.

The work in China during the summers of 2012 and 2013 would not have been possible without assistance from Professor Yan Shanchun and members of her research group. Zhou Yantao provided valuable field assistance throughout the field season.

Funding for these research projects were made possible by grants from the Alphawood Foundation, the Horticultural Research Institute, USDA Forest Service, and State and Private Forestry Northeastern Area Technology & Methods Development Grant 10-CA-11420004-316.

I would especially like to thank my major professor and co-advisor, Dr. Kelli Hoover, for her support, guidance and mentoring over the 3 years. I would also like to thank my co-advisor, Dr. Thomas Baker, for his mentoring and guidance with regards to the neurophysiological portion of my research. Finally, I would like to thank the other members of my Master’s committee: Dr. Melody Keena of the US Forest Service and Dr. James Marden from the Biology Department.
Chapter 1

Introduction

Insects utilize an array of semiochemicals to locate mates, identify food sources or signal the presence of threats (Morgan 2009, Vandermoten et al. 2012). Humans have exploited insect pheromones in agriculture to disrupt mating, attract predators and monitor pest populations (Butenandt 1959, Aldrich et al. 1984). Pheromones can also be used to detect pests in low-density situations (Gage et al. 1990). There has been a growing interest in developing low cost, pheromone-baited traps as a detection tool for cerambycid species (Hanks et al. 2012). The Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) 1853 (Coleoptera: Cerambycidae), is an invasive, polyphagous wood-boring insect that is capable of destroying 30.3\% of the urban trees in the U.S. at an economic loss of $669 billion (Nowak et al. 2001).

Early, accurate detection of this invasive pest is crucial for detecting infestations before they become unmanageable.

*A. glabripennis* was first discovered in the U.S. in Brooklyn, New York during August 1996 (Haack et al. 1997, Cavey et al. 1998). Following this initial discovery, it has also been found in the greater New York City area, New Jersey, Massachusetts, Illinois and Ohio (Poland et al. 1998, Haack 2006, Haack et al. 2007, Dodds and Orwig 2011). The most recent infestation was found in Babylon Township (USDA 2013b). *A. glabripennis* has been declared eradicated from Islip, NY; Manhattan, NY; Staten Island, NY; Jersey City, NJ; and Chicago, IL.

Infestations have also been found in other countries where eradication has been more elusive including Austria, Canada, Belgium, Denmark, France, Germany, Great Britain, Italy, Japan, the Netherlands, and Switzerland (Makihara 2002, Tomiczek 2003, Takahashi and Ito 2005, Hérard et al. 2006, Chapin and Chauvel 2009, IPPC 2009, Scheel 2009, Williams et al. 2010, Morall
2011, Bräsicke and Hommes 2013). Development of an effective, pheromone baited trapping system for *A. glabripennis* will allow for the rapid detection of this invasive pest at ports of entry or high-risk areas and reduce the amount of time the insect has to disperse and establish undetected.

**Host Range and Damage**

*A. glabripennis* is known to attack at least 48 different tree species in the wild with preference for those in the genera *Acer, Fraxinus, Populus, Salix* and *Ulmus* (MacLeod et al. 2002, Haack et al. 2007, Hu et al. 2009, Wang et al. 2009). In China, it is a major pest in monoculture *Populus* plantations. In the U.S., *A. glabripennis* has also been documented to infest tree species in the genera *Aesculus, Hibiscus and Betula* (Haack et al. 1996), but strongly prefers maples including *Acer negundo* (boxelder), *Acer platanoides* (Norway maple), *Acer saccharinum* (silver maple), *Acer saccharum* (sugar maple) and *Acer rubrum* (red maple) (Haack et al. 2007). The most recent new host record was recorded in Worcester, MA as Katsura in the genus *Cercidiphyllum* (USDA-APHIS-PPQ 2010).

Signs of *A. glabripennis* infestation include exit holes, oozing sap, frass, feeding damage and oviposition pits (Haack et al. 2010). *A. glabripennis* creates galleries in the sapwood and heartwood. Severe infestations compromise the structural integrity of a tree. Three year old *Populus euramericana* trees infested with *A. glabripennis* for 3 years had a 22-49% and 5-25% decrease in diameter and height, respectively (Gao et al. 1993). Degradation in wood quality as a result of infestation results in a 46% loss of economic value. A single *A. glabripennis* larva can consume 1000 cubic cm of wood (Yan and Qin 1992).
Life Cycle

*A. glabripennis* begins its life after a female chews an oviposition pit in the bark and lays an egg under the bark. Mortality is highest in the egg and first instar as these life stages occur close to the plant surface and leave *A. glabripennis* vulnerable to extreme temperatures and natural enemies (Tang et al. 1996). Larvae require 1-2 years to develop before reaching a critical weight and pupating. In colder, northern regions of China, *A. glabripennis* is more likely to take 2 years to develop (Hua et al. 1992). Adult beetles take 4-7 days to begin boring out of trees after eclosion and another 4-5 days to emerge from a tree at early summer temperatures (Sánchez and Keena 2013). In Ningxia, China, peak emergence occurs from late June to early July, with a smaller peak in mid-August (Zhang and Xu 1991). Several researchers are currently developing phenology models to predict *A. glabripennis* emergence in the U.S. Understanding *A. glabripennis*’ phenology is critical for proper timing of trap deployment and timing of infested tree removals.

Operational Detection and Eradication Methods for *A. glabripennis*

Several methods have been developed to detect and delimit *A. glabripennis* populations. The first, and most commonly used method utilizes specialized ground surveyors equipped with binoculars to locate signs of *A. glabripennis* damage on a tree. According to USDA environmental assessment reports, accuracy of detection by ground surveyors is about 30% as determined by quality assurance checks (USDA-APHIS 2013a). Tree climbers can also detect signs of *A. glabripennis* infestation with higher rates of accuracy, ranging from 60% to 75% (USDA-APHIS 2013a), but this method is more costly and slower than ground surveys (Hu et al.
Hydraulic lifts are also used to survey for evidence of *A. glabripennis* infestation and are more effective than ground surveys alone.

The only effective method to eradicate *A. glabripennis* is to completely remove and chip infested trees and grind stumps (Wang et al. 2000). From 1998 to 2006, the costs to detect, remove and prophylactically treat host trees with imidacloprid was $249 million dollars in the U.S. alone (GAO 2006). Full host removals of infested and healthy trees may prevent the spread of *A. glabripennis* but are controversial with the public. Early detection of invasive pests both increases the likelihood of eradication and decreases management costs (Mehta et al. 2007). The *A. glabripennis* infestation in Worcester, MA was not detected until 8-10 years after the beetle’s introduction (USDA-APHIS 2009). The quarantined area now spans 110 square miles in 6 jurisdictions. Thus, there is a strong need for developing sensitive *A. glabripennis* detection methods that can delimit the extent of the beetle population before it becomes unmanageable.

### *A. glabripennis* and Cerambycid Pheromones

Several pheromones have been identified that are produced by *A. glabripennis*, suggesting that chemical communication in this species is complex. A two component volatile male-produced pheromone (MP) consisting of 4-((n-heptyloxy)butanal and 4-((n-heptyloxy)butan-1-ol is produced in a 1:1 ratio (Zhang et al. 2002). Male antennae responded strongly to both constituents of the male-produced pheromone but female antennal responses were not reported. In bioassays, both sexes were attracted to the pheromone, suggesting that it may be an aggregation pheromone (Zhang et al. 2002). In follow-up lab bioassays, the male-produced pheromone was not highly attractive to male or female *A. glabripennis* (Nehme et al. 2009), but it was attractive to virgin females in combination with plant volatiles in the field in China (Nehme et al. 2010).

Pheromone production by some cerambycids has been predictable from the observation of pores
on the male but not female pronotum (Ray et al. 2006). A. glabripennis and some other cerambycids do not appear to have these pores, yet they still produce a volatile sex pheromone.

Female A. glabripennis produce a 5-component contact sex pheromone consisting of the cuticular hydrocarbons (Z)-9-tricosene, (Z)-9-pentacosene, (Z)-7-pentacosene, (Z)-9-heptacosene, and (Z)-7-heptacosene, which play a role in mate recognition (Zhang et al. 2003). When males contact this pheromone, copulatory behavior is initiated. In the lab, male A. glabripennis will attempt to copulate with Eppendorf™ tubes treated with this pheromone. Females of a closely related cerambycid, Anoplophora chinensis, produce a different contact sex pheromone composed of 8 hydrocarbons, 4 ketones and 3 lactones (Yasui 2009).

A. glabripennis females also produce a four-component trail pheromone consisting of 2-methyldocosane and (Z)-9-tricosene (major components), as well as (Z)-9-pentacosene and (Z)-7-pentacosene (minor components) (Hoover et al. 2014). Males, regardless of mating status, are highly attracted to the full trail pheromone blend, while virgin females are repelled. As female beetles become sexually mature, they produce 35- to 66- fold more of the major components that may be used by males to follow the females on the host tree. Few cerambycid trail pheromones have been described with the exception of the grey-black citrus longhorned beetle, Nadezhdiiellla cantori (Wang et al. 2002).

**A. glabripennis Host Plant Kairomones**

A number of plant volatiles have been tested for attractiveness to A. glabripennis. In a field trapping study in China, A. glabripennis was significantly more attracted to traps containing (Z)-3-hexen-1-ol than traps individually bated with 1-butanol, trans-2-hexen-1-al, 1-pentanol, 2-pentanol, trans-2-hexen-1-ol or hexanol (Li et al. 2003). A mixture of 1-butanol, 1-pentanol and 2-pentanol acted synergistically and was more attractive to beetles than any of the individually
released compounds. A similar synergistic effect was observed with the same compounds in a separate Chinese field trapping study (Jin et al. 2004).

More recently, Y-tube olfactometer bioassays showed that males were more attracted to plant kairomones compared to females (Nehme et al. 2009). Males were highly attracted to δ-3-carene and (E)-caryophyllene, but repelled by (Z)-3-hexenyl acetate. Females were not significantly attracted to any plant kairomones. Field and laboratory studies show that *A. glabripennis* may also be attracted to linalool oxide without the male-produced pheromone (Nehme et al. 2009, Wickham et al. 2012). In field trapping studies, traps baited with the male-produced pheromone caught significantly more females compared to control traps (Nehme et al. 2010). Addition of a plant kairomone mixture containing (-)-linalool, (Z)-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene and *trans*-pinocarveol to the male-produced pheromone significantly increased the number of beetles caught. This finding is consistent with several cerambycid trapping studies that demonstrated a synergistic effect when pheromone and host plant kairomones are released together, resulting in a larger number of beetles being captured compared to traps baited with pheromone or plant kairomones alone (Pajares et al. 2004, Reddy et al. 2005). For example, *Rhynchophorus ferrugineus*, the red palm weevil, can be captured in traps baited with its pheromone, ferrugineol and the plant volatile ethyl acetate, which is released at very high rates (57 to 350 mg/day) (Vacas et al. 2013).

*A. glabripennis* Traps

Pheromone- and plant kairomone-baited flight intercept panel traps have been used in the field in China to evaluate their ability to trap *A. glabripennis* and provide location information for eradication efforts (Nehme et al. 2010). Flight intercept panel traps and screen sleeve traps have been tested in the field but there are significant interactions between treatment and trap design.
(Nehme et al. 2009). Dark silhouettes also appear important by providing visual cues necessary for *A. glabripennis* to locate traps (Wang et al. 2002).

Over the past 5 years, our group has conducted *A. glabripennis* trapping studies in New York and Massachusetts. Since *A. glabripennis* is being eradicated in the U.S., it has not been possible to obtain large, statistically significant sample sizes in domestic field studies to determine the most attractive pheromone and plant volatile blend to capture this insect. Additionally, *A. glabripennis* clutch sizes are significantly smaller compared to most Lepidoptera and vary significantly by host plant species, which prevents large captures as seen in moth studies (Smith et al. 2002). While laboratory bioassays allow for screens of plant chemicals attractive to *A. glabripennis*, these compounds must be field-tested because lab bioassays with this insect produce highly variable results (personal observation).

Unlike lepidopterans, high pheromone release rates must be used to capture cerambycids. For example, the female eastern spruce budworm, *Choristoneura fumiferana*, releases 60 ± 50 ng (±S.D.) of pheromone per night (Morse et al. 1982). Females of the oriental fruit moth, *Grapholita molesta*, release a maximum of 25.3 ng/hour of pheromone (Lacey and Sanders 1992). In addition, moth antennal receptors can develop sensory adaptation and habituation to pheromone components at fairly low concentrations. Traps baited with 100 µg of pheromone caught more moths over a 4-week period compared to traps baited with 300 µg of pheromone (Kovanci et al. 2006). In contrast, the male cerambycid, *Monochamus galloprovincialis*, releases 1.3 µg/hour of pheromone, which is 50-fold more than *G. molesta* (Pajares et al. 2010).

**A. glabripennis Antennae and Mouthparts**

Insects utilize an extensive array of chemoreceptive sensilla to detect olfactory cues in the environment. *A. glabripennis* is frequently seen tasting the substrate it walks on or
antennating other beetles. For example, male antennal contact with the female contact pheromone is necessary to induce copulatory behavior (Zhang et al. 2003). Signals are transduced from sensilla on the antennae to the antennal lobes in the brain and undergo multiple rounds of processing before conversion to behavioral outputs. Cerambycids are notable for their unusually long antennae, although it is unclear if this trait enhances their chemoreception, mechanoreception or both.

*A. glabripennis* has 11 antennal segments, which consist of the scape, pedicel and 9 flagellomeres. To date, *A. glabripennis* antennal sensilla have been described but not quantified (Yan et al. 2010) and there is no published information on the morphology or sensilla distribution on their mouthparts. Describing *A. glabripennis* mouthpart and antennal morphology and determining where olfactory receptors are located on these structures will provide insight that can be integrated into our understanding of chemical communication in this economically important species.

**Conclusions**

Identification of attractants to monitor *A. glabripennis* and understanding the morphology of sensory structures will increase the knowledge regarding the insect’s behavior and potentially aid in detecting infestations. Additionally, knowledge on the sensory biology and chemical attractants applicable to *A. glabripennis* may lead to clues about chemical communication in *A. chinensis*, a closely related cerambycid that has an even broader host range. Early, accurate detection of invasive species will improve our ability to manage and ultimately eradicate these pests.
Objectives/Null Hypotheses

1. Identify the optimal ratio of the two-component male-produced pheromone.
   \( H_0: \) Different ratios of the two-component male-produced pheromone will be equally attractive to \( A. \) glabripennis.

2. Determine the optimal release rate of the two-component male-produced pheromone for trapping beetles.
   \( H_0: \) All male-produced pheromone release rates will be equally attractive to \( A. \) glabripennis.

3. Determine if a low or high release rate of plant kairomones is more effective in capturing \( A. \) glabripennis.
   \( H_0: \) \( A. \) glabripennis will be equally attracted to low and high release rates of plant kairomones.

4. Quantify and describe \( A. \) glabripennis sensilla on male and female antennae.
   \( H_0: \) There will not be sexual dimorphism in the distribution of sensilla on the antennae.

5. Quantify and describe \( A. \) glabripennis sensilla on the mouthparts.
   \( H_0: \) There will not be sexual dimorphism in the distribution of sensilla on the mouthparts.

Approaches

**Objective 1.** To determine the optimal ratio of the two-component male produced pheromone, a field trapping study was performed in China with different ratios of the alcohol and aldehyde components. Ratios of 1:1, 1:4, 1:8, 4:1 and 8:1 of the alcohol to the aldehyde were tested. All treatments contained a 3-component plant kairomone blend with (-)-linalool, \( trans \)-caryophyllene
and (Z)-3-hexen-1-ol in a 9:8:1 ratio. The treatment that captures the most *A. glabripennis* should have the optimal ratio of alcohol to aldehyde.

**Objective 2.** To determine the optimal release rate of the male-produced pheromone, a field trapping study was performed in China with different release rates of the male-produced pheromone at a 1:1 ratio. In 2012, release rates of 1 mg/day, 4 mg/day and 8 mg/day were tested. The optimal pheromone release rate should capture the most *A. glabripennis*.

**Objective 3.** To determine if high or low release rates of plant kairomones are more attractive to *A. glabripennis*, blends of 3 or 4 plant kairomones in conjunction with a 2 mg/day release rate of the male-produced pheromone with the components at a 1:1 ratio were tested in a field trapping study in China. The plant kairomones (-)-linalool, *trans*-caryophyllene and (Z)-3-hexen-1-ol were released at a low rate of 9 mg/day, 8 mg/day and 1 mg/day (1X), respectively, or a 10-fold higher rate of 90 mg/day, 80 mg/day and 10 mg/day (10X). The 4-component plant kairomone blend contained the previous 3 components as described plus linalool oxide that was released at 1 mg/day or 10 mg/day, depending on if a low or high release rate was desired.

**Objectives 4 and 5.** Scanning electron and light microscopy was performed in order to quantify the sensilla on *A. glabripennis* antennae and mouthparts. Some scanning electron micrographs and sensilla counts have been obtained from Kathleen Shields, a retired USDA Forest Service entomologist. *A. glabripennis* sensory organs will be cleaned in a 10% Triton X-100 solution, dried, degassed and sputter coated with a gold-palladium alloy prior to imaging with the scanning electron microscope. A light microscope will be used to quantify large, fragile sensilla that project from the mouthpart cuticle.
Chapter 2

Effects of pheromone and plant volatile release rates and ratios on trapping *Anoplophora glabripennis* in China

Abstract

Native to China and Korea, the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), is a polyphagous wood-boring pest that has established in several locations in the Northeastern U.S. and Europe. Development of a trapping system for *A. glabripennis* would greatly benefit eradication/management programs in the introduced and native ranges. In the summers of 2012 and 2013, a total of 160 flight intercept panel traps were deployed in Harbin, China and 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 mg/day 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, or no lure controls; 87% of trapped females were virgins. Males were caught primarily in traps baited with plant volatiles only. In 2013, 10-fold higher release rates of these plant volatiles were tested, in combination with the male-produced pheromone designed to release at 2 mg/day in a 1:1 ratio, and included linalool oxide as an additional plant volatile component in some treatments. Significantly more females were trapped using the pheromone with the higher plant volatile release rates compared to the plant volatile only and no lure controls. Our findings show that the male-produced pheromone in combination with plant volatiles can be used to detect *A. glabripennis*, which will facilitate survey efforts by eradication programs.

**Keywords.** *Anoplophora glabripennis*, male-produced pheromone, plant volatiles, kairomones, monitoring traps, detection, invasive species, lure release rates.
摘要

原产于中国和韩国的亚洲长角天牛-光肩星天牛，是一种杂食性的蛀干害虫，目前分布在欧洲和美国东部多个地区。针对光肩星天牛开发一种新型的诱捕器，对光肩星天牛的防控和根除有着重要意义。2012年和2013年夏季，在哈尔滨一共挂了160个飞行拦截挡板诱捕器，共诱捕到65只光肩星天牛。2012年，我们使用以雄性天牛释放的2种信息化合物（4-N-庚氧基-丁醛和4-N-庚氧基-1-丁醛）和3种植物挥发物（芳樟醇、反式石竹烯、顺-3-己烯-1-醇）做诱芯，检验了其林间诱捕效果。结果证明，以雄性2种信息化合物（比例为1:1，释放率为1mg/天或4mg/天）与3种植物挥发物的混合物为诱芯的诱捕效果最好，其对雌性光肩星天牛的诱捕效果明显好于信息素其它混合比例、释放率、或单独植物挥发物以及空白对照。在诱捕到的雌性光肩星天牛中，87%是未交配的。雄性天牛主要只被单独以植物挥发物为诱饵的诱捕器捕获。2013年，我们检测了高于原释放率10倍的植物挥发物与雄性信息素（比例为1:1，释放率为2mg/天）的混合物的引诱效果，同时，在一些实验中又额外添加了芳樟醇氧化物。与单独的植物挥发物和空白对照相比，这种较高释放率的植物挥发物和雄性信息素的组合可以诱捕到更多的雌性天牛。我们的实验结果表明光肩星天牛雄性信息素与植物挥发物的混合物可以用来监测光肩星天牛，这将有利于对光肩星天牛的调查和防控。
Introduction

Introduced invasive species pose a serious threat to ecosystem diversity and stability (Simberloff 1997). Over 450 species of exotic invasive forest insects can be found across the U.S. and cost municipal governments $1.7 billion to control annually (Aukema et al. 2011). On average, from 1860 to 2006, 2.5 new exotic, invasive species established in U.S. forests every year (Aukema et al. 2010). Of these, the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) 1853 (Coleoptera: Cerambycidae), a polyphagous wood-borer first discovered in North America in 1996 (Haack et al. 1997), could destroy 30.3% of the urban trees in the U.S. This level of destruction would result in an economic loss of $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, New York, 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). *A. glabripennis* attacks over 48 species of apparently healthy trees with preferences for 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 U.S., it primarily attacks maples, including *Acer negundo* (boxelder), *Acer platanoides* (Norway maple), *Acer saccharinum* (silver maple), *Acer saccharum* (sugar maple) and *Acer rubrum* (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.

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
are only about 30% accurate (USDA-APHIS 2013a). Tree climbers can maneuver through a tree 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 to 2006, the costs to survey and remove infested trees and prophylactically treat host trees with imidacloprid in quarantine zones as part of eradication efforts was $249 million dollars in the U.S. alone (GAO, 2006). Thus, a cost effective detection method is 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 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 and plant volatiles can act synergistically 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 in China, the effect of using different ratios of the male-produced pheromone components, pheromone release rates, plant volatile combinations, and plant volatile release rates on *A. glabripennis* trap catches was investigated. The results provide evidence of the utility of the combination of male-produced pheromone and plant volatiles for detecting this beetle.

**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 forest research plots. In 2012 and 2013, 66.7% and 45.7% of traps were hung in host trees, respectively (Table 2-2). Traps not hung in host trees were always adjacent to host tree stands.

**Traps.** All traps used in this experiment were flight intercept panel traps equipped with twist-off bottoms (ChemTica USA, Durant, OK). Traps were coated with 10% Fluon to increase slipperiness (Graham et al. 2010). Fluon can be diluted up to 1:20 on traps and the adults still slip off the trap panels in their operational position (unpublished data). 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 to paracord so they could be raised and lowered for servicing. Distances between traps varied with plot size but ranged from 5 – 15 m. Although the draw distance of these lures is estimated to be about 80 m (Nehme et al. 2014), distances between traps was limited by the size of the field site. Trap height, tree species and diameter breast height (DBH) were recorded.

**Experimental Design.** Traps were arranged in a Latin square design to ensure spatially balanced interactions. Each treatment was next to every other treatment an equal number of times. Because the traps were close together, they were checked and rotated sequentially within their block every 3 days to minimize positional effects. During each check, collection cups were emptied and filled with fresh water and detergent. To prevent accumulation of lure residues from multiple treatments, traps, not lures, were always rotated with the same lure set for the entire season. Each plot contained the same number and types of treatments. Trapped females were dissected to determine mating status as described previously (Nehme et al. 2010).

**Effects of Pheromone Component Ratios and Release Rates on Number of Trapped Beetles.** In 2012, our goal was to determine the most attractive ratio and release rate of the two male-produced pheromone components. 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 pheromone component was placed in a separate emitter designed to release at 1 mg/day, which allowed the number of emitters to be modified in order to achieve different release rates and ratios. Ratios and release rates in mg/day (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 3-component plant volatile blend, comprised of (-)-linalool, trans-caryophyllene, and (Z)-3-hexen-1-ol released at 9, 8 and 1 mg/day, respectively, from separate emitters (Tables 2-1 and 2-3). Each replicate plot also contained a 3-plant-volatile-only treatment and a no-lure control (Table 2-1). Thus, there were 8 lure types and a no-lure control for a total of 9 treatments in each of 10 plots, producing a total of 90 traps (Table 2-1). All emitters were manufactured by ChemTica Internacional S.A. (Heredia, Costa Rica). Traps were rotated through their respective plots fully once.

**Effect of Plant Volatiles on Number of Trapped Beetles.** The effects of high plant volatile release rates on herbivore trap efficacy are not fully understood. In some cases, the volatiles can enhance the performance of a pheromone used in traps (von Arx et al. 2012). Thus, in 2013, in combination with the male-produced pheromone, trap catches were compared using a low (1X) and high (10X) release rate of the 3-component plant volatile blend and added a fourth plant volatile component to the blend as another treatment (Table 2-1). To create the high plant volatile release rates, 10 low release rate emitters per chemical component were used. To determine if the plant volatiles alone could trap *A. glabripennis*, the 3-plant-volatile and 4-plant-volatile combinations alone without the pheromone were tested at low release rates. Six lure types and a no-lure control were tested for a total of 7 treatments in each of 10 replicate plots (Table 2-1). All treatments that included the male-produced pheromone were designed to release each pheromone component at 2 mg/day.

An additional goal of this experiment was to compare lures made by two different pheromone companies (ChemTica Internacional and Synergy Semiochemicals Corp., Burnaby,
B.C.). To do this, a paired block design was used in which five blocks with emitters made by ChemTica were placed adjacent to 5 blocks with emitters made by Synergy in an alternating pattern. There were a total of five paired blocks in the forest research plots at the Northeast Forestry University. Blocks within the pair and traps within the plots were both rotated every 3 days to insure spatially balanced interactions between treatments and lure manufacturers. Traps and plots were completely rotated through plots and the forest 1.5 times, respectively.

The male-produced pheromone alone was not used as a treatment in either 2012 or 2013 because space was limited and previous field studies showed 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, 2-3 of each lure type were placed in a parallel field trial in a flight intercept panel trap and weighed every 3 days 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 was collected in 5-minute intervals with a HOBO H8 Pro Series Data Logger (Onset Computer Corporation, Bourne, MA).

**Gas Chromatography quantification of lure release rates. Chemical analysis.** 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 were purchased from Synergy and ChemTica were hermetically sealed and stored at -20°C prior to testing.

Lure release rates were measured in a laboratory fume hood at 23°C for a ten-week 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 L/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 hexanes 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 x 0.25 µm x 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 co-injection 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 impact 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 4 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 minute. In the forest, larger trees were inspected with binoculars. Infestation levels were approximated using the 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 was used to calculate standard errors. Data were fitted to a general linear model using a Poisson distribution and the Firth-bias estimation method followed by orthogonal contrasts to compare treatment groups. Trap height, tree DBH and treatment were used as predictors. 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. Treatment had a significant effect on the number of female beetles caught ($\chi^2 = 19.6$ df = 8, $P = 0.012$, Fig. 2-1), of which 87% were virgins. The 1:1 and 4:4 male-produced pheromone + plant volatile treatments were significant linear predictors of the number of female beetles caught (Fig. 2-1). Orthogonal contrasts revealed that significantly more female beetles were caught in the 1:1 and 4:4 male-produced pheromone + plant volatile treatments compared to the 8:8, 1:4, 1:8, 8:1 male-produced pheromone + plant volatiles, plant volatiles only treatment and control treatments ($\chi^2 =$...
11.7, df = 1, P = 0.001). There was no statistically significant difference in the number of females trapped when the pheromone was released at 1:1, 4:4 or 4:1 with the plant volatiles. Two female beetles were caught in control traps, which were about 10 m away from the nearest chemical lure traps.

Treatment did not have a significant effect on the number of male beetles caught in the pheromone + plant volatile treatments compared to the controls ($\chi^2 = 12.5$, df = 8, $P = 0.130$, Fig. 2-1). However, significantly more male beetles were caught in the treatment comprised of only 3 plant volatiles compared to the control or treatments with 3 plant volatiles + pheromone at any ratio ($\chi^2 = 6.33$, df = 1, $P = 0.012$). Treatment did not affect the total number of beetles caught, i.e., males plus females, although it was close to significance at $\alpha = 0.05$ ($\chi^2 = 15.3$, df = 8, $P = 0.054$).

Peak male catches occurred during the second trap check from 26 July to 28 July (Fig. 2-2), 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 peaked slightly 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. 2-3); 83% of these females were virgin. The high release rate of 3 plant volatiles + male-produced pheromone, high release rate of 4 plant volatiles + male-produced pheromone, and low release rate of 3 plant volatiles + male-produced pheromone treatments were significant linear predictors of the number of females trapped and as a group, caught significantly more females than the control ($\chi^2 = 8.16$, df = 1, $P = 0.004$, Figure 2-3). Treatments without the male-produced pheromone, and the low release rate of 4 plant volatiles + male-produced pheromone did not catch significantly more female beetles than the control.
(Figure 2-3). Treatment did not have a significant effect on the number of male beetles caught ($\chi^2 = 5.55$, df = 6, $P = 0.475$).

There was no clear pattern in the timing of when beetles were trapped in 2013 for either sex, but trap catches declined during the last 3 sampling dates (Fig. 2-4).

**Effect of Trap Height and Diameter Breast Height (DBH) on Beetle Catches.** In the 2012 analysis, traps were hung at a mean height of 3.47 ± 0.06 m (Table 2-2); 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 2-2); 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 regardless of sex ($\chi^2 = 16.6$, df = 1, $P < 0.001$). Trap height was not a significant linear predictor of male trap catches ($\chi^2 = 0.063$, df = 1, $P = 0.427$) in 2013. 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.

**Effect of Different Lure Manufacturers on Beetles Caught.** In 2013 when lures from different companies were compared, a significant treatment effect was observed for female and total beetles trapped in plots with ChemTica lures ($\chi^2 = 18.5$, df = 6, $P = 0.005$; $\chi^2 = 20.6$, df = 6, $P = 0.002$, respectively; Fig. 2-3) 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 2-3). 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 (Figs. 2-5, 2-6 and 2-7). Gas chromatography measurements suggest the true pheromone release rates were far lower than the desired release rates (Table 2-4). Fluctuating temperatures paralleled fluctuating lure release rates (Fig. 2-8).
**Estimation of Beetle Population in Field Sites.** Plots were located in different areas of the campus in 2012 and 2013, which may explain the increase in the number of uninfested trees observed in 2013 (Table 2-2). Fewer trees with oviposition pits were observed in 2013 compared to 2012, while a higher percentage of trees had exit holes. The differences in these parameters suggest inter-location differences in population structure and dynamics may be related to the differences in the numbers of beetles caught in the two years.

**Discussion**

The results indicate that emitters designed to release a 1:1 ratio of the two male-produced pheromone components at release rates of 1 or 4 mg/day, but less than 8 mg/day, in conjunction with a higher release rate of plant volatiles (10-fold higher than was used in 2012) trapped the most beetles, and that this effect is 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 appear to significantly increase trap catches, indicating the addition of this compound may not add to the traps efficacy (Figure 2-3). Males, but not females, were significantly more attracted to traps baited with plant volatiles without the pheromone in 2012 (Figure 2-1).

Male produced sex and aggregation pheromones have been discovered in several cerambycid species, such as *Hedypathes betulinus, Neolytus acuminatus, Tectropium fuscum, and Monochamus galloprovincialis* (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 female-emitted, the described volatile sex pheromones in the Lamiinae subfamily are male-produced. However, in this group of beetles, the pheromones alone are often not sufficient
for optimal trapping. Improved attraction to pheromones by combining them with plant volatiles has been observed in several insect taxa (Reddy and Guerrero 2004). When floral odors were released with the male-produced pheromone of the cerambycid *Anaglyptus subfasciatus*, more females were caught in the traps with both components than in traps baited with either component alone (Nakamuta et al. 1997). Without host plant volatiles, a generic Lamiinae attractant pheromone, fuscumol, was unattractive or only slightly attractive to *Tetropium* spp. (Sweeney et al. 2010). Our results show that the male-produced pheromone plus plant volatiles significantly improved the capture of female *A. glabripennis*. On the other hand, when plant volatiles were released without the male-produced pheromone, the traps were attractive to male beetles.

Pheromone component ratios are often critical for attracting conspecifics, especially in the Lepidoptera. For example, male tortrix moths, *Adoxophyes orana*, were caught in traps baited with a 9:1 ratio of its two component female-produced pheromones (Minks and Voerman 1973), but slight deviations from the 9:1 ratio caused a rapid decrease in the number of moths trapped. Elm bark beetles, *Scolytus mutistraitus*, have also been shown to require a specific ratio of their three pheromone components to maximize trap catches (Cuthbert and Peacock 1978). In the pheromone ratio experiment reported herein, several *A. glabripennis* were also caught in the treatments that did not emit pheromone components in a 1:1 ratio (Fig. 2-1), although treatments that released pheromone components in a 1:1 ratio did trap significantly more females and total beetles overall. Among the lures designed to release at ratios other than 1:1, the 4:1 male-produced pheromone + plant volatiles treatment released pheromone components closest to the 1:1 ratio based on gravimetric measurements.

Traps with the highest pheromone release rate (8 mg/day) did not catch *A. glabripennis* until the end of the experiment when release rates declined substantially (Figs. 2-1, 2-5). Earlier in the experiment when lures were fresh, the towards-trap progress of beetles may have become arrested at some distance downwind because the concentration was too high and sensory
adaptation or habituation was occurring. It is well known that high pheromone release rates can be used to disrupt mating in moths (Cardé and Minks 1995). In mating disruption plots, male moths become habituated to their own pheromone after following a stronger-than-normal pheromone plume for some time from a long distance downwind. They then become incapable of responding to conspecific calling females in the area (Cardé and Minks 1995).

In trapping studies, knowledge of the range of effective pheromone release rates is important for cost considerations. High pheromone release rate emitters tend to be more expensive and may interfere with an insect’s ability to find traps. Low pheromone release rate emitters may be cheaper but have an insufficient draw distance or may be undetectable by insects. Results from the 2012 study suggest that a pheromone release rate between 1 and 4 mg/day was more effective for trapping *A. glabripennis* than higher rates. However, GC volatile collections suggest that the real pheromone release rate was far lower than the design specifications after the initial burst period (Table 2-4). Previous field trapping studies 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 very low (Nehme et al. 2010).

In 2013, traps baited with ten-fold higher release rates of plant volatiles in combination with the pheromone caught significantly more beetles than the other treatments. The higher plant volatile release rates may allow traps to successfully compete against background plant volatiles. 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 detrimental to attraction. For *Ips pini*, higher release rates of α-pinene increased trap catches, but the response was not linear and trap catches declined when α-pinene release rates exceeded 110.5 mg/day (Erbilgin et al. 2003). Similarly, for the red palm weevil, *Rhynchophorus ferrugineus*, beetles were captured optimally in traps baited with its pheromone ferrugineol and the plant volatile ethyl acetate when it was released at 57 to 350 mg/day (Vacas et al. 2013).
However, when the same amount of pheromone was released with 2,200 mg/day of ethyl acetate, female trap catches decreased. These results suggest that, like pheromones, plant volatiles may cause arrestment of upwind progress via sensory adaptation or habituation and thus interfere with attraction to sources of otherwise attractive volatile blends.

The low-rate, 3-plant-volatile treatment without pheromone was significantly attractive to males in the 2012 experiment, which is consistent with a previous field study in China showing that males are attracted to plant volatiles alone (Nehme et al. 2010). In our 2013 experiment, although this 3 plant volatile only treatment caught only males, it was not significantly more attractive than the control, potentially due to a small sample size. Alternatively, in 2013, traps were tested with tenfold higher plant volatile release rates, which may have outcompeted the low plant volatile only treatments. Interestingly, the low 4 plant volatile only treatments with and without male-produced pheromone caught significantly fewer beetles than the high release rate 4 plant volatile + pheromone. This suggests the addition of linalool oxide at low release rates may be ineffective for capturing *A. glabripennis*. In 2013, we experienced a reduction in sample size as some parts of our study area were treated with pyrethroids and these plots were omitted from the analysis. Traps were often placed within 10 m of one another, making it possible for a beetle that was attracted to a different trap to fly into and be caught in a nearby trap before making it to the odor source.

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. This may have been due to traps being hung in a combination of small urban-site trees and large forest trees in 2012, which increased the variability in height, while in 2013 traps were only hung in large forest trees. The lower variability in trap heights in 2013 may have reduced the sensitivity of our ability to detect height effects. However, the 2013 results suggest that hanging
traps higher up in the canopy may increase the number of *A. glabripennis* caught, perhaps by reducing the distance adults must 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 days 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 with *A. glabripennis* due to males having a smaller body size than females. The faster 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 highlights the importance of hanging traps early in the season before beetles are active to maximize catches. In both experiments, traps primarily captured virgin females, which is consistent with results from a previous field study (Nehme et al. 2010). Fewer virgin females may be present at the end of the season, which could explain the decrease in beetles caught since it appears that virgins are more attracted to the pheromone than mated females. In areas with denser *A. glabripennis* infestations, females may have more mating opportunities, 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 2-4). About 80-90% of the measured lure weight loss could not be attributed to 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 pheromone aldehyde component. Aldehydes are prone to oxidation and often require the addition of antioxidants or other adjuvants in order to prevent formation of carboxylic acids and prevent trimerization (Ishihara et al. 1986). Although just prior to the field season the lure formulations were evaluated by the manufacturers and by our group
using gravimetric methods at a constant 23 °C to obtain specified release rates, this approach is only an approximation. Temperatures in the field vary on an hourly basis, making it difficult to predict the overall temperature-dependent release rates prior to field deployment. Lure release rates will also burst for the first week of use while the vapor pressures of components equilibrate with the external environment, which was observed and expected in our experiments. Gravimetric monitoring of lure release rates is an easy, convenient method to determine if lures are depleted. This method allows researchers to adjust 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/volatile component.

This study demonstrated that traps baited with 1:1 ratios of *A. glabripennis* pheromone components released at less than 8 mg/day together with high release rates of plant volatiles can be used to detect *A. glabripennis* in forest and urban environments, even when the beetle populations are fairly low. Although traps caught primarily females, electroantennograms (unpublished data) indicate both sexes can detect each of the pheromone components. The addition of linalool oxide to the trans-caryophyllene, (-)-linalool and cis-3-hexen-1-ol did not significantly increase trap catches. Traps baited with trans-caryophyllene, (-)-linalool and cis-3-hexen-1-ol alone without pheromone are highly attractive to male beetles, but are unlikely to trap females. The findings from this study should be useful to improving the lures that will be used in the future to detect *A. glabripennis* in fly-in traps.

**Acknowledgements**

Y. Zhou, K. Zhang, J. Wang and N. Fang in the Yan research group at the Northeast Forestry University in Harbin, People’s Republic of China provided field assistance. Nate McCartney and Liz McCarthy performed the GC and gravimetric analyses of pheromone release
rates. Jacob Wickham at the Institute of Chemistry, Chinese Academy of Sciences also provided field assistance. The Penn State Statistical Consulting center provided statistical advice. This work was supported by grants to K. Hoover from the Alphawood Foundation, the Horticultural Research Institute, USDA Forest Service, State and Private Forestry Northeastern Area, Technology & Methods Development 10-CA-11420004-316.

Footnotes

Disclaimer: The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.
Table 2-1: Lure sets used in field experiments in China in 2012 and 2013. 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.

<table>
<thead>
<tr>
<th>2012 Treatment</th>
<th>Alc</th>
<th>Ald</th>
<th>L</th>
<th>CA</th>
<th>Z3</th>
<th>LO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 MP PV</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4:4 MP PV</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8:8 MP PV</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1:4 MP PV</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1:8 MP PV</td>
<td>1</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4:1 MP PV</td>
<td>4</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8:1 MP PV</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>PV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2013 Treatment</th>
<th>Alc</th>
<th>Ald</th>
<th>L</th>
<th>CA</th>
<th>Z3</th>
<th>LO</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 PV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 PV 1X MP</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3 PV 10X MP</td>
<td>2</td>
<td>2</td>
<td>90</td>
<td>80</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4 PV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 PV 1X MP</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4 PV 10X MP</td>
<td>2</td>
<td>2</td>
<td>90</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2-2: Percentage of traps that were hung in host trees and percentage of trees with insect signs on the trap tree and the nearest 4 host trees to the trap in 2012 and 2013. 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).

<table>
<thead>
<tr>
<th>Percentage</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Traps in Host Trees</td>
<td>66.7</td>
<td>45.7</td>
</tr>
<tr>
<td>% Uninfested Trees</td>
<td>69.7</td>
<td>79.6</td>
</tr>
<tr>
<td>% trees with oviposition pits</td>
<td>22.8</td>
<td>3.86</td>
</tr>
<tr>
<td>% trees with 1-10 Exit Holes</td>
<td>7.22</td>
<td>13.4</td>
</tr>
<tr>
<td>% trees with 11-100 exit holes</td>
<td>0.28</td>
<td>0.00</td>
</tr>
<tr>
<td>% trees with 101+ exit holes</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Avg. Trap Height (m)</td>
<td>3.47 ± 0.06</td>
<td>3.77 ± 0.13</td>
</tr>
<tr>
<td>Avg. Tree DBH (cm)</td>
<td>18.8 ± 0.38</td>
<td>21.7 ± 1.10</td>
</tr>
</tbody>
</table>
Table 2-3: Lures used in field experiments with desired and actual release rates mean (± SEM) measured gravimetrically in mg/day. No extra ChemTica aldehyde lures were available to be weighed in 2013 in China. *In 2013, two ChemTica alcohol lures were used in each trap to achieve a desired release rate of 2 mg/day.

<table>
<thead>
<tr>
<th>2012 Lures</th>
<th>Manufacturer</th>
<th>Desired</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB Alcohol</td>
<td>ChemTica</td>
<td>1</td>
<td>0.52 ± 0.03</td>
</tr>
<tr>
<td>ALB Aldehyde</td>
<td>ChemTica</td>
<td>1</td>
<td>1.48 ± 2.00</td>
</tr>
<tr>
<td><em>trans</em>-Caryophyllene</td>
<td>ChemTica</td>
<td>8</td>
<td>10.22 ± 4.15</td>
</tr>
<tr>
<td>(Z)-3-Hexen-1-ol</td>
<td>ChemTica</td>
<td>1</td>
<td>1.30 ± 0.10</td>
</tr>
<tr>
<td>(-)-Linalool</td>
<td>ChemTica</td>
<td>9</td>
<td>5.70 ± 0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2013 Lures</th>
<th>Manufacturer</th>
<th>Desired</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB Alcohol</td>
<td>ChemTica</td>
<td>1*</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>ALB Alcohol</td>
<td>Synergy</td>
<td>2</td>
<td>1.80 ± 0.24</td>
</tr>
<tr>
<td>ALB Aldehyde</td>
<td>ChemTica</td>
<td>2</td>
<td>Not weighed</td>
</tr>
<tr>
<td>ALB Aldehyde - Bag</td>
<td>Synergy</td>
<td>2</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td>ALB Aldehyde - No Bag</td>
<td>Synergy</td>
<td>2</td>
<td>1.60 ± 0.11</td>
</tr>
<tr>
<td><em>trans</em>-Caryophyllene</td>
<td>ChemTica</td>
<td>8</td>
<td>14.2 ± 0.54</td>
</tr>
<tr>
<td><em>trans</em>-Caryophyllene</td>
<td>Synergy</td>
<td>8</td>
<td>11.2 ± 0.26</td>
</tr>
<tr>
<td>(Z)-3-Hexen-1-ol</td>
<td>ChemTica</td>
<td>1</td>
<td>1.78 ± 0.25</td>
</tr>
<tr>
<td>(Z)-3-Hexen-1-ol</td>
<td>Synergy</td>
<td>1</td>
<td>2.51 ± 0.18</td>
</tr>
<tr>
<td>(-)-Linalool</td>
<td>ChemTica</td>
<td>9</td>
<td>14.4 ± 0.38</td>
</tr>
<tr>
<td>(-)-Linalool</td>
<td>Synergy</td>
<td>9</td>
<td>14.9 ± 0.88</td>
</tr>
<tr>
<td>Linalool Oxide</td>
<td>ChemTica</td>
<td>1</td>
<td>2.07 ± 0.13</td>
</tr>
<tr>
<td>Linalool Oxide</td>
<td>Synergy</td>
<td>1</td>
<td>2.26 ± 0.08</td>
</tr>
</tbody>
</table>
Table 2-4: Mean ± SEM (mg/day) 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. Numbers following company names indicate if lures were produced in 2012 or 2013. ChemTica alcohol pheromone lures were used for the 2012 and 2013 field seasons.

<table>
<thead>
<tr>
<th>Day</th>
<th>ChemTica 12</th>
<th>Synergy 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.66 ± 0.02</td>
<td>1.69 ± 0.15</td>
</tr>
<tr>
<td>14</td>
<td>0.09 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>21</td>
<td>0.08 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>28</td>
<td>0.06 ± 0.00</td>
<td>0.08 ± 0.00</td>
</tr>
<tr>
<td>35</td>
<td>0.05 ± 0.01</td>
<td>0.10 ± 0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>ChemTica 12</th>
<th>ChemTica 13</th>
<th>Synergy 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>3.42 ± 0.50</td>
<td>2.35 ± 2.07</td>
<td>3.85 ± 0.94</td>
</tr>
<tr>
<td>14</td>
<td>0.21 ± 0.04</td>
<td>1.45 ± 0.86</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>21</td>
<td>0.08 ± 0.02</td>
<td>0.49 ± 0.11</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>28</td>
<td>0.04 ± 0.00</td>
<td>0.29 ± 0.06</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>35</td>
<td>0.03 ± 0.00</td>
<td>0.26 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
</tbody>
</table>
Figure 2-1: Cumulative number of beetles trapped by each lure treatment by sex in 2012. Ten of each lure type were hung in 10 different plots for 27 days. An asterisk over the bar indicates treatments that were significant linear predictors of the number of female beetles trapped from the GLM analysis. Different letters over a bar indicate statistically significant differences in female trap catches among treatments based on orthogonal contrasts at $P < 0.05$. MP = male-produced pheromone, PV = 3 plant volatiles: (-)-linalool, trans-caryophyllene and (Z)-3-hexen-1-ol.
Figure 2-2: Number of beetles caught by sex during each trap check in 2012. Dates correspond to the duration between trap checks.
Figure 2-3: Cumulative number of beetles trapped by each lure treatment and company by sex in 2013. Ten of each lure type were hung in 10 different plots for 35 days. Asterisks indicate treatments that were a significant linear predictor of beetles trapped from the GLM analysis. Different lowercase letters over the bars indicate statistically significant differences among treatments (both manufacturers combined) compared to the control at P < 0.05 using orthogonal contrast tests. Table indicates how many beetles of each sex were caught using lures produced by each manufacturer. 3 PV = 3 plant volatile mix: (-)-linalool, *trans*-caryophyllene and (Z)-3-hexen-1-ol, 4 PV = 4 plant volatile mix: (-)- linalool, *trans*-caryophyllene, (Z)-3-hexen-1-ol and linalool oxide. MP = male produced pheromone, 1X = low plant volatile release rates, 10X = high...
plant volatile release rates. Synergy = all emitters were manufactured by Synergy. ChemTica = all emitters were manufactured by ChemTica.
Figure 2-4: Number of beetles caught during each trap check in 2013. Dates correspond to the duration between trap checks.
Figure 2-5: Mean (± SEM) release rates of pheromone and plant volatile lure components in mg/day during the trapping study in 2012 measured gravimetrically. Some standard errors are too
small to be visible. Horizontal dashed lines indicate desired plant volatile release rates of 9, 8, 1 and 1 mg/day for (−)-linalool, trans-caryophyllene, (Z)-3-hexen-1-ol and pheromone components, respectively.
Figure 2-6: Mean (± SEM) pheromone aldehyde and alcohol lure release rates given in mg/day.
during the 2013 study measured gravimetrically. Heavy rain accumulation caused erroneous weight measurements during days 24-27 of the experiment. Some standard errors are too small to be visible. Horizontal dashed line indicates desired pheromone release rates of 2 mg/day. Ald = pheromone aldehyde, Alc = pheromone alcohol.
Figure 2-7: Mean (± SEM) plant volatile lure release rates given in mg/day during the 2013 study measured gravimetrically. Horizontal lines indicate desired plant volatile release rates of 9, 8, 1 and 1 mg/day for (-)-linalool, trans-caryophyllene, linalool oxide and (Z)-3-hexen-1-ol.
respectively.
Figure 2-8: Daily field site temperatures in 2012 and 2013. In 2012, Temperatures were obtained from the National Climatic Data Center. In 2013, temperatures were collected in the field using a HOBO H8 data logger. Day 1 corresponds to 7/23 and 7/21 in 2012 and 2013, respectively.
Chapter 3

Sensory Biology and Neurophysiology of *Anoplophora glabripennis*

Abstract

The composite of thousands of chemoreceptive sensilla comprises the antennae of an insect and serves as its nose. The Asian longhorned beetle, *Anoplophora glabripennis* is a polyphagous, invasive, wood-boring pest capable of destroying 33% of North America’s hardwoods. In this study, quantities and descriptions of the distribution of sensilla on the *A. glabripennis* antennae and mouthparts are provided. No extreme cases of sexual dimorphism (absence or reduction of sensilla types on one sex) were observed. However, females had significantly more chetiform-1 and Böhm sensilla compared to males on some antennal segments. Males had significantly more short basiconic sensilla compared to females on some antennal segments. *A. glabripennis* behaviors may explain these sexually dimorphic differences in sensilla distribution. Trichoid sensilla were the dominant olfactory sensilla observed on antennae. Electroantennogram responses from male and female antennae to both components of the male-produced pheromone are also presented. The findings from these studies lay the groundwork for future olfaction studies in *A. glabripennis*.

Introduction
Insects must differentiate between semiochemical cues in the environment in order to locate food, avoid hazardous situations and find mates. Plants release an array of kairomones that can attract parasitoids and herbivores. The Asian longhorned beetle (ALB), Anoplophora glabripennis (Motschulsky) 1853 (Coleoptera: Cerambycidae) is a polyphagous, wood-boring pest that threatens to destroy 33.3% of the urban trees in the U.S. if left uncontrolled (Nowak et al. 2001). A. glabripennis was first discovered in New York and has since been found in Illinois, Ohio, New Jersey and Massachusetts (Hu et al. 2009). This beetle attacks 47 known species of apparently healthy trees (Hu et al. 2009). Despite the beetle’s broad host range and potential threat, little is known about its sensory biology and how it locates host plants.

Several types of pheromones have been discovered in A. glabripennis including a volatile, two-component male-produced sex pheromone (Zhang et al. 2002), a female-produced contact pheromone (Zhang et al. 2003) and a female-produced trail pheromone (Hoover et al., 2014). Additionally, several plant kairomones that can be used in trapping have been identified including (Z)-3-hexen-1-ol, (-)-linalool, linalool oxide, trans-pinocarveol and trans-caryophyllene (Nehme et al. 2009, 2010, Wickham et al. 2012). Observations indicate A. glabripennis is constantly antennating and contacting surfaces with its mouthparts as it explores its environment. Although several behaviorally active semiochemicals have been described for A. glabripennis, no quantitative studies exist that describe any sex-biased numerical distribution of various types of sensilla on mouthparts and antennae to allow researchers to begin to surmise how these structures allow A. glabripennis to perceive semiochemical cues in the environment.

Such studies of sensillar distribution across sexes have not been performed for any of the cerambycid species in the subfamily, Lamiinae. In this study, the relative abundance and distribution of sensilla is reported from work performed via scanning electron microscopy (SEM) on A. glabripennis mouthparts and antennae. Male and female antennae are also shown to respond to both of the male-produced sex pheromone components. Describing the morphology
and sensilla distribution on *A. glabripennis* antennae should help pave the way for future single cell recordings.

**Methods**

**Beetles.** Antennae used in this study were obtained from beetles reared at the USDA-Forest Service quarantine facility in Ansonia, CT. Adult beetles were collected from infested logs obtained from the New York City, NY and Chicago, IL infestations. Adult beetles were kept individually in 950-mL glass jars and fed *Acer saccharum* twigs.

Mouthparts used in this study were obtained from *A. glabripennis* reared at the Penn State quarantine facility in University Park, PA. Insects were reared in artificial diet as described by Keena (2005). All beetle life stages were kept at 25°C, 60% humidity and L:D 16:8 h. All beetles were kept in individual 750-mL glass jars and fed with *Acer platanoides* L. twigs.

**Microscopy.** For SEM, antennae were fixed in a 1:3 mixture of dimethoxypropane and absolute ethanol and air-dried or fixed for 1 hour in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, postfixed for 1 hour in 2% osmium tetroxide and critical-point dried in a Sorvall critical-point dryer (Sorvall Model 49300, Newtown, CT). Antennae were then mounted, sputter coated with 20 nm of gold and examined with an ETEC Autoscan scanning electron microscope (ETEC, Hayward, CA) at an accelerating voltage of 20 KV.

Antennae were moved and rotated inside the scanning electron microscope in order to quantify visible sensilla. It was not possible to quantify all basiconic and trichoid sensilla as some were hidden between scales (Fig. 3-9D). The short basiconic and trichoid sensilla were initially classified as the same sensilla type and counted together. To tease apart the two sensilla types, voxels were created across all antennal segments on 3 males and 3 females. Within these voxels,
only short basiconic and trichoid sensilla were quantified in order to create ratios and adjust the number of previously quantified structures.

Mouthparts used for SEM were air-dried for at least 5 days at room temperature. The maxillary and labial palps were carefully detached from the stipes and labium, respectively. An Olympus SZX10 light microscope (Olympus America, Center Valley, PA) with a 1X DF PLAPO objective lens (Olympus Corporation, Tokyo, Japan) was used to inspect mouthparts and quantify the large, fragile, chetiform sensilla that project from the cuticle. Mouthparts were then mounted on carbon tape and grounded with colloidal silver (Electron Microscopy Sciences, Hatfield, PA) and sputter coated with 10 nm of gold-palladium (3 min at 10 mA). Mouthparts were imaged on a JEOL JSM 5400 scanning electron microscope (JEOL USA, Peabody, MA) at acceleration voltages of 20 and 25 KV. Mouthparts were moved and rotated inside the scanning electron microscope in order to quantify visible sensilla. Sensilla on 5-6 antennae and palps of each sex were quantified and averaged.

Sensilla were classified according to Zacharuk (1985) with attempts made to harmonize nomenclature for *A. glabripennis* with that of Yan et al. (2010). Sensilla diameters were always measured at the base.

**Electroantennograms (EAGs).** *A. glabripennis* used for EAGs were reared at the Penn State quarantine facility as described above. Two antennae from different virgin beetles of each sex were used in this experiment. Antennae were carefully removed from beetles with MicroPoint scissors (Electron Microscopy Sciences, Hatfield, PA) by making a cut at the base of the first flagellomere next to the pedicel. The base of the antenna was inserted directly into a sharpened silver ground electrode. The tip of the antenna was inserted into a glass microcapillary tube with a 0.86 mm inner diameter (A-M Systems, Carlsborg, WA) that contained a silver recording electrode and was filled with modified Tuscon Ringer’s solution, pH 7. EAGs were sampled at 100 Hz. The AC signals from the recording electrode were amplified with the IDR-2 recording
unit (Syntech, Kirchzarten, Germany), passed through a Hum Bug 50/60 Hz noise eliminator (Quest Scientific, North Vancouver, BC, Canada) and acquired with the Syntech IDAC-4-USB intelligent data controller (Syntech, Kirchzarten, Germany). Acquired signals were digitally recorded onto the Autospike 3.2 software package (Syntech, Kirchzarten, Germany).

The *A. glabripennis* pheromone components 4-(n-heptyloxy)butanal and 4-(n-heptyloxy)butan-1-ol, and the plant volatile (-)-linalool were used individually to elicit responses from antennae. The synthetic compounds were purchased from Bedoukian Research Inc. (Danbury, CT). Compounds were diluted in gas-chromatography-grade hexane (≥98.5% purity, EMD Millipore Corp., Billerica, MA) to 10 µg/µl and 1 µg/µl and stored at -20°C. Compounds were delivered into a continuous airstream blown over the antenna using odor cartridges consisting of 2.5 ml glass Pasteur pipettes. One hundred micrograms and 1 µg of the pheromone components and 1 mg and 10 µg linalool were impregnated onto a 0.7 x 2.5-cm paper strip and inserted into a glass pipette. Odor cartridges were stored at -20°C and covered with aluminum foil until ready to use.

A stream of purified, humidified air was continuously blown across the center of the antenna at 10 ml/s (linear velocity ~ 0.3 m/s). A 50-ms air pulse at a 40 ml/s flow rate was injected through the odor cartridge into the airstream using a SFC-2 digital controller (Syntech, Kirchzarten, Germany). Antennae were puffed after the EAG signal repolarized.

To account for differences in each antenna, EAG responses to pheromone components were standardized to responses to 10 µg of (-)-linalool. An empty odor cartridge was used as a blank control to confirm EAG responses were from olfactory and not mechanoreceptive sensilla.

**Statistics.** Statistics were performed using R (R Core Team 2013). Data was analyzed using Welch’s t-test. Results are expressed as the mean (number of sensilla or length) ± standard deviation.

**Terminology.** Flagellomeres will be referred to as “segments” throughout this chapter.
Results

General Structure of Antennae and Palps. Antennae from males and females are filiform, with a scape, pedicel and 9 flagellomeres. White, flattened scales (Fig. 3-1A-D) on the basal half of each flagellomere create the familiar alternating white-black banding pattern on the antennae (Fig. 3-1A) of this beetle. Female antennae on a beetle at rest extend slightly beyond the posterior abdomen whereas those of males are longer, and may extend 1 cm beyond the abdomen. Antennal segment lengths ranged from 0.45 mm to 2.91 mm in males and 0.41 mm to 2.41 mm in females. All male antennal segments were significantly longer than female antennal segments with the exception of the scape and pedicel (Table 3-1). The pedicel and Segments 4, 7, and 11 ranged in width from 0.17 to 0.44 mm in males and 0.14 mm to 0.42 in females and were significantly wider in males compared to females (Table 3-1).

Sensilla. White, flattened, sculpted scales. Thousands of swollen, flattened, fine-tipped, curved scales that appear white under natural light are present on the antennae (Fig. 3-1B-D). These broadly flattened scales resemble those that form white spots on the elytra (Fig. 3-2) and seem unlikely to function as olfactory receptors. These scales were observed to be 70.17 ± 11.16 µm long and 8.97 ± 1.63 µm wide. On most segments, the scales were white and densely packed on the proximal end and then became sparsely spaced and transparent towards the distal end. The proximal and distal ends of antennal Segment 11 are densely packed with scales but they become sparsely packed and transparent at the center of the middle portion of the segment. No obvious sexual dimorphism in scale abundance or distribution was observed between the sexes. A greater relative proportion of each female antennal segment is covered with white scales compared to males. These scales thus seem to be solely decorative and were not quantified.
**Böhm bristles.** These sensilla were only observed on the pedicel, are lightly longitudinally grooved, dull tipped and highly variable in height (Fig. 3-3A-C). Most Böhm bristles are straight and orthogonal to the cuticle and come into contact with the walls of the scape when the antenna rotates (Fig. 3-3A). The bristles average $41.73 \pm 24.03 \, \mu m$ in length and $6.95 \pm 3.56 \, \mu m$ in diameter. Approximately $74.00 \pm 3.65$ and $53.17 \pm 5.08$ Böhm bristles can be found on the base of female and male pedicels respectively (Fig. 3-4). There are significantly more Böhm bristles on female *A. glabripennis* pedicels than on males (Fig. 3-4).

**Chetiform-1 sensilla.** Chetiform-1 sensilla are long and slightly curved. They are highly variable in length with sharp tips and longitudinal grooves (Fig. 3-5). Chetiform-1 sensilla averaged $103.39 \pm 96.53 \, \mu m$ in length and $2.42 \pm 0.48 \, \mu m$ in diameter. These sensilla were the most numerous on Segment 3 and decreased in number towards Segment 11 (Fig. 3-6). Chetiform-1 sensilla were primarily found along the distal portion of segment junctions (Fig. 3-5A). A few, sparsely spaced, convex curved chetiform-1 sensilla could also be found along the mid-region of each antennal segment and on the scape (Fig. 3-5B, C). The location at the distal end of segment junctions and distribution across the entire antenna suggests a role in proprioception. There were significantly more chetiform-1 sensilla on female Segments 3 – 5 compared to males (Fig. 3-6).

**Long basiconic sensilla.** The shorter, recurved, dull tipped long basiconic sensilla are shown (Fig. 3-7A-C). These sensilla measure $56.03 \pm 14.09 \, \mu m$ in length and $5.97 \pm 1.12 \, \mu m$ in diameter. Long basiconic sensilla are most numerous on Segment 3 and decrease in number towards Segment 11 (Fig. 3-8). There were few differences between the sexes with regard to numbers of long basiconic sensilla, although significantly more long basiconic sensilla were found on the scape of males compared to females. Significantly more long basiconic sensilla were found on Segment 7 of females compared to males.
**Trichoid sensilla.** Numerous small, curved, smooth walled, sharp tipped trichoid sensilla (Fig. 3-9) are distributed along *A. glabripennis* antennae. These are the most numerous sensilla often numbering more than 500 per segment (Fig. 3-10), and are likely to contribute significantly to olfaction in this species. The sockets of trichoid sensilla are slightly raised and do not appear to permit articulation. Trichoid sensilla averaged $18.62 \pm 3.04 \, \mu m$ in length and $1.94 \pm 0.14 \, \mu m$ in diameter. They are distributed primarily across Segments 4 to 11 and are interspersed between larger, flattened, white scales (Fig. 3-9A, B). Few trichoid sensilla were found on the scap, pedicel and Segment 3 (Fig. 3-10). The number of trichoid sensilla were found to roughly increase beginning on Segment 3 through Segment 11 where they were the most abundant and sometimes were clustered together in patches (Fig. 3-9C). Trichoid sensilla were typically found on the distal end of antennal segments. No significant sexual dimorphism was observed in the abundance or distribution of trichoid sensilla along the antenna.

**Short basiconic sensilla.** Numerous small, straight, smooth walled, blunt tipped basiconic sensilla are were found along Segments 3 – 11 of *A. glabripennis* antennae (Fig. 3-9B, D). Basiconic sensilla were found in non-articulating sockets that, like those of the trichoids, were raised (Fig. 3-9A, B). Short basiconic sensilla averaged $16.24 \pm 0.83 \, \mu m$ in length and $2.42 \pm 0.48 \, \mu m$ in diameter. The number of basiconic sensilla were found to increase beginning on Segment 4 until Segment 9 whereupon the number diminished, especially for females (Fig. 3-11). The abundance of short basiconic sensilla can rival trichoid sensilla in abundance on a few segments (Figs. 3-10, 3-11). Short basiconic sensilla are difficult to quantify because they are often found in dense clusters hidden beneath the flattened scales on the proximal ends of antennal segments (Fig. 3-9D). At the distal ends of antennal segments, they are usually found in the same patches as trichoids but were slightly outnumbered by them (Fig. 3-9C). Male antennal Segments 5, 8 and 11 were found to have significantly more basiconic sensilla than the corresponding female segments (Fig. 3-11).
Campaniform sensilla. Campaniform sensilla appear to be recessed into the cuticle surface within raised sockets (Fig. 3-12A, B). At the top of the socket, a domed, circular nipple-like sensillum was observed (Fig. 3-12B). These sensilla appear to have inner and outer regions (Fig. 3-12B). Campaniform sensilla were measured to have a base diameter of $5.84 \pm 0.79 \mu m$ and center diameter of $2.53 \pm 0.52 \mu m$. They were concentrated mainly at the distal portion of the antennae and the distal portion of Segment 11 (Fig. 3-13). These were the least common sensilla that were found on the antennae that were examined. No sexual dimorphism in abundance of campaniform sensilla was observed (Fig. 3-13).

Palps. Both sexes have 2- and 3-segmented labial and maxillary palps, respectively (Fig. 3-14A, B). Male palps ranged from 0.67 mm to 1.01 mm in length whereas female palps were slightly longer and ranged from 0.80 mm to 1.28 mm in length (Table 3-2). The first two segments of the maxillary and first segment of the labial palps were wider at the distal end compared to the proximal end. Maxillary palp Segment 3, the most distal segment, was narrowest at the proximal and distal ends and widest at the center. Segment 2 of the labial, and 3 of maxillary palp, terminate in a concave pit across the width of the entire tip and are populated by an abundance of very short basiconic sensilla (Fig. 3-14C). The tips of the female maxillary palps were significantly longer and wider at the center compared to those of males (Table 3-2). On the other hand, the second segment of the female labial palps was significantly shorter and narrower at both ends compared to those of males (Table 3-3). Segment 3 of the female maxillary palps was significantly longer and wider on the distal end than that of males. The tip of female labial palps was significantly shorter and narrower at the distal end compared to males (Table 3-3). The first Segment of the female labial palp was significantly shorter and narrower at the proximal end compared to that of males.

Sculpted scales. Flattened, sculpted scales similar in shape and sculpting to those found on the antennae, yet slimmer and more pointed, were also found on the palps (Fig. 3-15A, B).
These scales averaged from 51.13 ± 10.02 µm in length and 5.65 ± 0.31 µm in width and had a variable distribution along both sets of palps, yet being sometimes absent from the proximal ends. There was no sexual dimorphism in their abundance or distribution. Because these scales seemed unlikely to serve a mechanoreceptive or olfactory function, they were not quantified further.

**Chetiform sensilla.** Two types of chetiform sensilla were found on *A. glabripennis* palps. Chetiform-2 sensilla are very slim, short, sharp-tipped and longitudinally grooved sensilla (Fig. 3-16A-C). They averaged 64.33 ± 9.59 µm in length and 4.33 ± 1.00 in diameter. They were located primarily on Segments 3 of the maxillary and 2 of the labial palps (Fig. 3-17). No sexual dimorphism in distribution or abundance was observed.

Chetiform-3, are extra-long, extremely narrow and sharp-tipped sensilla with longitudinal grooves (Fig. 3-18A, B). In this study they were extremely brittle sensilla that were prone to detachment from the cuticle. They averaged 227.39 ± 62.32 µm in length and 6.94 ± 2.33 µm in diameter and were found mostly on the posterior of the second labial palp segment. There were significantly more chetiform-3 sensilla on the first labial palp segment in females compared to males (Fig. 3-19).

**Coeloconic sensilla.** Coeloconic sensilla were found in slightly recessed sockets on the mouthparts (Fig. 3-20A). Some coeloconic sensilla were observed to have an opening or were slightly flattened. They averaged 2.55 ± 0.31 µm in length and 1.43 ± 0.16 µm in diameter. These sensilla were only found on Segments 2 and 3 of the labial and maxillary palps, respectively (Fig. 3-21). No sexual dimorphism in distribution and abundance was observed.

**Basiconic sensilla.** Approximately 100 - 140 smooth walled basiconic sensilla were found in the terminal concave tips of *A. glabripennis* maxillary and labial palps (Figs. 3-14C, 3-22). Some basiconic sensilla had a clearly raised, circular base (Fig. 3-14C). It was not clear if the other basiconic sensilla lack raised bases or if these were concealed by waxes or other cuticle
secretions. These sensilla average $5.29 \pm 0.74$ µm in length and $2.62 \pm 0.22$ µm in diameter. No sexual dimorphism in distribution and abundance was observed.

**EAGs.** *A. glabripennis* antennae showed clear responses to the alcohol and aldehyde (Fig. 3-23) pheromone components at 1 and 100 µg doses (Table 3-4). Antennae also responded to 1 mg and 10 µg of the (-)-linalool standard (not shown). The amplitude of the depolarization in female EAGs were greater than those of males (Table 3-4). When the tip of the antenna was cut, EAGs could not be collected. As a result of using antennae with uncut tips, the baseline did not remain constant. Antennae did not respond at all or responded slightly when puffed with a blank.

Male and female antennal responses to pheromone components appear to be dosage dependent (Table 3-4). After repeated stimulation with the same compound within a 10 second interval, antennae became desensitized and less responsive.

**Discussion**

This study attempted to find whether or not there were any differences in the abundances and distributions of different types of setae (scales, sensilla) on male versus female *A. glabripennis*. Few differences were found, and these were only on particular antennal segments.

It was clear that most of the lengths and widths of the antennal Segments 3 - 11 of males were significantly greater than those of females, accounting for the ca. 1 cm longer total length of the antennae of males compared to females. The decorative white scales having no apparent sensory function on the basal portion of each antennal segment of both sexes were the most obviously abundant setae on the antennae of both sexes, and these did not differ in abundance in any apparent way between the sexes.

One trend that emerged was with regard to sex-specific differences in the abundance of antennal sensilla having a putative mechanosensory function, including Böhm and chetiform.
When certain antennal segments displayed sexual dimorphism, it was always females that were endowed with greater numbers of mechanoreceptive sensilla, never males. This was true for Böhm sensilla on the pedicel (Fig. 3-4) and chetiform sensilla on Segments 3, 4, and 5 (Fig. 3-6). No differences in campaniform sensillar abundance between the sexes were found, even on the tips of the antennae (Fig. 3-13). Females may have a greater need for antennal proprioception and mechanoreception related to the use of antennae for determining host-tree bark-crevice suitabilities for oviposition, and thus require greater abundances of mechanoreceptors than males. The greater numbers of mechanoreceptive sensilla observed on female antennae may also be used by females to sense movements by male antennae during mating. Males alternate touching one female antenna after the other in order to gain acceptance after mounting and before or during copulation attempts.

Trichoid sensilla, which are known in insects to have primarily an olfactory function, were present in greater numbers on *A. glabripennis* antennal segments than any other type of sensillium. Surprisingly, there were no significant differences in their abundance on any segment of male antennae compared to those of females (Fig. 3-10). This lack of sexual dimorphism may indicate the need by both sexes to sense pheromones equally. However, both sexes may respond to pheromone components differently. For example, male beetles, regardless of mating status, are highly attracted to the female-produced trail pheromone (Hoover et al. 2014). Virgin females, on the contrary, avoid the trail pheromone, and may use it as a spacing signal.

Basiconic sensilla, however, another sensillar type having a known olfactory function in insects, were more often found in greater abundance on certain male antennal segments than on those of females (Fig. 3-11). There were no instances of female basiconic sensilla on any antennal segments significantly outnumbering those of males; when there were significant differences, it was always the basiconic sensilla of males outnumbering those of females, such as on Segments 5, 8, and 11 (Fig. 3-11).
From *Drosophila* to moths, trichoid sensilla are known to be usually involved with sex pheromone olfaction (Dickens et al. 1993, Kurtovic et al. 2007), yet despite the use of a male-produced *A. glabripennis* sex pheromone for mate-finding by females, no greater abundance of trichoids was found in females compared to males. Of course, the tuning profiles of neurons within these sensilla might be quite different between females and males, and both sexes do have antennal responses to the male-produced pheromone.

In this regard the EAG results indicated that in fact female antennae give higher EAG amplitudes in response to the two male-emitted *A. glabripennis* sex pheromone components than do male antennae (Table 3-4). These results are what would be expected with regard to females being the receivers of the male signal. If, as in other insect species, the trichoid sensilla of *A. glabripennis* house olfactory receptor neurons tuned to sex pheromone components, then these neurons’ response profiles and overall sensitivities in females seem to be much more tuned and sensitive to sex pheromone components than those of males, despite the equal abundance of trichoids between the sexes. Further exploration of these sensilla via single-cell neurophysiological recordings may reveal much more about any possible functional, not merely morphological, sexual dimorphism.

Male attraction to traps baited with plant volatiles alone may be an explanation for the slight, but significant, male bias in the abundance of basiconic sensilla. Males, who have more short basiconic sensilla compared to females, may be able to detect and locate plant volatile plumes more easily compared to females as evidenced in the field trapping study. Basiconic sensilla of *Drosophila* and moths usually house olfactory receptor neurons that are tuned to a wide variety of general odorants such as leaf and flower volatiles (Anderson et al. 2000), as well as volatiles resulting from the decay of organic material. Detection and discrimination of such volatiles would presumably be equally or more important to females compared to males, with regards to locating suitable oviposition sites; thus one might expect female *A. glabripennis* would
have equal or greater numbers of basiconic sensilla than males. The distribution of short basiconic sensilla on the antennae, labial and maxillary palps does not support this assertion. However, in the Lepidoptera, it is not uncommon to find reports of male moths of several species having greater numbers of basiconic sensilla than females or else greater neuronal sensitivity to plant-related volatiles than females (Hansson et al. 1989). Thus at this stage, our understanding of sex biases in sensillar abundance may not be great enough to allow conjecture with much assurance.

One caveat that must be inserted here is that the numbers of trichoid and basiconic sensilla that lie within the bands of white scales at the basal portion of each antennal segment could not be accurately counted (Fig. 3-9D). The conclusions might be quite different had these sensilla been accurately quantified. Nevertheless, it is assumed that the relative male-female abundances of these two types of olfactory sensilla that were counted in the more distal portion of each segment are reflective of the ratios within the white scale-bands and that our counts are reflective of the true relative ratios across all segments unless further studies from within the white scale bands indicate otherwise.

Examination of the labial and maxillary palps of *A. glabripennis* reveals that there are at least two types of sensilla that might provide olfactory inputs to adults with regard to host selection, acceptance, and perhaps pheromonal communication. Coeloconic sensilla on the tips of the labial and maxillary palps may be hygroreceptive or contain ionotropic receptors tuned to amines and other hydrophilic molecules (Guo et al. 2013) which could be used by beetles to evaluate host plant quality. The dense array of basiconic sensilla in the recessed concave pits at the tips of the terminal segments of both types of palps (Fig. 3-14) would seem to indicate that olfactory information on or near the substrate must be of primary importance to these beetles. A trail pheromone has been found in this species in which beetles are able to follow the paths on bark that have been taken by other adults. The basiconic sensilla in the recessed areas at the tips
of the palps (Fig. 3-14) might be good targets for neurophysiological explorations of the tuning profiles of any olfactory neurons housed in these sensilla, whether it be for trail pheromone communication or for food or oviposition site olfactory assessment.

Acknowledgements

K. Shields and D. Mikus devoted countless hours to collecting scanning electron micrographs and quantifying sensilla on *A. glabripennis* antennae. The Penn State Microscopy and Cytometry Facility provided the training and resources necessary to image *A. glabripennis* mouthparts. M. Keena and D. Long maintained the insect colonies used in this study. Funding for these research projects were made possible by grants to K. Hoover from the Alphawood Foundation, the Horticultural Research Institute, USDA Forest Service, and State and Private Forestry Northeastern Area Technology & Methods Development 10-CA-11420004-316.
Table 3-1: Antennal segment lengths (mm) and diameters (mm) ± SD. The lengths of male antennal Segments 3 - 11 are significantly longer than the corresponding female antennal segments. The male pedicel, Segments 4, 7 and 11 are significantly wider in males compared to females.

<table>
<thead>
<tr>
<th></th>
<th>Scape</th>
<th>Pedicel</th>
<th>Segment 3</th>
<th>Segment 4</th>
<th>Segment 5</th>
<th>Segment 6</th>
<th>Segment 7</th>
<th>Segment 8</th>
<th>Segment 9</th>
<th>Segment 10</th>
<th>Segment 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.32 ±</td>
<td>0.45 ±</td>
<td>2.91 ±</td>
<td>2.56 ±</td>
<td>2.22 ±</td>
<td>1.99 ±</td>
<td>1.93 ±</td>
<td>1.84 ±</td>
<td>1.70 ±</td>
<td>1.55 ±</td>
<td>2.15 ±</td>
</tr>
<tr>
<td>Length</td>
<td>0.12</td>
<td>0.07</td>
<td>0.25</td>
<td>0.08</td>
<td>0.17</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
<td>0.15</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Female</td>
<td>1.28 ±</td>
<td>0.41 ±</td>
<td>2.41 ±</td>
<td>2.09 ±</td>
<td>1.62 ±</td>
<td>1.43 ±</td>
<td>1.39 ±</td>
<td>1.30 ±</td>
<td>1.22 ±</td>
<td>1.15 ±</td>
<td>1.46 ±</td>
</tr>
<tr>
<td>Length</td>
<td>0.07</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>T Statistic</td>
<td>-0.52</td>
<td>-0.61</td>
<td>-4.37</td>
<td>-10.22</td>
<td>-7.55</td>
<td>-13.01</td>
<td>-10.70</td>
<td>-12.60</td>
<td>-7.10</td>
<td>-7.21</td>
<td>-9.17</td>
</tr>
<tr>
<td>df</td>
<td>6.70</td>
<td>7.23</td>
<td>4.47</td>
<td>7.96</td>
<td>4.77</td>
<td>6.16</td>
<td>7.28</td>
<td>6.35</td>
<td>4.16</td>
<td>5.02</td>
<td>4.35</td>
</tr>
<tr>
<td>P value</td>
<td>0.619</td>
<td>0.559</td>
<td>0.009</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.010</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Scape</th>
<th>Pedicel</th>
<th>Segment 3</th>
<th>Segment 4</th>
<th>Segment 5</th>
<th>Segment 6</th>
<th>Segment 7</th>
<th>Segment 8</th>
<th>Segment 9</th>
<th>Segment 10</th>
<th>Segment 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.74 ±</td>
<td>0.44 ±</td>
<td>0.46 ±</td>
<td>0.40 ±</td>
<td>0.33 ±</td>
<td>0.30 ±</td>
<td>0.28 ±</td>
<td>0.26 ±</td>
<td>0.23 ±</td>
<td>0.20 ±</td>
<td>0.17 ±</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.04</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Female</td>
<td>0.69 ±</td>
<td>0.42 ±</td>
<td>0.42 ±</td>
<td>0.35 ±</td>
<td>0.31 ±</td>
<td>0.28 ±</td>
<td>0.26 ±</td>
<td>0.24 ±</td>
<td>0.21 ±</td>
<td>0.18 ±</td>
<td>0.14 ±</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.08</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>T Statistic</td>
<td>-1.24</td>
<td>-3.40</td>
<td>-2.42</td>
<td>-3.11</td>
<td>-1.03</td>
<td>-1.11</td>
<td>-2.74</td>
<td>-1.11</td>
<td>-1.35</td>
<td>-1.70</td>
<td>-2.80</td>
</tr>
<tr>
<td>df</td>
<td>6.13</td>
<td>5.67</td>
<td>5.37</td>
<td>7.44</td>
<td>6.58</td>
<td>6.65</td>
<td>5.65</td>
<td>5.61</td>
<td>5.43</td>
<td>6.36</td>
<td>6.50</td>
</tr>
<tr>
<td>P value</td>
<td>0.259</td>
<td>0.016</td>
<td>0.056</td>
<td>0.016</td>
<td>0.337</td>
<td>0.307</td>
<td>0.036</td>
<td>0.311</td>
<td>0.229</td>
<td>0.137</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Table 3-2: Maxillary palp segment lengths (mm) and diameters (mm) ± SD. The tip of female maxillary palp is significantly longer and wider at the center compared to males (t = 4.85, df = 9.35, p = 0.001) and (t = 3.76, df = 8.27, p = 0.005) respectively. The second Segment of the female labial palp is significantly shorter and narrower at both ends compared to males (t = 32.5, df = 5, p < 0.001), (t = 5.48, df = 6.3, p = 0.001) and (t = 2.3, df = 9.24, p = 0.049) respectively. Segment 3 is significantly longer and wider on the distal end on females compared to males (t = -3.4, df = 9.0, p = 0.007) and (t = 4.66, df = 7.00, p = 0.002), respectively. Segment 3 is the most distal maxillary palp segment.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Dia Max</td>
<td>Dia Min</td>
<td>Length</td>
</tr>
<tr>
<td>S3</td>
<td>1.01±0.08</td>
<td>0.33±0.04</td>
<td>0.15±0.02</td>
<td>1.28±0.11</td>
</tr>
<tr>
<td>S2</td>
<td>0.67±0.07</td>
<td>0.33±0.04</td>
<td>0.19±0.02</td>
<td>0.80±0.06</td>
</tr>
<tr>
<td>S1</td>
<td>0.74±0.09</td>
<td>0.36±0.02</td>
<td>0.19±0.05</td>
<td>0.94±0.11</td>
</tr>
</tbody>
</table>
Table 3-3: Maxillary palp segment lengths (mm) and diameters (mm) ± SD. The tip of the female labial palp is significantly shorter and narrower at the distal end compared to males (t = 6.6, df = 7.87, p < 0.001) and (t = 8.1, df = 9.4, p < 0.001) respectively. The first Segment of the female labial palp is significantly shorter and narrower at the proximal end compared to males (t = 46.0, df = 5, p < 0.000) and (t = 2.7, df = 9.8, p = 0.029), respectively. Segment 2 is the most distal labial palp segment.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Dia Max</td>
<td>Dia Min</td>
<td>Length</td>
</tr>
<tr>
<td>S2</td>
<td>0.95±0.04</td>
<td>0.33±0.03</td>
<td>0.17±0.02</td>
<td>1.19±0.08</td>
</tr>
<tr>
<td>S1</td>
<td>1.01±0.20</td>
<td>0.34±0.08</td>
<td>0.19±0.03</td>
<td>1.01±0.05</td>
</tr>
</tbody>
</table>
Table 3-4: Ratio of EAG responses standardized to 10 µg of linalool ± SD. EAG responses to pheromone components appear to be dosage dependent. Female antennal response ratios are were greater, but not statistically different compared to males.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alc 1 µg</td>
<td>0.56±0.59</td>
<td>1.26±0.51</td>
</tr>
<tr>
<td>Ald 1 µg</td>
<td>0.39±0.39</td>
<td>1.35±0.45</td>
</tr>
<tr>
<td>Alc 100 µg</td>
<td>0.78±0.03</td>
<td>1.98±0.07</td>
</tr>
<tr>
<td>Ald 100 µg</td>
<td>1.01±0.28</td>
<td>1.63±0.28</td>
</tr>
</tbody>
</table>
Figures

A

B

C
Figure 3-1: SEM images of flattened white scales on various segments of male *A. glabripennis* antennae. Under natural light, these appear as decorative bands encircling the proximal 1/3 of each antennal segment on both males and females. Dense clumps of morphologically similar, if not identical, scales form decorative spots on *A. glabripennis* male and female elytra (see Fig. 3-2). A) Male *A. glabripennis* antenna under natural light with visible black–white banding pattern. Scale bar = 2 mm. B) A group of scales (arrows) on segment 2. Note their typical swollen mid-section (arrow) and narrowed proximal and distal ends. Scale bar = 100 µm. C) Higher magnification of a single scale on Segment 5. Note the swollen, ovoid mid-region and the pointed tip. Scale bar = 10 µm. D) Still higher magnification of a scale’s tip. The scale appears to be hollow (note what appears to be a hole), and this may contribute to the luminous, bright-white appearance of these scales under natural light. Scale bar = 1 µm. Panel D image taken by Kathleen Shields.
Figure 3-2: Cluster of white, broadly flattened scales on female elytra (arrows). These scales bear similarities to the scales located on the antennae. On older females, the scales are sometimes missing. Scale bar = 100 µm. Image taken by Kathleen Shields.
Figure 3-3: Böhm sensilla on the pedicel of male and female *A. glabripennis*. A) Böhm sensilla (arrows) on the female pedicel. As the pedicel rotates inside of the scape, sensilla come into
contact with the side of the scape. Ped = Pedicel, Sca = Scape. Scale bar = 50 µm. **B)** Shorter Böhm sensilla on the male pedicel (arrows). Fine sculpting of the pedicel cuticle is visible on the surface. Scale bar = 50 µm. **C)** Close-up of Böhm sensilla on a female pedicel. Scale bar = 10 µm. Images taken by Kathleen Shields.
Figure 3-4: Distribution of Böhm sensilla on antennae ± SD. There are significantly more Böhm bristles on female *A. glabripennis* compared to males. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females).
Figure 3-5: Long chetiform-1 sensilla from male and female *A. glabripennis*. These sensilla were primarily found toward the distal end of antennal segments. **A)** Distal end of female Segment 3 showing chetiform-1 sensilla (arrows). Scale bar = 100 µm. **B)** Chetiform-1 sensilla on in the middle of male antennal Segment 2 (arrow). Sculpted scales can also be seen across the entire segment. Scale bar = 100 µm. **C)** Chetiform-1 sensilla on the male scape (arrows). Ped. = Pedicel Sca. = Scape. Scale bar = 100 µm. **D)** Close-up of chetiform-1 sensillium on female Segment 4 showing longitudinal striations. Scale bar = 10 µm. Panel **A** and **C** images taken by Kathleen Shields.
Figure 3-6: Distribution of chetiform sensilla on antennae ± SD. There are significantly more chetiform sensilla on Segments 3 - 5 of females than males. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females).
Figure 3-7: Long basiconic sensilla on male and female *A. glabripennis*. These sensilla were observed on the midsegments of flagellomeres. A) Long basiconic sensilla on the tip of male antennal segment 11 (arrows). Sculpted scales can also be seen. Scale bar = 50 µm. B) Long basiconic on female antennal Segment 3 (arrow) between several sculpted scales. Scale bar = 50 µm. C) Long basiconic on male antennal Segment 6 projecting away from cuticle surface. Grooves are possible slit-pores on long basiconic sensillum. Scale bar = 50 µm.
Figure 3-8: Distribution of long basiconic sensilla on antennae ± SD. There are significantly more long basiconic sensilla on the scape and Segment 7 of male beetles compared to females. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females).
Figure 3-9: Trichoid and short basiconic sensilla on *A. glabripennis* antennae. These sensilla may have an olfactory function. **A)** Trichoid sensilla (arrows) interspersed between sculpted scales on male antennal Segment 6. A long basiconic sensilla can also be seen. Scale bar = 100 µm. **B)** Sharp tipped trichoid (Tr) and blunt short basiconic sensilla (Ba) interspersed between sculpted scales on female antennal Segment 9. Trichoid sensilla have raised sockets (arrows) Scale bar = 10 µm. **C)** Cluster of short basiconic and trichoid sensilla (dotted black line) in the middle of female antennal Segment 11. Scale bar = 100 µm. **D)** Hidden pocket of short basiconic sensilla (arrows) at proximal end of a male antennal segment. Scale bar = 100 µm. Panel A, C and D images taken by Kathleen Shields.
Figure 3-10: Distribution of trichoid sensilla on antennae ± SD. Trichoid sensilla are present primarily on Segments 4 – 11. No sexual dimorphism in distribution or abundance was observed.
Figure 3-11: Distribution of short basiconic sensilla on antennae ± SD. Short basiconic sensilla are present primarily on Segments 4 – 11. Male Segments 5, 8 and 11 have significantly more short basiconic sensilla compared to the corresponding female segments. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females).
Figure 3-12: Campaniform sensilla. Campaniform sensilla are circular, raised, structures recessed into Segment 11 of both sexes. These pictures are from female beetles. A) Campaniform sensilla (arrows) hidden between sculpted scales. Scale bar = 10 µm. B) Close-up of campaniform sensilla. The inner and outer diameters can be seen. Scale bar = 5 µm. Images taken by Kathleen Shields.
Figure 3-13: Distribution of campaniform sensilla on antennae ± SD. Basiconic sensilla are primarily found on the tip of antennae. No sexual dimorphism in distribution was observed.
Figure 3-14: Labial and maxillary palps of *A. glabripennis*. Arrows denote Segment 2 and 3 of labial and maxillary palps, respectively on panels A and B. **A)** Posterior female labial palp. Scale bar = 1 mm. **B)** Posterior male maxillary palp. Scale bar = 1 mm. **C)** Close-up of female labial palp basiconic sensilla (arrows) inside the cone on Segment 2. Raised sockets are visible. Scale bar = 50 µm.
Figure 3-15: Sculpted, flattened scales on *A. glabripennis* maxillary palps. These scales closely resemble the sculpted scales on the antennae. **A**) Segment 3 of the female maxillary palp mostly covered with sculpted scales (arrows). Small chetiform sensilla can be seen orthogonal to the cuticle surface. Scale bar = 50 µm. **B**) Close-up of sculpted scales on the Segment 3 of a male maxillary palp. Scale bar = 10 µm.
Figure 3-16: Short mechanoreceptive chetiform-2 sensilla are abundant on Segments 2 and 3 of the labial and maxillary palps, respectively. A) Chetiform-2 (Ch 2), chetiform-3 (Ch 3) and coeloconic sensilla (arrows) are visible. Scale bar = 100 µm. B) Short chetiform-2 sensilla on Segment 2 of a female labial palp. Scale bar = 10 µm. C) Close-up of chetiform-2 sensilla on the male maxillary palp Segment 2. Longitudinal grooves are clearly visible. Scale bar = 1 µm.
Figure 3-17: Distribution chetiform-2 sensilla on *A. glabripennis* mouthparts ± SD. No sexual dimorphism in abundance and distribution was observed. L = Labial palp, M = Maxillary palp. Segments 2 and 3 are the most distal segments of the labial and maxillary palps, respectively.
Figure 3-18: Long chetiform-3 sensilla are concentrated on the proximal palp segments of *A. glabripennis*. A) Long chetiform-3 sensilla on Segment 2 of a female labial palp. Longitudinal grooves are clearly visible. Scale bar = 1 µm. B) Long chetiform-3 sensilla on posterior of male maxillary palp Segment 2. Scale bar = 50 µm.
Figure 3-19: Distribution of chetiform-3 sensilla on the labial and maxillary palps ± SD. Significantly more chetiform-3 sensilla were found on the Second labial palp segment of females compared to males. Asterisks above the bars indicate significant difference in the number of sensilla between the sexes (t-test; P < 0.05; n = 6 males, 6 females). L = Labial palp, M = Maxillary palp. Segments 2 and 3 are the most distal segments of the labial and maxillary palps, respectively.
Figure 3-20: Coeloconic sensilla were observed on the most distal segments of *A. glabripennis* mouthparts. Above photos are from Segment 3 of a female maxillary palp. A) Close-up of coeloconic sensilla. Distribution can be seen in Fig. 3-16A (arrows) Scale bar = 5 µm.
Figure 3-21: Distribution of coeloconic sensilla on *A. glabripennis* mouthparts ± SD. No sexual dimorphism in distribution was observed. Tip is most distal mouthpart segment.
Figure 3-22: Distribution of basiconic-2 sensilla on *A. glabripennis* mouthparts ± SD. No sexual dimorphism in distribution was observed. The tip is the most distal mouthpart segment.
Figure 3-23: Female EAG response after exposure to 100 μg of aldehyde pheromone. Female antennae exhibited a clear depolarization immediately after stimulation by individual male-produced pheromone components. Red bars indicate when the pheromone was puffed over the antenna.
Chapter 4

Conclusions

My work has provided new information concerning the development of improved blends of sex pheromone and plant volatiles to optimize the attraction and capture of this beetle pest to create a more sensitive detection and monitoring tool. In addition, my survey of the sensilla on both male and female antennae will help guide future neurophysiological studies that can assess the neuronal activity of some of the same pheromone components and plant volatiles that comprise some of the attractive blends that I showed to be most active in the field. I initiated neurophysiological assessment of the activity of the pheromone components on both male and female antennae via the electroantennogram (EAG) recording technique and showed that the EAG amplitudes from females in response to the sex pheromone components were greater than those of males.

There are many elements that go into the development of an optimal trap for monitoring and detection of a pest species. The semiochemical lure, of course, is of paramount importance. However, if the trap design is not optimal, then a less sensitive detection tool will be the result.

In 2012, a video recording in Harbin, China, showed an adult female *A. glabripennis* attempting to walk into a trap (Fig. A-3). Only after several minutes and failure to locate a direct path to the lure did she finally give up and fly into the trap. Despite her poor flight skills, she managed to grab the bottom of the trap and eventually climb to the top. During her ordeal, several design flaws became apparent:

1. Beetles would prefer to walk, rather than fly, into traps.
2. Beetles can exploit trap edges and exposed corrugation to maneuver about a trap and avoid slippery areas coated with Fluon.
3. In addition to their poor flight skills, the momentum acquired by large cerambycids in flight allows them to bounce off of traps, which increases the difficulty in trapping them. Modification of the flight intercept trap design could increase trapping efficiency. A larger bottom funnel on traps could increase the chances of catching insects that bounce off of panels while flying. Increasing trap contact with branches, leaves or even adding projections onto the trap could increase the number of direct walking paths to a trap and increase captures of beetles unwilling to fly. Minimization of trap edges and exposed corrugation via a circular or dome shaped trap design could reduce places that *A. glabripennis* could place its tarsi and avoid Fluon treated areas.

Males are not highly attracted to traps baited with a combination of male-produced pheromone and plant volatiles. Instead, males prefer traps baited with plant volatiles alone. To save money and increase the likelihood of capturing males, traps could be deployed in an alternating plant volatile, plant volatile + pheromone pattern. A recently described trail pheromone has been shown to be highly attractive to males when used in its entirety (Hoover et al. 2014). Trail pheromone coated tape could be applied to trees and used to lead male beetles to traps.

The EAG results are a good beginning for understanding how the *A. glabripennis* olfactory system is set up to respond to semiochemicals of importance to this species. However, much more now needs to be done. The receptors tuned to plant volatiles and *A. glabripennis* pheromone components have not been identified. The distribution of trichoid and short basiconic sensilla suggests an olfactory function. Short basiconic and trichoid sensilla form pads on Segment 11 of the antennae on both sexes. It is possible that the trichoid sensilla may be tuned to *A. glabripennis* pheromone components, while the basiconic sensilla may be tuned to plant volatiles, but these hypotheses can only be tested with single sensillum recordings.
The function of the “campaniform” sensilla located on Segment 11 of the *A. glabripennis* antennae is not clear. Campaniform sensilla are generally regarded as mechanoreceptive, but the small size and recessed location I observed seems counterintuitive. The “campaniform” sensilla may actually be extremely short basiconic sensilla, like those seen in *Psylloides affinis* (Ricey and Mciver 1990). *A. glabripennis* taps the tree surface as it walks, which could be indicative of the presence of contact chemoreceptors.

Like the antennae, *A. glabripennis* mouthparts play an important role in olfaction. Beetles constantly taste their substrate while walking. The basiconic sensilla located inside broad, recessed pits at the tip of the maxillary and labial palps may be tuned to trail pheromone components. The coeloconic sensilla located on the exterior palp surface may be used to assess water content or the presence of amines (Guo et al. 2013). Water content and amines could be indicators of host plant quality and used by females to locate optimal oviposition sites.

The scanning electron micrograph study was limited by the quality of the microscope. I was unable to obtain images that could resolve the presence of pores on the basiconic and trichoid sensilla. Future studies could investigate if basiconic sensilla on the mouthparts have terminal pores, which are characteristic of “taste” receptive sensilla (Steinbrecht 1997). Additionally, the presence or absence of pores on antennal sensilla could be investigated. A transmission electron microscope could also be used to search for the presence of sensillar pores.

These two research studies on attractive pheromone and plant volatile blends and *A. glabripennis* sensilla will advance our understanding of cerambycid sensory biology and chemical ecology. By improving our ability to monitor *A. glabripennis*, we can effectively allocate resources to control the beetle’s spread. Morphological studies will provide the foundation for future neurophysiological studies. Together, these studies bring us a step closer to understanding olfaction in *A. glabripennis*. 
Appendix

Field Site Maps of Harbin, China

The following figures illustrate where traps were placed in Harbin, China during the summers of 2012 and 2013. In 2012, trap locations were collected with a Trimble Juno GPS unit. In 2013, trap locations were estimated on the map. Maps were created using ArcGIS 10.1. The forest research plots (left side of maps) contain distinct sections with *Quercus* spp., *Betula* spp. and *Larix* spp.
Figure A-1: Map of trap locations and plots in Harbin, China in 2012. Numbers denote plot number. Red dots indicate trap locations.
Figure A-2: Map of trap locations in Harbin, China in 2013. Different colored dots indicate lures from different manufacturers. Black (data not presented) = combination lures in which all plant volatiles and the alcohol pheromone component were manufactured by ChemTica. Aldehyde pheromone lures were manufactured by Synergy and placed in a pill bag. Pink = lures manufactured by Synergy. Yellow = lures manufactured by ChemTica. ChemTica and Synergy trap lines were rotated in an alternating pattern through the forest every 3 days. Numbers denote plot number.
Still Frames of Beetle Flying into Flight Intercept Trap

The following still frames are from a video of an adult female *A. glabripennis* trying to locate a flight intercept panel trap in Harbin, China on August 1, 2012 in Plot 2. The trap was baited with a 4:1 ratio of alcohol : aldehyde with the 3-plant volatile blend. The following set of events occurred before the video below was captured:

1. Beetle landed on my hand and then flew to the ground.
2. Beetle remained motionless on the ground for a few minutes.
3. The beetle began climbing over blades of grass and located the trunk of the tree that the trap was hung from.
4. The beetle proceeded to walk up the tree trunk and down the branch towards the trap.

This video was taken over a 5-minute interval with a Sony Cybershot DSC-T99 digital camera. Preceding videos (not shown) show the beetle climbing up the tree trunk and wandering on other branches before finally locating the branch closest to the trap.
Figure A-3: Still frames of a female *A. glabripennis* flying into a flight intercept panel trap in Harbin, China. The beetle spends several minutes searching for a direct, walkable path to the trap at the end of the branch before conceding and flying into the trap. Blue boxes indicate the beetle’s location.
References


