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# Host Volatile Percepts of Two Sympatric Longhorned Beetles, Anoplophora Chinensis and Anoplophora Glabripennis

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# HOST VOLATILE PERCEPTS OF TWO SYMPATRIC LONGHORNED BEETLES, *ANOPLOPHORA CHINENSIS* AND *ANOPLOPHORA GLABRIPENNIS*

by

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# ABSTRACT

**L.E. Hansen.** Host Volatile Percepts of Two Sympatric Longhorned Beetles, *Anoplophora Chinensis*, and *Anoplophora Glabripennis*, 151 pages, 20 tables, 11 figures, 2020. APA style guide used.

*Anoplophora chinensis* (CLB) and *Anoplophora glabripennis* (ALB) are sympatric sibling species of pest lamiine cerambycids. Both are destructive invasives under strong domestic and international focus. Monitoring lures for both species need improvement. Under the current ratio hypothesis of insect host detection, insects orient towards their hosts via identification of a host-indicative, attractive blend of multiple volatile compounds. I evaluated multivariate statistical comparison of host versus non-host hardwood volatiles as a method for simultaneously identifying host-indicative compounds for both species. Statistical methods determined the commercially unavailable (*E*)-4,8-dimethyl-1,3,7-nonatriene was indicative of CLB hosts and a multicomponent blend including benzyl acetate, α-humulene, (*E*)-nerolidol, (*E*)-caryophyllene, isoamyl benzoate, and 6-methyl-5-hepten-2-ol was indicative of ALB hosts. I hypothesized that the CLB host indicative blend is inclusive of the ALB host indicative blend and tested the six ALB host-indicative compounds for attraction to both species in Bengbu, China. Field trapping treatments were host volatiles only, male pheromone only, host volatiles + pheromone, and isopropanol control. Pheromone containing treatments captured significantly greater numbers of CLB with the host volatile + pheromone treatment capturing the greatest number of CLB. To further examine intraspecies chemical communication between ALB and CLB, cuticular hydrocarbon extracts from ALB and CLB were collected. Stepwise discriminate analysis showed differences in samples by species and sex, illustrating that ALB and CLB males and females can be identified by their cuticular extracts. In addition, principle component analysis indicated ALB cuticular hydrocarbon samples collected from beetles from Hunchun, Jilin, diverged from the rest of the samples. This research found supporting evidence for the ratio hypothesis of insect host detection, characterized the cuticular hydrocarbons of ALB and CLB, and identified potential geographic variation in ALB cuticular hydrocarbons.



Key Words: *Anoplophora chinensis*, *Anoplophora glabripennis*, cerambycidae, insect host detection, host percepts, plant volatiles, gas chromatography electroantennography, kairomones, semiochemicals, field trapping



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# CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

## Historical Background

The miraculous ability of male moths to track female conspecifics over long distances and the sudden appearance of beetles at temporal host resources has long puzzled scientists. Although insect chemical repellent use stretches back to pre-history, chemical attractants were less noted, and the strong chemosensory nature underlying insect conspecific communication and insect-host attraction is a modern consensus (Dethier 1947). The chemical compounds involved in this intra- and interspecific communication, termed semiochemicals, are the major focus of insect chemical ecology. Behavioral observations, physiological discoveries, and advances in analytical chemistry preceded the adoption of volatile insect semiochemicals in integrated pest control strategies.

One of the first behavioral observations linking insect-host attraction to an underlying volatile chemical basis was the observation of *Pieris brassicae* and *Pieris rapae* orientation towards mustard oil producing botanical garden plants (Verschaffelt 1910, Dethier 1947). Further studies continued to support the then novel theory of chemotropism, the instinctual orientation towards or away from chemical odorants (Loeb 1918), including a 1919 physiological verification that Lepidopteran larvae possess olfactory organs and an olfactory sense, in which the publication immediately notes the immense pest control possibilities (McIndoo, Dethier 1947). Following the discovery of attractants for two invasive agricultural pests – a *Graphiolita molesta* attracting molasses-yeast bait in 1925 (Peterson), and a *Popillia japonica* attracting geraniol bait in 1927 (Richmond, Dethier 1947), the use of long-range volatile attractants as lures for pest insects gained even more interest. However, physiological understanding was still preliminary, and experiments designed to counter disbelief about the

centrality of the insect antenna in insect-host orientation were still being performed well into the 1930's (reviewed in Marshall 1935) when Wigglesworth and Gillett published their experiments comparing the ability of blinded, antenna-less, and proboscis-less *Rhodnius prolixus* to detect their hosts (1934).

Despite increasing physiological and behavioral understanding, the limited development of analytical chemical techniques delayed the discovery of insect-produced volatile conspecific attractants until the 1960's, when the chemical characterization of bombykol, the *Bombyx mori* long-range volatile female sex pheromone, was published (Schneider 1984). The decades of effort spent identifying bombykol inspired a search for an expedited method, and electroantennography, a technique that applies knowledge of insect physiology to identify biologically important odorants by measuring the electrical signals transduced through insect antenna when presented with a detectable, or antennally-active odorant, was developed. Electroantennography was later paired with gas chromatography, and GC-EAD, a technique for identifying the individual antennally active compounds in a mixture resulted in an explosion in the number of identified pheromones and other insect attractants (Moorhouse et al. 1969). Further research on insect chemical ecology with the aim of identifying attractants for practical use in pest control must continue to explain behavioral observations, translate new discoveries in insect physiology, and take advantage of novel analytical chemistry techniques and advances.

**The Physiology of Insect Olfaction and the Ratio Hypothesis**

Recent insight on the molecular physiology of insect olfaction has greatly informed knowledge on insect-host detection mechanisms. While much of this research has been performed on model organisms with sequenced genomes, such as *Drosophila melanogaster*

(Stocker 1994, Carlson 1996), integral gene families and basic mechanisms are widely conserved across class Insecta (Krieger et al. 2003, Fleischer et al. 2018, Robertson 2018).

The translation of external, chemical odorant signals into internal, peripheral neurobiological signals occurs within chemoreceptor olfactory sensilla located on insect antenna, palps, or other sensory organs (Steinbrecht 1997). Although sensilla have a variety of different morphologies including trichoid and basiconic shapes (Keil 1999), the physiological importance of these different morphologies remains poorly understood (Yuvaraj et al. 2018). Olfactory sensilla are innervated by one to several olfactory sensory neurons (OSNs) that extend into the insect's central nervous system, where they form visually distinct neural clusters termed glomeruli (Carlson 1996, Gao et al. 2000, Vosshall et al. 2000). Exposure to a detectable chemical odorant at a physiologically relevant dose triggers OSN firing, relaying an electrical signal containing information on the chemical identity, temporal variation, spatial variation, and dosage of the odorant signal for further interpretation by higher neural processes (Vickers et al. 2001, Hallem and Carlson 2006).

The chemical sensitivity and selectivity of OSNs depends on the expression of several different classes of proteins, including soluble odorant binding proteins (OBPs) and odorant-degrading enzymes (ODEs) (Leal 2013, Pelosi et al. 2017) as well as OSN-membrane-bound olfactory receptors (ORs), gustatory receptors (GRs), and ionotropic receptors (IRs) (Clyne et al. 1999, Gao and Chess 1999, Vosshall et al. 1999, Kwon et al. 2007, Benton et al. 2009). Odorants first enter olfactory sensilla through odorant pores and dissolve in the sensillum lymph (Vogt and Riddiford 1981) where they interact with soluble proteins, including OBPs and ODEs. Although OBPs and ODEs were among the first characterized insect olfactory proteins (Vogt and Riddiford 1981), their physiological importance remains poorly understood (Leal 2013, Pelosi et

al. 2017). Work in empty neuron models (Hallem, Fox, et al. 2004, Hallem, Ho, et al. 2004) and non-insect models (Wetzel et al. 2001) has shown OBPs are not necessary for signal transference, although they may influence odorant detection by increasing the solubility of key odorants or activating OSNs as ligand-bound odorant complexes (Pelosi et al. 2017). ODEs are believed to clear the lymph of dissolved odorants, accelerating signal termination (Leal 2013). After dissolving in the sensillum lymph, odorant molecules interact with the OSN-membrane-bound receptors (Leal 2013). IRs and ORs/GRs are believed to be two independent, evolutionary distinct lineages of volatile odorant-detecting receptors (Silbering et al. 2011, Robertson 2018). GRs, while primarily taste-receptors, have been implicated in $CO_2$ detection (Jones et al. 2007), and IRs are believed to be an ancestral lineage that detects specific classes of odorants including carboxylic acids and amines (Silbering et al. 2011).

The role of ORs and their respective olfactory receptor neurons (ORNs) in volatile odorant detection has received more attention than either IRs or GRs (Fleischer et al. 2018). Most commonly, each type of ORN expresses two ORs, a highly-conserved olfactory co-receptor (Orco) and a variant, non-conserved, ligand-specific OR (Clyne et al. 1999, Gao and Chess 1999, Vosshall et al. 1999, Vosshall and Hansson 2011, Robertson 2018). The odorant specificity of ORNs is primarily determined by their expressed ligand-specific OR (Larsson et al. 2004). The structure of Orco homotetramer ligand-gated calcium-ion channels has recently been resolved and it is hypothesized that Orco and ORs bind to form similar heterotetramer channels, allowing for simultaneous conservation of OR function by the conserved Orco and quick response to evolutionary pressures by the poorly-conserved ligand-specific OR (Butterwick et al. 2018, Zufall and Domingos 2018).

OR tuning and "public" versus "private stimuli" are two critically important concepts in the understanding of insect peripheral nervous system interpretation of odors (de Bruyne et al. 2001, Leal 2013, Wicher 2018). ORs may be narrowly tuned, responding to only one chemical odorant, or broadly tuned, responding to multiple chemical odorants. For example, a narrowly tuned OR may respond only to a pheromone molecule or a broadly tuned OR may respond to multiple different host volatile terpenes. Private stimuli refers to a response to an odorant by one ligand-specific OR only, while public stimuli are recognized by multiple different ligand-specific ORs. For example, representing its critical importance, a pheromone molecule may be recognized by one type of OR only, providing a direct, "labeled line", private stimulus, while a host volatile terpene may be recognized by multiple different ORs (Leal 2013, Haverkamp et al. 2018). This combination of broad tuning, narrow tuning, private stimuli, and public stimuli provides the central nervous system with a complex signal representing any given odor and allows for the existence of "combinatorial code", where a relatively small number of ORNs are, in theory, capable of producing distinct peripheral neurobiological signals representing a massive number of different odorants (Vosshall et al. 2000, Bruyne and Baker 2008). Many attempts have been made to interpret the patterns of firing neurons representing pheromone odors, attractive odors, and repulsive odors in the antennal lobe of the insect brain, especially in *Drosophila*, where numerous different maps have been published (Vosshall et al. 1999, Knaden et al. 2012). However, understanding of higher neural processing, which involves glomeruli-connecting lateral neurons and projection neurons extending in the mushroom body/lateral horn, has not progressed enough to fully link current behaviorally supported, top-down hypotheses about the detection of host plants by phytophagous insects to these associated neurobiological

physiological processing mechanisms (Ng et al. 2002, Wilson 2004, Bruyne and Baker 2008, Seki et al. 2017, Haverkamp et al. 2018).

Initially, there were two main behavioral hypotheses for how phytophagous insects detect host plants: the ratio and single-compound hypotheses. Current evidence supports the ratio hypothesis, where insects detect their hosts by identifying host indicative mixtures of common odorants whose temporal pattern and relative ratio combined with the absence of non-host indicative compounds distinguishes them from habitat odor, rather than the single-compound hypothesis, where insects detect unique, host-specific compounds (Bruce et al. 2005, Bruce and Pickett 2011). For example, *Cydia molesta* orient strongly towards a host-imitating synthetic blend of 4:1:1 (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol and benzaldehyde, all extremely commonly produced green plant volatiles, rather than any compound alone (Natale et al. 2003, Bruce et al. 2005). In another example, although *Aphis fabae* strongly orient towards a host-imitating mixture of 15 volatiles, when those volatiles were tested individually, 10 were repellent (Webster et al. 2010, Bruce and Pickett 2011). Although the complete odor profile of any host plant may be cost-prohibitive to replicate, complex odorant mixtures appear to have redundant compounds, and are reducible to a handful of important odorants (Riffell et al. 2009, Bruce and Pickett 2011, Gregg et al. 2018, Haverkamp et al. 2018). Successful trap-and-kill or other attractant volatile pest control strategies are hypothesized to be effective using only four volatiles in an appropriate ratio (Szendrei and Rodriguez-Saona 2010, Gregg et al. 2018). These behavioral data are well explained by the previously discussed physiological discoveries – host odorants are apparently recognized by combinations of ORNs whose tandem firing into the insect brain produces a host-indicative percept that triggers orientation towards the host (Bruce and Pickett 2011).

Genetic mutation of olfactory genes has been implicated in host shift by phytophagous insects, followed by subsequent development of host races, reproductive isolation, then speciation, providing further insight on insect host-detection (Forbes et al. 2017, Vertacnik and Linnen 2017). For example, *Drosophila sechellia*'s preference for its host morinda fruit is linked to a loss-of-function mutation in OBP56e, whose knockdown in *Drosophila melagonaster* significantly decreases the repellency of morinda fruit (Dworkin and Jones 2009). However, this remains a unique example, as very few organisms are well-understood enough to produce similar elegant, interdisciplinary explanations of behavioral phenomena. More research is needed on closely related species of insects to further benefit from these types of analyses.

**Cerambycids: Importance, Life History, and Phylogeny**

Cerambycids (Order: Coleoptera, Superfamily: Chrysomeloidea), commonly known as longhorned beetles, are a family of over 35,000 species documented on all continents apart from Antarctica (Tavakilian 2019). Their common name refers to their characteristically long antennae, which may be several times greater than their body length. The functional significance of these long antennae has been under debate, with suggestions that they may be used for balance, long-distance olfaction, male-male competition, or ambulatory mate-seeking by males (Hanks et al. 1996, Kariyanna 2017). Cerambycids are also notable as one of the few animals to have evolved cellulases, allowing their xylophagous larva to directly digest cellulose from woody tissues, rather than relying on symbiotes, taking advantage of a poorly occupied ecological niche (Watanabe and Tokuda 2010).

Cerambycid research is prompted by the many notorious agricultural and forest pest species prone to outbreaks and invasions into new geographic regions. Ten of the 86 most

damaging Chinese forest pests are cerambycids (Ji et al. 2011), two of the ten most costly invasive insect species globally are cerambycids (*Tetropium fuscum* and *Anoplophora glabripennis*) (Bradshaw et al. 2016) and, as of 2019, the CABI Invasive Species Compendium database maintains datasheets on approximately 40 invasive cerambycids (CABI 2019). In addition, cerambycids are valued for their cultural and aesthetic importance and several cerambycids of conservation concern have been ranked as vulnerable to critically endangered by the IUCN (2018), For example, significant efforts are underway to conserve the previously endangered and now vulnerable *Rosalia alpina*, a beautiful beetle with a striking blue-grey and black striped coloration (Kosi et al. 2017). Increasing understanding of cerambycid chemical communication is necessary for the conservation of vulnerable species as well as the control of destructive pests.

Cerambycid life-history has made their management difficult. Typically, after fertilization by a sexually mature male, female adults oviposit near or on the woody surfaces of host plants. Depending on the species, healthy, stressed, recently deceased, or partially decomposed plants as well as specific host parts such as roots, branches, or trunks may be preferred or necessary for successful larval development. Newly eclosed larvae tunnel into woody tissues, forming larval galleries that permanently damage or degrade the host and protect the larvae from predation, parasitism, environmental stressors, and anthropogenic attempts at pest control. After pupation in an incubation chamber, the adult beetle ecloses, then emerges from the host through a chewed exit hole, further damaging host tissues. Some cerambycid species are obligate adult feeders and do not reach sexual maturity until after days to weeks of maturation feeding, while others do not feed as adults. Many species are good adult fliers and readily disperse in search of new host resources, while others are flightless. Although most

cerambycids are univoltine to semivolitine, overwintering as eggs or larvae, bivolitine and multivolitine generation times are commonly reported. Due to this life history the timing of management strategies including the introduction of predators such as woodpeckers, parasitoids such as wasps or beetles, egg niche hammering, pesticide injection into larval tunnels, mating disruption using pheromones, and/or pesticide application are crucial and mistimed efforts may miss the appropriate life stage window. Thus, accurate understanding and monitoring of beetle populations is necessary for successful pest control. (Wang 2017)

There are eight recognized subfamilies of cerambycids (Švácha et al. 2014, Wang 2017). A recent attempt to resolve the subfamily phylogeny suggests that the Prioninae + Parandrinae are most closely related to the potentially polyphyletic Cerambycinae + Dorcasominae. Together, these four subfamilies are most closely related to the Lepturinae + Necydalinae, followed by the + Lamiinae and the Spondylidinae (Figure 1-1) (Haddad et al. 2018). Cerambycid subfamilies differ in their morphology, host selection, and life history patterns. Importantly, the Spondylidinae, Prioninae, and Parandrinae do not feed as adults, the Lamiinae feed primarily on bark, stems, leaves, and/or needles, and the Lepturinae and Cerambycinae are divided, with representative feeding and apparently non-feeding species (Wang 2017).

## Cerambycid Long-Range Volatile Attractants: General Research Directions and Established Themes

Discoveries of cerambycid long-range volatile attractants parallel the progression of insect attractant knowledge in general. Initially, orientation towards hosts and other indicators of host availability were recorded, including the attraction of *Hoplocerambyx spinicornis* to fallen Green Sál Trees and the attraction of several species to smoke and pine oils (Stebbing 1914,

Beeson 1930, Becker 1942, Gardiner 1957, Linsley 1959). Early reports suggesting attractants as solely chemical in nature included the capture of several cerambycid species using *Grapholita molesta* molasses-yeast traps (Champlain and Kirk 1926, Frost and Dietrich 1929, Champlain and Knull 1932) and observed orientation towards synthetic paints and watermelon rinds (Gardiner 1957, Chemsak 1958). However, the chemical identity of the volatile attractants was limited by the available analytical technology. It was not until 1983, after the fermentation product ethanol was shown to be produced by stressed and decaying trees, and following its discovery as a bark beetle attractant, that it was reported as one of the first single-compound cerambycid attractants (Montgomery and Wargo 1983). Similarly, conifer monoterpenes found in pine oils, including α-pinene, especially in synergy with ethanol, were identified as attractive to many species of conifer-feeding and decaying-host-feeding cerambycids (Ikeda et al. 1980, Chénier and Philogène 1989).

The first long range volatile sex pheromone was discovered in the cerambycine *Xylotrechus pyrrhoderus* using GC-EAD (Sakai et al. 1984). Since then, hundreds of cerambycid pheromones and attractive volatile semiochemicals have been reported, and attempts to reveal general rules governing cerambycid chemical ecology suggest the critical importance of subfamily, life history, and host choice (Hanks 1999). The following pages briefly summarize known cerambycid chemical attractants by subfamily, highlighting seven general trends that have greatly influenced research directions. Although acceptance of these trends has led to the discovery of numerous attractants, they may not be as widely applicable as assumed.

Chemical attractants have been identified for cerambycids in six subfamilies: the Spondylidinae, Prioninae, Lepturinae, Cerambycinae, and Lamiinae. Although there is evidence for a female sex pheromone in Necydalinae, it has not been identified (Curkovic and Ferrera

2012). The following review draws heavily from the chapter "Chemical Ecology of Cerambycids" by Hanks and Wang published in Cerambycidae of the World: Biology and Pest Management (Wang 2017).

*Spondylidinae*

Research on Spondylidinae pheromones and host volatile attractants provide excellent examples of seven general trends that have greatly influenced research on cerambycid long-range chemical attractants.

**(1) Pheromone components and motifs are often conserved and used by multiple closely related species** (Hanks and Millar 2016)**.** The first discovered spondylidine long-range pheromone, the *Tetropium fuscum* male aggregation pheromone, (*S*)-fuscumol ((*2S,5E*)-6,10-dimethyl-5,9-undecadien-2-ol) (Silk et al. 2007) was later found to attract five species of *Tetropium* (Silk et al. 2007, Sweeney et al. 2010, Hanks and Millar 2013, Halloran et al. 2018). Geranyl acetone has also been identified as both the single component long-range pheromone of *Asemum caseyi*, and an integral part of the *Asemum nitidium* pheromone blend of geranyl acetone and (*S*)-fuscumol (Halloran et al. 2018).

**(2) Long-range attractant pheromones are more readily discovered in species of cerambycids with life history patterns that stress quick adult reproduction** (Hanks 1999)**.** Spondylidinae do not feed as adults, a life history pattern that stresses quick reproduction and is hypothesized to increase the importance of pheromone use in mate seeking. Considering the small size of the subfamily, approximately 100 species (Tavakilian 2019), the short list of Spondylidinae with identified pheromones is a very high success rate. Spondylidinae appear to

be readily attracted to lure blends containing their male-produced pheromones, making their use in monitoring traps effective (Sweeney et al. 2010)

**(3) Cerambycid pheromone-producing and pheromone-attracting sexes show subfamily conservation** (Hanks and Millar 2016)**.** All verified spondylidine pheromones are male-produced aggregation pheromones (Silk et al. 2007, Sweeney et al. 2010, Hanks and Millar 2013, Halloran et al. 2018).

**(4) Long-range volatile pheromones may be recognized only as blends of multiple compounds in a specific ratio rather than as single chemical compounds**. The two *A. nitidium* pheromone components are not additive, rather, lure formulations containing host volatiles must include both (*S*)-fuscumol and geranylacetone if they are to capture greater numbers than a host volatile control (Halloran et al. 2018).

**(5) Conifer and or dead/stressed tree feeding cerambycids are attracted to ethanol and/or conifer-produced terpenes. Host attractants for healthy-tree angiosperm feeders are poorly understood** (Collignon et al. 2016)**.** Prior to the discovery of any attractive pheromones, blends of spruce-produced terpenes in synergy with ethanol were known to be good *Tetropium* attractants (Sweeney et al. 2004). In addition, various other conifer-feeding Spondylidinae are known to be attracted to pine terpenes, ethanol and smoke (Chénier and Philogène 1989, Suckling et al. 2001, Jurc et al. 2012).

**(6) Cerambycid pheromones may only be attractive in the presence of host volatiles or other host-indicative compounds**. During *Tetropium fuscum* pheromone identification, when potential pheromone compounds were tested as lures in the field with and without the previously discovered spruce-produced terpene and ethanol attractants, it was discovered that although (*S*)-fuscumol is an effective attractant in synergy with host compounds, capturing greater numbers of

individuals than host compounds alone, as a single compound it is incapable of capturing more *Tetropium* than a blank trap (Silk et al. 2007, Collignon et al. 2016). Particularly in ALB, attempts at pheromone identification have failed or uncovered only weakly attractive compounds despite decades of research efforts (Hanks and Millar 2016). The difficulty of simultaneously discovering integral host volatile synergists along with a potential multi-component pheromone blend may explain this difficulty.

**(7) Compound stereochemistry has critical biological importance.** (*S*)-fuscumol stereochemistry is crucial, with the lure formulations containing the (*R*) enantiomer capturing no more beetles than the corresponding controls (Sweeney et al. 2010).

*Prioninae*

Research in the Prionidae provides good examples of five of the previously discussed seven trends. Multi-component pheromone blends **(4)** and host volatile-pheromone synergy **(6)** has not been noted in the Prioninae.

**(1: Pheromone conservation)** Following its discovery in *Prionus californicus* (Rodstein et al. 2009), the female-produced pheromone prionic acid was quickly discovered to attract more than 10 additional species of Prionini in multiple continents (Barbour et al. 2011, Wickham, Lu, et al. 2015, Hanks et al. 2018). In addition, the two other known prionine pheromones, the *Megopis costipennis* pheromone (*2R, 3S*)-2,3-octanediol (Wickham, Millar, et al. 2015), and the *Tragosoma depsarium pheromone* (*2R, 3R*)-2,3-hexanediol pheromone (Ray, Barbour, et al. 2012) are also used by several other species in the Cerambycinae. **(2: Life history importance)** Prioninae adults are non-feeding and *P. californicus* females are poor fliers with short adult lifespans (Rodstein et al. 2009). Considering the relatively small size of the Prioninae

(approximately 1,000 species (Tavakilian 2019), the current number of species with known pheromone attractants is relatively high. Prionic acid is also so attractive that baited traps can be used for mating disruption (Maki et al. 2011). **(3: Pheromone-producing sex)** All pheromones in the Prionidae are female-produced sex pheromones (Rodstein et al. 2009, Ray, Barbour, et al. 2012, Wickham, Millar, et al. 2015). **(5: Host volatile attractants)** a conifer-feeding *Prionus* species (Beutenmuller 1896) is known to be one of the many pine-feeding cerambycids attracted to α-pinene (Miller 2006) but little is known about host volatile attractants for angiosperm feeding Prioninae including *P. californicus*. **(7: Stereochemistry)** *P. californicus* males are only attracted to the (*3R, 5S*) enantiomer of prionic acid (Rodstein et al. 2011).

*Lepturinae*

As in the Prioninae, multi-component pheromone blends **(4)** and host volatile-pheromone synergy **(6)** are not noted in the Lepturinae, although the subfamily provides good examples of the other five trends.

**(1: Pheromone conservation)** Although the first discovered lepturine long-range volatile sex attractant, the *Ortholeptura valida* female sex pheromone, *cis*-vaccenyl acetate ((*Z*)-11-octadecen-1-yl acetate) (Ray et al. 2011), is only known to attract a single species. The *Desmocerus californicus californicus* produced (*R*)-desmolactone ((*4R,9Z*)-hexadec-9-en-4-olide) was later found to attract multiple *Desmocerus* species (Ray et al. 2014). **(2: Life history importance)** The Lepturinae are a mix of adult-feeders and apparently non-feeding adults. For example, *Desmocerus californicus californicus* are adult-feeders that feed on the flowers and foliage of living elderberry (Ray et al. 2014) while *Ortholepura valida* oviposit on stressed to deceased conifers and may not feed as adults (Ray et al. 2011). Although at approximately 2,000

species (Tavakilian 2019), the Lepturinae are twice the size of the Prioninae subfamily and many times larger than the Spondylidinae, fewer than half of the species have known sex attractants. **(3: Pheromone producing sex)** Both Lepturinae pheromones are female-produced sex pheromones (Ray et al. 2011, Ray, Swift, et al. 2012). **(5: Host attractants)** Several conifer-feeding species of Lepturinae are known to be attracted to pine terpenes, ethanol, and smoke (Montgomery and Wargo 1983, Chénier and Philogène 1989, Sweeney et al. 2014). Although their effectiveness compared to a control has not been consistently evaluated, the floral compounds benzyl acetate, methyl phenylacetate, linalool, and methyl benzoate have captured numerous lepturine individuals and are among the few known attractants for non-conifer feeding cerambycids (Sakakibara et al. 1996, 1997, 1998, Shibata et al. 1996). **(7: Stereochemistry)** (*R*)-desmolactone stereochemistry is vital and alternate stereoisomers are unattractive (Ray, Swift, et al. 2012, Ray et al. 2014).

*Cerambycinae*

The subfamily Cerambycinae provides good examples of all seven general trends.

**(1: Pheromone conservation)** The vast majority of long-range volatile cerambycine pheromones have hydroxyketone and/or 2,3-alkanediol motifs, including the first discovered cerambycid volatile pheromone, the *Xylotrechus pyrrhoderus* male-produced blend of (*2S, 3S*)-octanediol and (*2S*)-hydroxy-3-octanone (Sakai et al. 1984, Wang 2017). These compounds are so widely used by cerambycines that field trapping experiments using mixtures of conserved compounds can simultaneously attract multiple species, including previously unstudied species in novel geographic areas (Hanks et al. 2007, 2018, Wong et al. 2012, Hanks and Millar 2013, Imrei et al. 2013, Sweeney et al. 2014, Wickham et al. 2014, Handley et al. 2015, Hayes et al.

2016, Miller et al. 2017, Silva et al. 2017, Wang 2017, Fan et al. 2018, Millar et al. 2018, Rassati et al. 2018). **(2: Life history importance)** The Cerambycinae (approximately 12,000 species (Tavakilian 2019)) do not feed as adults and many species, the highest number of any subfamily, have known, highly-attractive, long range pheromones. However, it is important to note that volatile pheromones in some species remain unidentified despite a great deal of research effort (Hanks and Millar 2016). **(3: Pheromone-producing sex)** All known long-range volatile pheromones in the Cerambycinae are male-produced and **(4: Pheromone blends)** Cerambycine pheromones are often blends of multiple compounds. For example, a 80:20 to 95:5 diol to ketone ratio is the most attractive to *Xylotrechus pyrrhoderus*, while either compound alone is unattractive (Sakai et al. 1984). It is hypothesized from cerambycine research that the addition of minor compounds allows cerambycids to discriminate their pheromone from other species with similar blends, a phenomenon that has been also observed in other species of insects (Mitchell et al. 2015).

**(5: Host attractants)** Conifer and stressed/dead host-feeding cerambycines are found among insects captured in pine terpene / ethanol baited traps. In addition, field trapping using various antennally-active host volatiles found that *Anaglyptus subfasciatus* and *Demonax transilis* were significantly more attracted to methyl phenylacetate or benzyl acetate than a control (Ikeda et al. 1993, Nakashima et al. 1994, Mizota 1997, Nakamuta et al. 1997), an additional rare example of host volatile attractants for floral feeding cerambycids. Multiple attempts have been made to identify volatiles attractive to oak feeding cerambycids (Dunn and Potter 1991). Although indicators of fermentation were able to significantly trap *Cerambyx welensii*, a dead-oak feeder, the addition of oak terpenes did not synergize attraction (Sánchez-Osorio et al. 2015). An additional attempt to develop a synthetic, oak-imitating volatile blend did

not synergize attraction to a blend of cerambycid pheromones (Collignon et al. 2016), highlighting the apparent difficulty in reproducing angiosperm-indicating host volatile blends.

**(6: Synergy)** Trapping experiments including host volatiles in combination with potential cerambycine attractants have led to the identification of new attractant lures for several species of cerambycines (Hanks et al. 2012, Hanks and Millar 2013). In addition, field trapping using the previously mentioned *Anagylptus subfasciatus* host volatile attractants in combination with sex pheromone was able to capture significantly more female beetles than either host volatiles or sex pheromone alone (Ikeda et al. 1993, Nakashima et al. 1994, Mizota 1997, Nakamuta et al. 1997). **(7: Stereochemistry)** pheromone stereochemistry has repeatedly been shown to be critically important to the extent that incorrect isomers may be inhibitory.

*Lamiinae*

Research on ALB and CLB is discussed in detail in a following section. Pheromone identification in the Lamiinae has shown to be particularly tricky. The assumption that the previously discussed trends will remain consistent within the Lamiinae has been both advantageous and problematic. Although the widespread application of general trends in cerambycid chemical ecology has proven immensely practical, it is prone to confirmation bias, and in some cases, trends may not be as widely applicable as they first appear.

**(1: Pheromone conservation)** The Lamiinae provide several examples of multispecies attraction to a conserved pheromone component. The male-produced Lamiinae pheromone monochamol was found to attract many species of *Monochamus* as well as several other lamiines across multiple continents (Pajares et al. 2010, Teale et al. 2011, Macias-Samano et al. 2012, Wickham et al. 2014, Ryall et al. 2015). Fuscumol and geranyl acetone have also been identified

as *Hedypathes betulinus* male-produced pheromone components (Fonseca et al. 2010). Fuscumol and fuscumol acetate have been found to attract many species of lamiines and the compounds are currently used as part of screening field bioassays (Mitchell et al. 2011, Wong et al. 2012, Hanks and Millar 2013, Sweeney et al. 2014, Wickham et al. 2014, Handley et al. 2015, Fan et al. 2018, Hanks et al. 2018, Millar et al. 2018, Rassati et al. 2018).

**(2: Life history importance)** Although the Lamiinae are the largest Cerambycid family (approx. 21,000 species (Tavakilian 2019)), relatively few sex pheromones have been discovered and the chemical ecology of many species remains cryptic. Lamiines feed as adults and may undergo a period of post-eclosion maturation prior to sexual maturity. This life history may explain why so few long-range volatile pheromones have been discovered in what is the largest cerambycid subfamily (Hanks 1999). In addition, a relatively early publication based on observations of *Tetraopes tetrophthalmus* suggested the species relies entirely on host volatiles and does not use long-range pheromones (Reagel et al. 2002). This paper contributed to an early hypothesis that cerambycids only weakly rely on pheromones and that host volatile-mediated communication predominates (Hanks 1999, Allison et al. 2004). Although the discovery of numerous cerambycid pheromones has since contradicted this idea (Hanks and Millar 2016), it should be remembered that pheromones have been identified for an extremely small percentage of cerambycid species, many of which have been discovered through screening trapping that is inherently biased towards pheromone identification and this early hypothesis stressing host volatile importance has not been disproven.

**(3: Pheromone-producing sex)** In contrast to other cerambycid subfamilies, the long-range pheromone producing sex is less clear in the Lamiinae. Although the ALB and CLB long-range pheromone (Zhang et al. 2002, Hansen et al. 2015) and monochamol (Pajares et al. 2010)

are both male-produced aggregation pheromones, research on ALB has also suggested that female produced long-range pheromones may also be important (Wickham et al. 2012, Xu 2018).

**(4: Pheromone blends)** Several lamiine pheromones are blends. For example, *Hedypathes betulinus* is only attracted to its pheromone blend of fuscumol acetate, fuscumol, and geranyl acetone, rather than either compound alone (Fonseca et al. 2010).

**(5: Host attractants)** The attraction of conifer-feeding or dead-wood feeding lamiines to conifer produced terpenes and ethanol is well documented. Interestingly, many species of Lamiinae are attracted to bark beetle pheromones, appearing to recognize the compounds as indicative of a temporally limited host resource (Allison et al. 2001). Host volatile attractants for healthy-tree or angiosperm lamiines are poorly understood.

**(6: Synergy)** Several of the screening tests using conserved pheromone components have tested for synergy using host volatiles. In a 2012 study, ethanol and/or α-pinene synergized the attraction of *Astylidius parvus*, *Astyeiopus variegatus*, *Graphisurus fasciatus*, *Lepturges angulatus* and *Monochamus carolinus* to a pheromone mixture. Notably, although ethanol significantly synergized *Graphisurus fasciatus* attraction to the pheromone blend, a mixture of ethanol and α-pinene inhibited attraction (Hanks et al. 2012). In further studies, α-pinene, β-pinene, and/or ethanol either inhibited attraction or synergized the attraction of many lamiines to a pheromone blend containing fuscumol, monochamol and/or fuscumol acetate (Hanks and Millar 2013, Handley et al. 2015, Hayes et al. 2016, Hanks et al. 2018). As previously mentioned for cerambycines, attempts to develop synthetic, oak-imitating volatile blend did not synergize the attraction of oak-feeding lamiines to a fuscumol-containing blend of cerambycid pheromones (Collignon et al. 2016).

**(7: Stereochemistry)** Pheromone stereochemistry is also critically important in the Lamiinae. For example, *Astylidius variegatus* is attracted to (*R*)-fuscumol, while *Astylopsis macula* is attracted to (*S*)-fuscumol (Hughes et al. 2016). It is hypothesized that stereochemistry differences may allow cerambycids to discriminate between similar pheromone blends (Meier et al. 2016).

**ALB and CLB: Taxonomy, Hosts, Life History, and Pest Status**

Asian Longhorned Beetle (ALB, *Anoplophora glabripennis*) and Citrus Longhorned Beetle (CLB, *Anoplophora chinensis*) (Subfamily: Lamiinae, Tribe: Lamiini) are sympatric cerambycids native to East Asia. ALB is native to both mainland China and the Korean Peninsula (CABI 2019) with the yellow-spotted form found in Northern China historically recognized as *Anoplophora nobilis* currently junior to *A. glabripennis* (Lingafelter and Hoebeke 2002). CLB is native to mainland China, Japan, Korea, the Philippines, Myanmar, and Taiwan, with isolated populations in Malaysia, Vietnam, and Indonesia (CABI 2019) and with the Japanese CLB historically recognized as *Anoplophora malasiaca* currently synonymized under *A. chinensis* (Lingafelter and Hoebeke 2002). Both species are polyphagous pests of woody trees with ALB infesting a wide range of hardwood trees from at least 15 families including maple, elm, willow, and poplar species (Haack et al. 2010) and the even more broadly polyphagous CLB infesting trees from at least 36 families including maple, elm, willow, and poplar, and agriculturally important trees such as citrus, fig, pecan, and plum (Haack et al. 2010). CLB is even known to infest conifers such *Cryptomeria* species (Wang and Chen 1984). However, host list information is incomplete and different reports are sometimes contradictory. Both species are found in diverse geographic areas, have been misidentified as each other or other similar species,

and there is a tendency towards host inclusion based on suspect reports because of management objectives. A review of ALB and CLB host lists by Sjöman et al. (2014) includes a host list table highlighting contradictory information (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018).

ALB and CLB both display the typical lamiine life cycle. In most geographic areas, ALB and CLB are univoltine, with larvae overwintering within their hosts, pupating, then emerging as adults during a spring or summer flight period depending on the local climate. Adults from both species require a 10-15 day period of maturation feeding prior to sexual maturity, and both prefer healthy to stressed host trees. Host damage is two-fold, with larval xylophagous feeding damaging woody tissues and adult feeding damaging host leaves and twigs. Within their native ranges, both species are serious pests and outbreaks have caused massive amounts of damage (Ji et al. 2011). In China, ALB is estimated to cause at least $1.5 billion USD annual damage and has contributed to the death of millions of non-native poplars planted as part of the Three-Norths shelterbelt region afforestation project (Cao 2008, Hu et al. 2009), while CLB infestation has complicated similar attempts to halt dune erosion using *Casuarina* monocultures (Ge et al. 2014). In Japan, CLB is a major agricultural pest of citrus (Adachi and others 1990). Both ALB and CLB are readily invasive. North American and European trees lack evolved resistance to either species and may prove to be suitable hosts. In addition, larval presence within woody tissues hides their presence and facilitates accidental transport. (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018)

The first detected ALB population in North America was reported in New York City in 1996 and is believed to have arrived from China via either ship dunnage or wooden crating (Haack et al. 1996). ALB are currently present in Austria, the UK, Montenegro, Ontario,

Massachusetts, New York, and Ohio, while introduced populations have been eradicated in Belgium, the Netherlands, Illinois, and New Jersey (CABI 2019). While ALB are often intercepted from woody materials transported from East Asia to other continents, CLB are more commonly intercepted on live hosts such as nursery trees or bonsai. According to a 2018 review, a total of 56 CLB infestations in 11 European countries have been reported, and eradication is still ongoing in Italy and Croatia (Hérard and Maspero 2018). Eradication of both species is costly and labor-intensive. Adult ALB and CLB are good fliers, with adult ALB dispersal potential estimated at 2,394 meters (females) and 2,644 meters (males) (Smith et al. 2004) . Anthropogenic transportation of firewood or other woody products may also facilitate dispersal. (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018)

Both ALB and CLB have been the focus of massive eradication efforts. Estimated failure to eradicate New York and Chicago infestations of ALB would have resulted in the mortality of 30.3% of urban street trees, estimated at a value of $669 billion (Nowak et al. 2001), making the cost of failure so high that eradication efforts have proceeded despite their immense cost and difficulty. Commonly, eradication involves the destruction of all host trees within a defined dispersal radius, a technique which is effective if the infested area is well understood, although may be complicated by poor detection of host trees, mistaken delimitation of the infested area, underestimation of dispersal ability, land owner resistance to the loss of valuable hardwoods, or human transport of infested material. To lessen these difficulties, it is critically important that invading ALB and CLB be detected early, if not at ports-of-entry, then as early infestations, and that established populations be accurately mapped. The current weakly-attractive monitoring traps (Nehme et al. 2014) and reliance on visual inspection and citizen-reports does not

accomplish this goal and there is a great need for improved, semiochemical-based monitoring traps. (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018)

**ALB and CLB: Research Progress on Long-Range Volatile Chemical Attractants**

Research efforts guided by the seven previously discussed trends has resulted in several compounds that elicit statistically significant attraction for both ALB and CLB (Tables 1-4). Monitoring traps baited with pheromone compounds were an initial primary major research focus (Tables 1-2). Following the identification of 4-(*n*-heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol (Zhang et al. 2002), the first identified lamiine pheromones, 4-(*n*-heptyloxy)butan-1-ol was also identified as a CLB male-produced aggregation pheromone and example of pheromone component and pheromone-producing sex conservation in the Lamiinae (Hansen et al. 2015). However, as male pheromone baited traps were poorly attractive to ALB (Fukaya 2003), researchers initiated alternative pheromone-based approaches including identification of female-produced ALB long range pheromones (Wickham et al. 2012, Xu 2018) and a search for minor pheromone components (Crook et al. 2014). An alternate pathway, research on host volatiles produced by preferential or stressed host plants (Luo et al. 1997, Huang et al. 1998, Francese 2005), also produced a list of weakly attractive host volatile blends and compounds. Pheromones and host volatiles were later tested in combination (Nehme et al. 2009, Wickham et al. 2012, Zhu et al. 2017), producing an additional list of weakly attractive blends. In addition, although there is little evidence for these effects in other species of Cerambycids, natal host, post eclosion feeding, and possible memory effects have been evaluated in both ALB and CLB (Wang et al. 2007, Yasui and Fujiwara-Tsujii 2016). The following is a

summary of research on attractive compounds organized by the seven previously discussed trends.

**(1) Pheromone components and motifs are often conserved and used by multiple closely related species (Hanks and Millar 2016).** This trend successfully guided the discovery of 4-(*n*-heptyloxy)butan-1-ol, the CLB male aggregation pheromone (Table 1-1) (Hansen et al. 2015). Following the discovery of a male ALB aggregation pheromone (Table 1-1) (Zhang et al. 2002), screening trapping experiments were performed in Nanjing, Jiangsu Province, China using the same compounds and a variety of other potentially conserved pheromone components as lures. Preliminary field experiments suggested that CLB were attracted to one or more ALB pheromone components and further field bioassays in Jiangsu confirmed significant CLB attraction to the alcohol component (Hansen et al. 2015). Currently, ALB and CLB are the only known cerambycids to use these compounds as a long-range pheromone, however, other species of *Anoplophora* were never observed at the Jiangsu trapping locations and additional testing in novel geographic locations may reveal that other species use these compounds.

**(2) Long-range attractant pheromones have been more readily discovered in species of cerambycids with life history patterns that stress quick adult reproduction (Hanks 1999).** Unfortunately, the discovery of highly attractive volatile pheromones for ALB and CLB appears to follow this trend. Both ALB and CLB feed as adults, have an adult life span of up to several months, and require a period of maturation feeding prior to sexual maturity (Hu et al. 2009, Haack et al. 2010). Prior to the discovery of an aggregation pheromone, CLB was reported to lack any long range pheromones based on caged-beetle trapping and behavioral observation (Wang et al. 1996). Although the ALB male-produced esters were attractive to both sexes of ALB in y-tube laboratory olfactometers, initial field trapping was not successful (Fukaya 2003),

and despite a great deal of testing and widespread use of male-pheromone based monitoring traps in infested areas in Massachusetts (Nehme et al. 2009, 2014, Meng et al. 2014), statistically significant ALB trap catch of females beetles was not reported until 2010 (Nehme et al.), and the first report of significant trap catch of both sexes was not reported until 2017 (Zhu et al.). Female pheromones reported by Wickham (2012) and Xu (2018) are currently not being used for monitoring purposes. Pheromone-based long-range attractants in CLB have received less attention. Currently the CLB male aggregation pheromone 4-(*n*-heptyloxy)butan-1-ol (with or without the corresponding aldehyde) is the only known attractive compound (Table 1-1) (Hansen et al. 2015, Zhu et al. 2017). Although significant bioassay results in both species suggest these pheromones have real biological influence, it is likely that mate-seeking by these adult-feeding species does not rely solely on pheromone compounds and that host compounds or other factors may be important synergists of the pheromone signal.

**(3) The pheromone-producing and pheromone-attracting sex shows subfamily conservation** (Hanks and Millar 2016)**.** The assumption that a long-range sex or aggregation pheromone would be produced by either male or female beetles was used to identify the Zhang 2002 ALB male-produced aggregation pheromone. GC-EAD comparison of separate male and female aerations identified two male-produced ALB antennally active compounds that were attractive to both males and females in laboratory bioassays (Zhang et al. 2002). Although subsequent examples of male aggregation pheromones in the Lamiinae suggested that lamiine long-range volatile pheromones are all male-produced aggregation pheromones (Wang 2017) there is increasing evidence that ALB use both female and male-produced volatile pheromones (Table 1-1). A volatile mixture of heptanal, nonanal and hexadecanal simulating oxidized virgin female cuticular hydrocarbons successfully captured significantly greater numbers of ALB in

field traps than controls (Wickham et al. 2012). Female-indicating α-longipinene also captured significantly greater numbers of ALB that controls (Xu 2018). Although female produced cuticular hydrocarbons have been investigated in CLB as short-range pheromones, their oxidation products have not been evaluated as long-range pheromones (Yasui et al. 2007). It should be noted that the discovery of a male-produced attractants does not exclude the possibility of additional female-produced attractants.

**(4) Long-range volatile pheromones may be recognized only as blends of multiple compounds in a specific ratio rather than as single chemical compounds**. As the omission of minor pheromone components would explain the poor attractivity of the Zhang 2002 ALB male pheromone, male ALB aerations have been investigated for additional male-produced antennally active compounds, resulting to the report of (3*E*,6*E*)-α-farnesene as the third component of the ALB male aggregation pheromone (Table 1-1) (Crook et al. 2014). However, significant field attraction to this compound or a three compound blend containing the male-produced esters has not been reported. Minor components of the CLB male pheromone (Hansen et al. 2015) have yet to be identified.

**(5) Conifer and or dead/stressed tree feeding Cerambycids are attracted to ethanol and/or conifer-produced terpenes. Host attractants for healthy-tree angiosperm feeders are poorly understood** (Collignon et al. 2016)**.** ALB and CLB are both highly polyphagous species that feed primarily on angiosperms. The preference of both species for certain host trees over others is very well-documented, to the extent that certain species, such as *Acer negundo* for ALB or *Melia azedarach* for CLB, have been used as trap trees (Li and Wu 1990, Wen et al. 1999, Li, Fan, et al. 2003). Although a great deal of effort has been spent identifying attractive host-indicative compounds, resulting in many different reported host volatile attractants (Tables 1-2

and 1-3), and in an extensive list of antennally active host volatile compounds (Tables 1-4 and 1-5), a highly attractive host volatile blend remains elusive.

Reported ALB antennally active host volatiles include a variety of fatty acid derived alcohols, esters, aldehydes, terpenes and other compounds (Table 1-4) (Li, Luo, et al. 1999, Fan et al. 2003, Li, Jin, et al. 2003, Jin et al. 2004, Francese 2005, Wickham 2009, Fan et al. 2012, 2013). The orientation of ALB toward many of these volatiles and other potentially attractive host indicative compounds has been evaluated. As early as 1997, significant ALB attraction in y-tube olfactometers to four undisclosed host *Acer negundo* produced synthetic monomers was reported (Luo et al. 1997). Subsequently, the *A. negundo* produced volatiles *trans*-2-hexen-1-al, *trans*-2-hexen-1-ol, or decanal were reported to be significantly attractive in y-tube olfactometer bioassays (Fan et al. 2003), and two lure formulations of antennally active compounds imitating drought-stressed *A. negundo*, a 1:1:1 mixture of 1-pentanol, 2-pentanol, and 1-butanol or the single compound *cis*-hexen-1-ol, were significantly attractive in the field (Li et al. 2003, Jin et al. 2004). However, further attempts to replicate this attraction were unsuccessful (Lund et al. 2005, Teale, unpublished research). Potentially attractive host volatile compounds have also been ascertained by statistically comparing levels of antennally active compounds in hosts and non-hosts (Francese 2005, Wickham 2009, Wickham et al. 2012) and these findings later informed the discovery of multiple host volatile based attractive lure formulations (Nehme et al. 2010). Additionally, non-statistical host vs. non-host comparison has also found that an *Acer negundo* imitating host volatile blend was significantly more attractive to ALB and mixture imitating *Melia azedarach* as significantly more attractive to CLB. (Zhu et al. 2017).

Reported CLB antennally active host volatiles are mainly terpenes (Table 1-5) (Liu and Xu 2014, Qian et al. 2018). The attractiveness of these compounds or other host indicative

compounds in laboratory bioassays or field traps is poorly explored (apart from Zhu et al. 2017). *Melia azedarach* is significantly attractive in the field to CLB, initial chemical analyses have been performed, and several antennally active host volatiles have been reported (Huang et al. 1998, 2000, 2001, Liu and Xu 2014). In addition, antennally active volatiles produced by *Citrus reticulata* cv. *Shiyue Ju* and *Melia azedarach* have been reported (Qian et al. 2018). However, none of these studies report bioassay results or attractive lure mixtures.

Yasui et al. (2007, 2008) has reported significant short-range attraction of CLB to several host volatiles in the context of host sesquiterpene sequestration by female beetles or natal / post-eclosion feeding host preference. Significant attraction to a β-caryophyllene, α-humulene, β-elemene, and α-farnesene, which are present in *Citrus unshiu* bark was reported, although bioassays confirming attraction to synthetic compounds without the addition of CLB hydrocarbons were not included (Yasui et al. 2007, 2008). Although similar fractionation of *Salix schwerinii* failed to provide interpretable results, male CLB attraction to wounded branches significantly decreased over time, corresponding to decreases in the release of the host volatile nerol. Male CLB were significantly more attracted to nerol or a nerol-containing three compound blend (Yasui et al. 2011). Finally, in 2012, significant male attraction to β-caryophyllene, sulfur, (*E)*-phytol, α-terpineol, and/or triterpene alcohol containing *Vaccinium* spp. bark fractions was reported. Males were significantly more attracted to synthetic β-caryophyllene, synthetic (*E*)-phytol, or a mixture of synthetic (*E*)-phytol, α-terpineol, and extracted terpinene alcohols than a control (Fujiwara-Tsujii et al. 2012).

**(6) Cerambycid pheromones may only be attractive in the presence of host volatiles**. As poor attraction to the ALB pheromones is readily explained by the necessity of host volatile

synergists, multiple attempts have been made to synergize existing pheromone-based or host-volatile-based lures, resulting in the report of several significantly attractive lure formulations (Table 1-3). Tests using the female oxidized hydrocarbon pheromone found that lures containing the six aldehyde blend plus a mixture of the host indicative antennally active volatiles *cis*-3-hexen-1-ol, camphene, δ-3-carene, linalool, and *trans*-caryophyllene captured significantly more ALB in the field than a control. In two additional field experiments, the three aldehyde female pheromone plus the previous host volatiles and linalool oxide captured significantly more male beetles than a control. However, trap catch using these lures was never significantly higher than trap catch using the female pheromones alone (Wickham et al. 2012).

Nehme et al. (2009) has also explored host volatile synergists for the dialkyl ether male pheromone. Laboratory tests showed significantly more virgin male ALB were attracted to pheromone plus (-)-linalool or pheromone plus (-)-linalool and (Z)-3-hexen-1-ol than pheromone alone (Nehme et al. 2009). Field testing then revealed significant field attraction to various lure formulations containing male pheromone and the host volatiles (-)-linalool, (Z)-3-hexen-1-ol, (-)-*trans*-pinocarveol, linalool oxide, and/or *trans*-caryophyllene (Nehme et al. 2010, Meng et al. 2014). Yu et al. (2017) also reported significant attraction to such lures and Zhu et al. (2017) have reported male pheromone combined with camphene, *cis*-3-hexen-1-ol, ocimene, and β-caryophyllene was significantly more attractive than male pheromone or host volatiles alone to both CLB and ALB.

**(7) Compound stereochemistry has critical biological importance**. As neither the ALB male dialkyl eithers or ALB female straight chain aldehydesare chiral compounds , compound stereochemistry in ALB and CLB has received relatively little attention, although care

was taken by Crook 2012 to identify the stereochemistry of the minor component (3*E*,6*E*)-α-farnesene. The importance of host volatile stereochemistry is established in other species of Coleoptera (Hobson et al. 1993). Although stereoisomers are usually treated as unique compounds, exact enantiomers of chiral compounds can be difficult to identify, and enantiomerically pure compounds can be difficult to synthesize. In many cases, this has complicated research efforts. For example, isomers may be substituted when the correct isomer is unavailable (Yasui et al. 2008) or electroantennography may fail to specify the exact stereoisomer (Jin et al. 2004). Although the effect of small quantities of impurities is unexplored, the use of the commercially available terpene mixtures with 97%-98% purity is common (Nehme et al. 2010).

**Research Directions**

Despite many statistically significant attractive pheromone and host-volatile based lures for ALB and CLB, trap catches remain impractically low for a monitoring lure. The Wickham 2012 host volatile female pheromone lures captured at most ~3 beetles per trap per week, and various male pheromone + host volatile lures have captured at most ~4 beetles per lure (Nehme et al. 2010), 9 beetles total (Meng et al. 2014), ~1 beetle per trap (~110 beetles total over a 2 year experiment) (Yu et al. 2017), or ~5 ALB per trap per week (Zhu et al. 2017), (Xu Tian 2018). However, despite this low attraction, the utility of such traps remains clear. Although four years of ALB trapping around Worcester, Massachusetts, with 800 traps baited with a variety of lure formulations was only able to capture 45 ALB, the experiment detected beetles in previously undetected locations and provided valuable management information (Nehme et al. 2014). More effective lures would greatly enhance detection surveys.

Identification of attractants for use in pest control must continue to stress behavioral observation, apply physiological understanding, and take advantage of existent analytical chemistry techniques. ALB and CLB are readily attracted to their host trees, especially in the case of the highly attractive species used as trap trees (Adachi 1990, Huang et al. 2000, Sjöman et al. 2014). According to current behaviorally and physiologically based hypotheses of insect attraction in general, this attraction is based on recognition of a host-indicative mixture of common odorants. The high level of attractiveness of α-pinene and ethanol to conifer-feeding cerambycids suggests that angiosperm-feeding cerambycids may be attracted to similar common volatiles, and the current failure to identify such is due to the difficulty of deriving a host indicative blend from a complex volatile mixture.

I suggest that the multivariate host vs. non-host statistical comparison used by Francese (2005) and Wickham (2009), which has already informed the discovery of several mildly attractive host volatile lures for ALB, is superior to methods based on compound selection from a single preferred host species. This method is as follows: **(1)** head-space volatile collection from host and non-host trees, **(2)** GC-MS of identified volatiles, **(3)** GC-EAD identification of antennally active volatiles, **(4)** multivariate statistic derivation of host indicative compounds, and **(5)** bioassay confirmation of the attractiveness of the host indicative blend. In the past decade, GC-MS and GC-EAD compound identification and techniques have improved, allowing for this method to be replicated with superior results. In addition, I expand this method to include CLB, providing a start to the comparison of host attraction by two closely related sister species of cerambycids with different host ranges. An overlapping selection of host and non-host trees enables host versus non-host comparison for both species simultaneously. ALB and CLB are not

only in need of study due to their pest risk, they may provide information of the evolution of pheromone systems and host attraction in polyphagous pest insects in general.

**TABLE 1-1:** Potential ALB and CLB Pheromones

| Compound | Pheromone Producing Sex | Attracted Sex | Verification Method |
|---|---|---|---|
| **ALB** | | | |
| 4-(*n*-Heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol | Males | | Laboratory y-tube olfactometer bioassays (Zhang et al. 2002), field trapping bioassays (Zhu et al. 2017) |
| 4-(*n*-Heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol | Males | Females | Field trapping bioassays (Nehme et al. 2010, Yu et al. 2017) |
| 4-(*n*-Heptyloxy)butan-1-ol | Males | Females | Laboratory wind tunnel bioassay (Nehme et al. 2009), field trapping bioassays (Nehme et al. 2010) |
| Heptanal, Nonanal, Tetradecanal, Hexadecanal, Octadecanal, and Eicosanal | Females | Males (females not tested) | Laboratory y-tube olfactometer bioassay (Wickham et al. 2012) |
| 3:11:1 Heptanal, Nonanal, and Tetradecanal | Females | Males (females not tested) | Laboratory y-tube olfactometer bioassay (Wickham et al. 2012) |
| 1:7:1 Heptanal, Nonanal, and Tetradecanal | Females | | Field trapping bioassays (Wickham et al. 2012) |
| (3E,6E)-α-Farnesene | Males | | Laboratory y-tube olfactometer bioassays (Crook et al. 2014) |
| (3E,6E)-α-Farnesene, 4-(*n*-heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol | Males | | Laboratory y-tube olfactometer bioassays (Crook et al. 2014) |
| α-longipinene | Female | | Laboratory y-tube olfactometer bioassays and field trapping (Xu 2018) |
| **CLB** | | | |
| 4-(*n*-heptyloxy)butan-1-ol | Males | | Field trapping bioassays (Hansen et al. 2015) |
| 4-(*n*-Heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol | Males | | Field trapping bioassays (Hansen et al. 2015, Zhu et al. 2017) |

**TABLE 1-2**: Potentially Attractive Host Volatiles to ALB or CLB

| Host Volatile-Type Attractants | Attracted Sex | Verification Method |
|---|---|---|
| **ALB** | | |
| Monomers A, B, E, or I | | Laboratory cross tube olfactometer bioassay (Luo et al. 1997) |
| *trans*-2-Hexen-1-al | | Laboratory y-tube olfactometer bioassay (Fan et al. 2003) |
| *trans*-2-Hexen-1-ol | | Laboratory y-tube olfactometer bioassay (Fan et al. 2003) |
| Decanal | | Laboratory y-tube olfactometer bioassay (Fan et al. 2003) |
| *cis*-3-Hexen-1-ol | | Field trapping bioassays (Li, Jin, et al. 2003*, Jin et al. 2004*) |
| 1-Butanol, 2-pentanol, and 1-pentanol | | Field trapping bioassays (Li, Jin, et al. 2003*, Jin et al. 2004*) |
| 3-Carene | Males | Laboratory y-tube olfactometer bioassay (Nehme et al. 2009) |
| (*E*)-Caryophyllene | Males | Laboratory y-tube olfactometer bioassay (Nehme et al. 2009) |
| Linalool | Females | Field trapping bioassays (Nehme et al. 2010) |
| Linalool, (-)-*trans*-pinocarveol, linalool oxide, (*Z*)-3-hexen-1-ol, *trans*-caryophyllene | Females | Field trapping bioassays (Nehme et al. 2010) |
| Linalool Oxide | Males | Field trapping bioassays (Wickham et al. 2012) |
| Linalool, *trans*-caryophyllene, *cis*-3-hexen-1-ol | | Field trapping bioassays (Yu et al. 2017) |
| Styrene, β-myrcene, *cis*-3-hexenyl acetate, acetophenone | | Field trapping bioassays (Zhu et al. 2017) |
| **CLB** | | |
| Nerol | Males | Laboratory bioassays (Yasui et al. 2011) |
| Nerol, 1,8-Cineol, and Geraniol | Males | Laboratory bioassays (Yasui et al. 2011) |
| β-Caryophyllene | Males | Laboratory bioassays (Fujiwara-Tsujii et al. 2012) |
| (E)-Phytol | Males | Laboratory bioassays (Fujiwara-Tsujii et al. 2012) |
| Camphene, cis-3-hexenylacetate, acetophenone | | Field trapping biaossays (Zhu et al. 2017) |

*Field trapping experiment reported twice.

**TABLE 1-3:** Potentially Attractive Pheromone and Host Volatiles Blends to ALB or CLB

| Pheromone + Host-Volatile-Type Attractants | Attracted Sex | Verification Method |
|---|---|---|
| **ALB** | | |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy butan-1-ol, linalool, and (-)-*trans*-pinocarveol | Females | Field trapping bioassays (Nehme et al. 2010) |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, linalool, (-)-*trans*-pinocarveol, linalool oxide, (Z)-3-hexen-1-ol, *trans*-caryophyllene | Females | Field trapping bioassays (Nehme et al. 2010) |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, linalool | Males | Field trapping bioassays (Nehme et al. 2010) |
| Heptanal, Nonanal, Tetradecanal, Hexadecanal, Octadecanal, Eicosanal, *cis*-3-hexen-1-ol, camphene, δ-3-carene, linalool, *trans*-caryophyllene | | Field trapping bioassays (Wickham et al. 2012) |
| Heptanal, Nonanal, Tetradecanal, *cis*-3-hexen-1-ol, camphene, δ-3-carene, linalool, *trans*-caryophyllene, linalool oxide | Males | Field trapping bioassays (Wickham et al. 2012) |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, linalool, *trans*-caryophyllene, (*Z*)-3-hexen-1-ol | Females | Field trapping bioassays (Meng et al. 2014) |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, linalool, *trans*-caryophyllene, (*Z*)-3-hexen-1-ol, linalool oxide | Females | Field trapping bioassays (Meng et al. 2014, Yu et al. 2017) |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, linalool, *trans*-caryophyllene, (*Z*)-3-hexen-1-ol | Males | Field trapping bioassays (Yu et al. 2017) |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, camphene, *cis*-3-hexen-1-ol, ocimene, β-caryophyllene | | Field trapping bioassays (Zhu et al. 2017) |
| **CLB** | | |
| 4-(*n*-Heptyloxy)butanal, 4-(*n*-heptyloxy)butan-1-ol, camphene, *cis*-3-hexen-1-ol, ocimene, β-caryophyllene | | Field trapping bioassays (Zhu et al. 2017) |

| **Alcohols:** | **Esters:** | **Terpenoids:** |
|---|---|---|
| Benzyl alcohol[a] | Butyl Acetate[a] | Camphene[e,f,g] |
| 1-Butanol[c] | Ethyl Acetate[f,g] | 3-Carene[e,f,g] |
| 2-Butoxy-ethanol[c] | Ethyl Butanoate[a] | *trans*-Caryophyllene[e] |
| 1-Ethyl-2-hexanol[d] | *cis*-3-Hexenyl Acetate[b] | Farnesene isomers[h*] |
| 2-Ethylhexanol[b] | *n*-Hexyl Acetate[b] | Limonene[c,f,g] |
| Hexanol[a] | Propyl Propionate[a] | Linalool[b,c,d,e] |
| 1-Hexenol[c] | | Linalool Oxide[c] |
| *trans*-2-Hexen-1-ol[a,b] | **Aldehydes:** | *cis*-Linalool Oxide[d] |
| *cis*-3-Hexen-1-ol[a,b,c,d,e,f,g] | Decanal[b] | *trans*-Linalool Oxide[d] |
| 1-Methyoxy-2-Propanol[b] | Hexanal[a,e] | β-Myrcene[f,g] |
| 1-Octanol[b] | *trans*-2-Hexen-1-al[a,b,e] | Ocimene[f,g] |
| 1-Octen-3-ol[c,d] | Furfural[a] | α-Pinene[c] |
| 1-Pentanol[c,d] | 5-Methyl Furfural[a] | S-α-Pinene[f,g] |
| 2-Pentanol[c] | Nonanal[b,e] | R-α-Pinene[f,g] |
| | Octanal[e] | S-β-Pinene[f,g] |
| ***n*-Alkanes:** | | α-Phellandrene[f,g] |
| Hexane[f,g] | | |

**TABLE 1-4:** Previously Reported ALB Antennally Active Host Volatiles

[a] First author affiliation Beijing Forestry University. Syntech brand system EAG dosage curves with purchased standard compounds. (Li, Luo, et al. 1999)

[b] First author affiliation Beijing Forestry University. Syntech brand system EAG with purchased standard compounds (Fan Hui et al. 2003)

[c]First author affiliation Beijing Forestry University. Noncommercial GC-EAD with standards. (Jin et al. 2004)

[d]Master's thesis author affiliation SUNY-ESF. Noncommercial GC-EAD with aerations and standards. (Francese 2005)

[e]Doctoral thesis author affiliation SUNY-ESF. Noncommercial GC-EAD with aerations. (Wickham 2009)

[f] First author affiliation Northeast Forestry University. Syntech brand system EAG with standards. (Fan et al. 2012)

[g]First author affiliation Northeast Forestry University. Syntech brand system EAG dosage curves with purchased standard compounds (Fan et al. 2013)

[h]First author affiliation USDA APHIS. Syntech brand system GC-EAD with standards. (Crook et al. 2014)

*Investigated as a male-produced pheromone compound.

| **Terpenes:** | **Others:** |
|---|---|
| β-Caryophyllene[b] | Benzothiazole[b] |
| Limonene[b] | Styrene butadiene[a] |
| Linalool[b] | |
| Myrcene[a] | |
| Myrtenol[a] | |
| α-Pinene[a] | |
| β-Pinene[a] | |
| 4-Terpineol[b] | |
| Terpineol[b] | |

**TABLE 1-5:** Previously Reported CLB Antennally Active Host Volatiles.

[a]First author affiliation Zhejiang A&F University. GC-EAD with aerations. (Liu and Xu 2014)

[b]First author affiliation Guangdong Academy of Forestry. GC-EAD with aerations. (Qian et al. 2018)

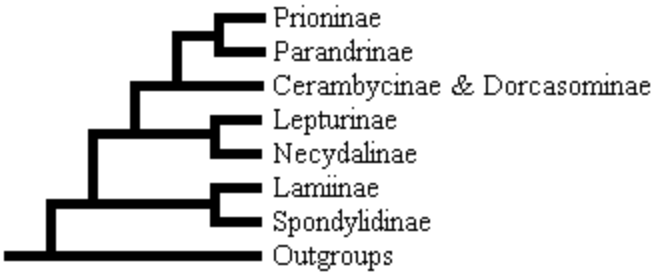



**FIGURE 1-1:** Cerambycid Subfamily Phylogeny According to Haddad et al. (2018).

## CHAPTER 2: DETERMINATION OF HOST PERCEPTS

### Introduction

The concept of odorant percepts is understandable through analogy with human experience. For example, although baking bread has a recognizable odor, there is an allowable variation in the types of scents perceived as baking bread. In addition, the scent of baking bread is not a single chemical odorant. Instead, it is a mixture of multiple odorants in an indicative ratio (Cho, 2010). Although the mixture has this recognizable identity, any single odorant extracted from the mixture alone will not. It follows that there is a mathematically representable hyperspace of odorant combinations that are perceived as baking bread – an odorant percept. This hyperspace would have a center and would allow some level of deviance away from this center before the recognizable identity of the mixture is lost. However, the addition of certain compounds, the removal of others, or too strong a change in the ratio would move the mixture outside of the space and outside of the odorant percept of baking bread.

Current research suggests that despite differing physiologies from humans, insect higher level interpretation of odorants operates in a similar way. Similar to how great effort has been made to imitate recognizable scents with a minimum of synthetic compounds in the flavor and perfumery industries, great effort has been made to design insect lures with attractive odors identifiable as mates, feeding hosts, or oviposition sites. However, as one cannot ask an insect what their interpretation of an odorant mixture is, data must be verified experimentally, substituting bioassays and behavioral information for a spoken interpretation. (Bruce et al. 2005, Bruce and Pickett 2011)

This difficulty is highlighted by two theories of insect odor interpretation. A “single odorant” theory, which suggested insects detected their hosts by identification of single, unique

odorants, and a “ratio hypothesis” which suggests that insects identify their hosts by identification of odorants in a unique ratio. Current understanding has converged on the “ratio hypothesis” of host detection. There are several reasons why the “single odorant” hypothesis was initially appealing. First, there are examples of insects being attracted to single compounds. Ethanol, an indicator of plant distress, is attractive to many insects by itself (Montgomery and Wargo 1983). Second, single compound lures are more easily experimentally verifiable as attractive or repellent, than multi-component lures. And third, single compound pheromones are not uncommon. However, the “single odorant” hypothesis as the main driver host attraction is easily rejected by basic chemical understanding. Phytophagous plants do not commonly produce compounds unique to their species (Dewick 2009) and there are many tens of thousands of insect host relationships. Additionally, as discussed in Chapter 1, more laborious experiments found insects were attracted to mixtures of common, ubiquitously produced compounds in host characteristic ratios. There is permissible variation in these ratios and specific compounds can be removed or added while the ratio as a whole remains attractive. (Bruce et al. 2005, Bruce and Pickett 2011)

Based on the current theory, I hypothesize that there is a mixture of volatiles compounds with allowable variation that ALB and CLB recognize as host odor. As ALB and CLB have similar but overlapping host lists, their host percepts will be slightly altered. I proposed to expand upon host versus non-host multivariate comparison method used by Wickham et al. (2009). As both species of longhorned beetles successfully detect their hosts in the field via olfaction, hosts must produce a recognizable odor that is within the host percept that is not produced by non-hosts.

Asian Longhorned Beetle (ALB, *Anoplophora glabripennis*) and Citrus Longhorned Beetle (CLB, *Anoplophora chinensis*) (Subfamily: Lamiinae, Tribe: Lamiini) are sympatric cerambycids native to East Asia. Both species are polyphagous pests of woody trees with ALB infesting a wide range of hardwood trees from at least 15 families including maple, elm, willow, and poplar species (Haack et al. 2010) and the even more broadly polyphagous CLB infesting trees from at least 36 families including maple, elm, willow, and poplar, and agriculturally important trees such as citrus, fig, pecan, and plum (Haack et al. 2010).Host damage is two-fold, with larval xylophagous feeding damaging woody tissues and adult feeding damaging host leaves and twigs. Within their native ranges, both species are serious pests and outbreaks have caused massive amounts of damage (Ji et al. 2011). In China, ALB is estimated to cause at least $1.5 billion USD annual damage and has contributed to the death of millions of non-native poplars planted as part of the Three-Norths shelterbelt region afforestation project (Cao 2008, Hu et al. 2009), while CLB infestation has complicated similar attempts to halt dune erosion using *Casuarina* monocultures (Ge et al. 2014). In Japan, CLB is a major agricultural pest of citrus (Adachi and others 1990). Both ALB and CLB are readily invasive. North American and European trees lack evolved resistance to either species and may prove to be suitable hosts. In addition, larval presence within woody tissues hides their presence and facilitates accidental transport. (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018)

ALB and CLB are broadly polyphagous insects that feed on a wide range of angiosperm trees. Due to the significant damage they have caused and continue to cause, several attempts have been made to determine host use by each species. Xiao (1992) compiled host lists from the Chinese literature. In a systematic revision of the genus *Anoplophora*, Lingafelter and Hoebeke

(2002) reviewed the English and Chinese literature available at the time. Wang (2012) reported on the known hosts and non-host in the United States. Lim et al. (2014) compiled a list of the Korean species susceptible to ALB, Gang and Loomans (2014) reviewed the ALB host and resistant species in Europe, while EPPO (2019) and CABI (2019) databases list hosts of ALB and CLB. The somewhat contradictory nature of host lists is highlighted by the review of Sjöman et al. (2014), which tabulates the host and non-host reports published by multiple different sources including several of the above.

Care was taken to choose a variety of taxonomic groups and clear hosts or nonhosts (Table 2-1). *Ailanthus altissima* and *Liriodendron sp.* were selected as representative nonhosts of both ALB and CLB. *Ailanthus* is not reported as a host of CLB and is extremely well documented as a highly resistant, ALB nonhost whose unattractive properties are a research focus (Hua et al. 1999), while *Liriodendron sp.* are reported as resistant to ALB and are not reported as hosts of CLB. *Citrus microcarpa*, *Melia azedarach*, and *Mora alba* were selected as ALB non-hosts and CLB hosts. *Citrus* species are a preferred host of the Citrus Longhorned Beetle, while they are not reported as hosts of ALB. Similarly, *Melia azedarach* is highly attractive to CLB to the extent that its attractive properties are a research focus and it has been evaluated as a trap tree (Sun et al. 1990, Huang et al. 1998, 2000, 2001), and this species is reported as an ALB nonhost by several sources. *Salix babylonica* and *Ulmus parvifolia* were selected as hosts of both ALB and CLB. Individuals from both species were personally observed infesting *S. babylonica*, while *Ulmus sp.* are well documented as hosts of both ALB and CLB. (Xiao 1992, Lingafelter and Hoebeke 2002, USDA-APHIS and WANG 2012, Gaag and Loomans 2014, Sjöman et al. 2014)

## Materials and Methods

Host and Non-Host Plant Static Headspace Aerations

*Ailanthus altissima* (N=11), *Liriodendron tulipiferus x chinensis* (N=6), *Melia azedarach* (N=6), *Morus alba* (N=7), *Salix babylonica* (N=7), and *Ulmus parvifolia* (N=7), headspace samples were collected from live foliage on the Nanjing Forestry University campus (Nanjing, Jiangsu Province, China (32°04'45.0"N 118°48'44.5"E)) in June and July 2017. *Citrus microcarpa* (N=10) aerations were collected from live foliage in the SUNY-ESF greenhouse (Syracuse, New York State, United States (43°02'06.7"N 76°08'09.4"W)) in May 2017 (Table 2-1). Clean activated coconut charcoal adsorbent from one ORBO$^{TM}$ -32 Standard Charcoal Tube (20/40), 100/50 mg, (Sigma-Aldrich, St. Louis, Missouri) broken immediately prior to volatile collection was poured into ~3 cm brass mesh packets. Packets were suspended in 2L glass Erlenmeyer flasks using brass wire and undamaged foliage was inserted into the flasks. Flasks and foliage were secured in position with white cotton string and the opening of each flask was sealed with Teflon tape. Control samples contained no foliage and were collected in close proximity to foliage aerations. As *Morus* and *Melia* aerations were collected in the same area, the same blank controls were used for both species. Volatiles were collected for approximately 7 hours during daylight hours. Immediately post-aeration, ORBO charcoal was poured from the packets into 2mL glass autosampler vials with PTFE coated septa (Thermo Fisher Scientific, Waltham, Massachusetts), then extracted with 0.5 mL chromatography-grade DCM after transport to the Nanjing Forestry University laboratory. Samples were stored in a subzero freezer prior to GC-MS analysis.

GC-MS Analysis

In Syracuse, New York, samples were concentrated to ~100 uL under grade 5.0 nitrogen (PurityPlus, Indianapolis, Indiana) prior to analysis on a 7890A-5976C VL EI MSD with triple-axis detector GC-MS system using a HP-5MS non-polar chromatography column (L 30 m, ID 0.250mm, F 0.25µm) (Agilent Technologies, Santa Clara, California). A 3 µL aliquot of concentrated sample was manually injected followed by a temperature program of 40°C for 1 minute, then 4°C/min to 210°C, then 210°C for 20 minutes (63.5 minute total runtime). Peaks were deconvoluted using open-source AMDIS Version 2.71 software (available at: https://chemdata.nist.gov/mass-spc/amdis/downloads/). Deconvolution component width was set to 32 and sensitivity was set to low. Compounds eluting between 5 and 60 minutes were closely examined in a representative sample from each species and its corresponding blank control. AMDIS libraries of all compounds detected in each representative sample and a corresponding blank control were created and used to match compounds in each sample based on their major ions and retention index based on a C7-C20 alkane standard (Sigma-Aldrich, St. Louis, Missouri). Major ions and tentative identifications using NIST Mass Spectral Search Program Version 2.0 f 2009 were recorded for each compound from the largest peak by integration in any sample or control.

GC-EAD Analysis

Adult male ALB for use in GC-EAD at SUNY-ESF were generously provided by the Sarkaria Arthropod Laboratory at Cornell University through cooperation with Dr. Ann Hajek with the help of Dr. Sana Gardescu. ALB were transported to SUNY-ESF under permit, kept in a secure incubator prior to use and fed *Acer pensylvanicum* twigs obtained from SUNY-ESF-

owned property at Heiburg Forest in Tully, NY. ALB were freeze killed after use in a -60°C freezer, pinned, then baked at ≥100°C then stored in an insect cabinet.

During each GC-EAD test, 3 uL of a representative concentrated sample was injected into a Hewlett Packard 5890 Series II GC-FID with nitrogen carrier gas fitted with a HP-5MS nonpolar chromatography column (L 30 m, ID 0.250 mm, F 0.25 µm**).** The oven temperature program was 40°C for 1 minute, then 10 or 15°C/min to 210°C, then 210°C until all host volatiles had eluted. Column effluent was split (1:1) between FID and EAD detectors with a glass y-tube and segments of deactivated capillary column. The EAD antenna holder, amplifier, and power source were a modified from Methods in Chemical Ecology (Millar and Haynes 1998). The tip of an adult male ALB antenna were excised with a razor blade and a 2 cm segment was placed in a saline (aqueous 140 mM NaCl, 10 mM KCl, mM $CaCl_2$, 2mM $MgCl_2$, and 2mM TES) filled plexiglass antennal holder with inserted conductive gold wire. EAD and FID signals were converted with a DataApex Colibrick A/D converter then analyzed with DataApex Clarity Lite Version 7.1.00.151 chromatography software. Antennal activity and chromatography were verified prior to sample runs by injecting a hexanal standard. GC-EAD was replicated until a minimum of three signal traces with repeatable responses were obtained for each sample. FID peaks were matched to their corresponding GC-MS peaks, and antennally active peaks were identified using GC-MS library matches (NIST Mass Spectral Search Program Version 2.0 f 2009), retention indices, and GC-MS and GC-EAD comparison to analytical standards. Chromatography traces were imaged using OpenChrom Lablicate Edition 1.4.0.

Statistical Analysis

Antennal responses to peaks detected by the FID or compounds found at the same magnitude in control samples were excluded from the analysis, as was *neo-allo* ocimene, which did not deconvolute from a co-eluting siloxane column contaminate. Raw AMDIS integration values for antennally active compounds (54 compounds) present in host volatiles from all hardwood samples (N=44) were converted to ratios, zeros were substituted with $2.02 \times 10^{-8}$ (the minimum value divided by 100), then transformed using the Aitchison transformation for compositional data (1986). Transformed data were evaluated for univariate normality using the Anderson-Darling test (Minitab™ 17 software (Minitab Inc. 2010)) followed by principle component analysis using the correlation matrix of the response variables (PCA, Minitab™ 17 software (Minitab Inc. 2010)), discriminate analysis using the CANDISC procedure with the distance option (SAS™ 9 software (SAS Institute Inc. 2013)), and random forests (RF, R-3.4 ((R Core Team 2014)).

PCA principle components (PCs) 1-4 were plotted using Stastitica™ 13 (Statsoft 2017) software and hardwood species groups were visualized using range ellipses. Significant differences between ALB or CLB host and non-host groupings in the first three PCs were evaluated using t-tests with Minitab™ 17 (Minitab Inc. 2010). Null hypotheses were that ALB host versus nonhost groups or CLB host versus nonhost groups were the same while the alternative hypotheses were that groups would show significant differences. The ten most indicative ALB host and ALB non-host compounds from PC3 were reported.

DA groupings included ALB hosts versus nonhosts, CLB hosts versus nonhosts, and all species as separate groups. D. Canonical functions 1 and 2 were graphed for each discriminate

analysis using Stastitica™ 13 (Minitab Inc. 2010) and groups were visualized using range ellipses. The ten most indicative ALB or CLB host and nonhost compounds were reported.

RF groupings were ALB host versus nonhosts and CLB hosts versus nonhosts. The 10 most important predictor variables according to %IncMSE were reported for each analysis. Compounds were selected for further analysis based on comparison of all three statistical tests.

## Results

### GC-MS Analysis

Representative GC-MS total ion chromatographs (TIC) of *Ailanthus ailanthus, Citrus microcarpa, Liriodendron tulipifera x chinensis, Melia azedarach, Morus alba, Salix babylonica,* and *Ulmus parvifolia* headspace samples along with their corresponding blank controls are shown in Figure 2-2. Foliage aerations were visually distinct from blank aerations. Compounds eluting after 5 minutes with integration values greater than 3000 are summarized in Table A-1, including the compound number (#), best NIST library match, major ions, average RT, average RI, and the integration value. NIST library match identification suggested most of these compounds were volatile hydrocarbons. AMIDS also detected approximately 200 additional compounds with integration values less than 3000.

At least 100 compounds were recorded in the *Ulmus* blank control (125), *Liriodendron* sample (110), and *Morus* sample (100), 99-60 compounds were detected in the *Ailanthus* sample (94), *Ulmus* sample (88), *Melia* sample (74), *Melia/Morus* blank control (69), and *Ailanthus* blank control (65), and fewer than 60 compounds were detected in the *Citrus* sample (51), *Liriodendron* blank control (47), *Salix* sample (46), *Salix* blank control (33), and *Citrus* blank control (16), corresponding to a total of 270 different compounds. Including the blank controls,

35 of these compounds were found in 8-13 samples, 81 compounds were found in 3-7 aerations, 45 compounds were found in 2 samples, and 109 compounds were unique to a single aeration. Of the compounds recorded in a single aeration, 32 were sizable peaks with integration values over 100,000. Twelve of these unique sizable peaks were detected in the *Liriodendron* sample, seven in the *Morus* sample, four in the *Citrus* sample, and one each in the *Ailanthus* sample, *Ailanthus* blank control, *Melia* sample, and *Melia/Morus* control

The most abundant 20 compounds by integration value in each representative hardwood sample and their abundance in the corresponding blank controls are shown in Table 2-2. All hardwood samples contained a set of highly abundant compounds not detected in the corresponding control, while several compounds were present in both hardwood sample and control but were found in much higher magnitudes in the hardwood sample. Several highly abundant compounds were close to being ubiquitously found in all samples, including compounds #19 and #157 (siloxane derivatives), compounds #263 and #296 (morpholine, 4-octadecyl-), and compound #238 (decyl ester decanoic acid).

GC-EAD Analysis

There were 85 ALB antennally active responses recorded from the representative samples (Figure 2-3). Additional compounds that were identified as antennally active during identification attempts but were not detected in samples based on GC-MS spectra and RT are included in Table 2-3. Thirteen compounds were commonly present in the controls: the straight chain aldehydes hexanal, heptanal, octanal, nonanal, and decanal, and *cis*-geranylacetone, acetophenone, 6-methyl-5-hepten-2-one, butyl ester acetic acid, 3-methyl 2-butenal, and three unidentified compounds. These compounds were removed from subsequent analyses. An

additional 16 compounds did not have a visible corresponding FID peak, could not be quantified, and were omitted from subsequent analyses. Finally, *neo-allo* ocimene, which co-eluted with a siloxane and could not be reliably detected by the AMDIS software was removed. The remaining 54 antennally active compounds are reported in Table 2-4 along with their CAS #, compound class, # of samples, RT, RI, and reference AI and KI. Most of these antennally active compounds were green leaf volatiles or terpenoids, although compounds such as 6-methyl-5-hepten-2-ol , methyl benzoate, benzyl acetate, and methyl salicylate were also detected. Although 41 compounds were successfully identified using reference standards and retention indices, thirteen compounds, including eleven terpenoid-like compounds, remained unidentified.

Statistical Analysis

Multiple variables violated the assumption of univariate normality (Anderson-Darling, $p<0.05$). PCA separated samples by species (Figure 2-4), with PC1 (22.9%) separating *Liriodendron* samples from others, suggesting that a large portion of the variance is due to the distinctness of *Liriodendron* volatiles. The most important *Liriodendron* indicating volatiles were unknown monoterpene (#26), unknown oxygenated terpenoid #70, unknown oxygenated terpenoid #73, unknown monoterpene (#49), and unknown compound (#62) (respective coefficients of the PC1 eigenvector: -0.261, -0.250, -0.240, -0.2.39, and -0.236). The most important indicators of non-*Liriodendron* samples were (*3Z*)-hexenyl acetate, (*3Z*)-hexenyl propionate, α-cubebene, hexyl acetate, (*3Z*)-hexenyl butanoate (respective coefficients of the PC1 eigenvector: 0.265, 0.172, 0.168, 0.165, and 0.160). PC2 separated the samples along a species gradient from *Citrus* to *Morus*. The most important *Morus*-leaning volatiles in the separation were *n*-hexanol, (*2E*)-hexenol, *allo*-ocimene, (*3Z*)-hexenyl benzoate, and hexyl

acetate (respective coefficients of the PC2 eigenvector: -0.287, -0.258, -0.229, -0.208, and -0.202), while the most important *Citrus*-leaning volatiles in the separation were bergamotene, germacrene D, (*E*)-caryophyllene, unknown sesquiterpene (#80), and γ-Elemene (respective coefficients of the PC2 eigenvector: 0.262, 0.256, 0.242, 0.200, and 0.179).

PC3 separated the ALB hosts *Ulmus* and *Salix* samples from ALB nonhosts. T-test analysis indicated that the ALB host versus nonhost separation in PC3 was highly significant ($p<0.000$), the CLB host vs. nonhost separation in PC1 and 2 were significant ($p<0.023$, $p<0.017$), and the CLB host vs. nonhost separation in PC3 was highly significant ($p<0.000$) (Table 2-5). PC3 was interpreted for ALB host indicative compounds. The ten compounds with the greatest PC3 positive coefficients of the eigenvector (indicating hosts compounds) and most negative coefficients of the eigenvector (indicating nonhost compounds) are reported in Table 2-7.

DA by ALB or CLB host versus nonhost groupings showed clear visual host versus non-host separation (Figure 2-6). At the multivariate level, the separation by ALB hosts versus non-hosts was not significant ($p=0.1805$ for all test statistics). At the univariate level, the separation by CLB hosts versus non-hosts was significant ($p=0.0066$ for all test statistics). For ALB and CLB, respectively, the ten compounds with the greatest pooled within-class standardized canonical coefficients (indicating hosts) and the most negative coefficients (indicating nonhosts) are reported in Table 2-6 and Table 2-7 along with their univariate significance. Nine out of ten of the most important ALB host indicative compounds identified via PC3 were also in the ten identified via DA. ALB nonhost indicative compounds were more disparate, with only five out of ten compounds indicated as most important by both PCA and DA.

RF returned a ranking of predictor variable importance (%IncMSE) for both the ALB and CLB analysis. The ten most important predictor variables in the ALB analysis, which are either host or non-host indicative compounds, are reported in Table 2-6 while the respective CLB predictor variables are reported in Table 2-7. Eight of the ten most important ALB predictor variables were host indicative compounds, all of which were among the ten most important PCA and DA host indicative compounds. For ALB non-host indicative compounds, β-pinene was PCA and DA non-host indicative compound, while *p*-mentha-2,4(8)-diene was also a DA non-host indicative compound. Three of the ten most important CLB predictor variables were among the ten most important DA compounds, while six were among the ten DA nonhost indicative compounds.

(*E*)-Nerolidol, (*E*)-4,8-dimethyl-1,3,7-nonatriene, 6-methyl-5-hepten-2-ol, isoamyl benzoate, benzyl acetate, α-humulene, (*E*)-caryophyllene, and δ-cadinene were identified by all three statistical methods as important ALB host-indicative compounds, while (*E*)-4,8-dimethyl-1,3,7-nonatriene was identified as the most important CLB host-indicative compound by DA and RF. The average GC-MS integration values of these compounds by tree species along with the average integration values of select non-host indicative compounds are given in Table 2-8.

## Discussion

### GC-MS Analysis

Classes of compounds known to be produced by higher plants in high quantities, including green leaf volatiles via the lipoxygenase pathway and terpenoids via the mevalonate and non-mevalonate pathways (Dewick 2009, Dudareva et al. 2013), were well represented. Among many others, NIST library search tentatively identified peaks as the green leaf volatiles

2-hexenal, (*Z*)-3-hexen-1-ol, and 3-hexen-1-ol acetate, the terpenes α-pinene, ocimene, and farnesene, and the lignin-related compound methyl salicylate (Teranishi and Kint 1993, Dewick 2009, Dudareva et al. 2013, Chern 2014, Loreto et al. 2014). Alkaloids, which are among the largest class of plant VOCs but are not known to be commonly produced by hardwoods, were appropriately poorly represented (Séquin 2015). Compounds expected to be co-expressed based on plant biochemistry included α-pinene, β-pinene, myrcene, and limonene, all produced by limonene synthase (Heldt and Piechulla 2004). Blank aerations were designed to correct for the presence of background volatiles, environmental pollutants, and contaminants introduced during sample collection and chromatography. Compounds detected in comparatively equal levels in hardwood samples and blank controls were typically those from classes unlikely to be plant volatiles.

GC-EAD Analysis

We provide the most extensive list of ALB antennally active compounds available. This is attributed to the high degree of sensitivity of the GC-EAD system, which is based on a custom amplifier (Cha et al. 2016). Apart from 3-carene (no response to a reference standard), furfural, and 5-methyl furfural (both not tested), antennal activity was confirmed towards the exact stereoisomer or a mixture of enantiomers for all previously reported antennally active terpenoids or aldehydes (Figure 2). Many previously reported antennally active esters and alcohols were also confirmed to be antennally active, including *cis*-3-hexen-1-ol. Many of the alcohols not confirmed as antennally active in this analysis are low molecular weight compounds. Due the presence of contaminates and solvent tailing (Figure 3), no GC-EAD responses prior to (*E*)-2-hexenal (RT 6.57, RI 849.6) could be matched to their corresponding peaks in the TIC.

GC-EAD was intended as a screening method to remove non-antennally active compounds from the analysis. Although this was effective, picking out only 54 peaks of interest from the hundreds of compounds detected using GC-MS, the relatively high number of antennally active compounds compared to previous reports was surprising. GC-EAD plant volatile screenings with Coleoptera commonly return single digit numbers of antennally active compounds. For example, GC-EAD of plant volatiles resulted in identification only seven *Pachnoda interrupta* (Family: Scarabaeidae) antennally active volatiles (Bengtsson et al. 2009), five *Rhynchophorus phoenicis* (Family: Curculionidae) antennally active volatiles (Gries et al. 1994), and five *Ips typhographus* (Family: Curculionidae) antennally active volatiles (Zhang et al. 2000). However, others have identified larger numbers, for example, forty-two *Dendroctonus brevicom*is (Family: Curculionidae) antennally active volatiles were identified from angiosperm extracts by Shepherd et al. (2007). Interestingly, while the Bengtsson report found only seven *Pachnoda interrupta* volatiles via GC-EAD, single sensillium recording using eighty-two potentially antennally active compounds determined fifty-seven were antennally active (2009). A high number of antennally active compounds is also consistent with the underlying physiology of insect olfaction derived from research on model organisms. *Drosophila melanogaster*, which has approximately fifty olfactory receptor neurons, each typically co-expressing Orco and a variant odorant receptor (Groschner and Miesenböck 2019), is known to detect in excess of a hundred chemical compounds (Knaden et al. 2012, Dweck et al. 2018). In theory, ALB, with 132 reported expressed OR genes (Hu et al. 2016, Mitchell et al. 2017), and CLB, with 53 expressed OR genes (Sun et al. 2018), are both capable of detecting at least the same number of compounds as *D. melanogaster*.

Although it has been noted that the antennally active / non-antennally active paradigm may be misleading, as OSNs may fire in response to high doses of any compound (Hansson and Stensmyr 2011), this is unlikely the reason for the observed high number of antennally active compounds in ALB. The sample collection method did not produce highly concentrated samples. Of the six volatiles quantified in *Ulmus* samples using analytical standards, the highest dose in any *Ulmus* sample was (*E*)-caryophyllene at 39 µg/mL. The current study also observed responses to compounds at concentrations below the baseline, further highlighting the high sensitivity of the EAD detector. Previous reports of low numbers of antennally active compounds in ALB, CLB, and insects with similar biology may be due to poor equipment sensitivity, resolution, or the type of sample collection.

Insect ORs operate under combinatorial code, meaning that one OR can be activated by multiple compounds (Haverkamp et al. 2018). Although the behavioral relevance of any compound cannot be inferred by the response strength or type due to higher level neural processing, observed ALB GC-EAD response type varied based on chemical identity and class. Straight chain aldehydes (hexanal through decanal) produced strong, sharp responses. Similar response types were seen towards linalool oxides, methyl salicylate, acetophenone, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and others. However, many terpenoids elicited only weak responses that were identifiable after replication, showing a difference in the way these signals are transmitted in the ALB peripheral nervous system.

Statistical Analysis

Host indicative compounds were investigated using multiple statistical methods due to the advantages and limitations of each when analyzing the multivariate, non-normal,

compositional data set collected. (Brückner and Heethoff 2017, Hervé et al. 2018). Similar results obtained from each test made final selection very convincing.

Principle component analysis (PCA) is an exploratory technique for condensing complex data sets from a single sample into descriptive principle components that maximize the within-sample variance. It is independent of normality, has no variable number limitation and is readily interpretable (Brückner and Heethoff 2017). The clear visual grouping of samples by species in PCA indicates species information was encoded in the antennally active volatiles. The significant difference between ALB hosts and non-hosts seen in PC3 (Figure 2-5) confirmed that a portion of the sample variance was explained by differences in quantities of host versus nonhost ALB antennally active volatiles and provided a list of ALB host indicative compounds. However, a large portion of the variance was due to other reasons other than host versus nonhost differences, and PC1 and PC2 did not show a significant difference. A portion of sample variance can be explained phylogenetically. The magnoliids, which includes *Liriodendron*, are the sister group to the eudicots, which the rest of the study species belong, and the monocots (Moore et al. 2010), suggesting that *Liriodendron* is an outgroup in the analysis. Although effort was made to avoid linking phylogeny with any host versus nonhost difference by excluding conifers from the analysis, selecting a diverse variety of hosts, and including the nonhost *Ailanthus altissima*, which shares an order with preferred hosts such as *Citrus* and *Acer* species, this factor cannot be entirely removed. Higher level grouping by phylogeny was not otherwise seen in the PCA. CLB host vs. nonhost PCA groups were significant in PC 1, PC 2, and PC 3, but no PC provided a clear interpretation.

Discriminate analysis (DA) separates samples into defined groups by linear combinations of variables. It is another standard, easily interpretable method for determining variable

importance in a multivariate group discrimination. However, the results are influenced by normality violations and the number of variables relative to group size (Brückner and Heethoff 2017). These violations influence rather than invalidate the results. Because normality violations bias the method's inherent significance test rather than influence the reported variable importance and host vs. nonhost groupings for both species were previously shown to be significant using PCA and MANOVA, therefore, DA results were included. The ALB DA host compounds closely matched the selection from PC 3 and DA also provided a list of CLB host compounds. While no host volatile was clearly the most important in the ALB host versus nonhost separation, DA with CLB host vs. nonhost groupings indicated that (*E*)-4,8-dimethyl-1,3,7-nonatriene was the major discriminating compound.

The machine learning method Random Forests (RF) creates decision trees that classify samples into defined groups. It has few to no assumptions. Groups can be specified, there are no normality or variable number limitations, and results are readily interpretable (Brückner and Heethoff 2017). Because RF is a new method that is not in common use, corroboration by other statistical methods would support findings by RF. ALB host compounds according to RF closely matched those determined by PCA and DA, and CLB host compounds closely matched those determined by DA. Although eight of the ten most important ALB host vs. nonhost predictor compounds were host indicative, only three of the ten most important CLB predictor variables were host indicative, suggesting the CLB host vs. nonhost split was dominated by nonhost compounds rather than host compounds and confirming that (*E*)-4,8-dimethyl-1,3,7-nonatriene was the most important CLB predictor variable.

Compound Selection

A decision to focus on ALB host indicative volatiles for further study rather than CLB host indicative volatiles was made for several reasons. (**1**) The dominate CLB host indicative volatile, (*E*)-4,8-dimethyl-1,3,7-nonatriene, is not commercially available. (**2**) Much of the CLB host vs. nonhost discrimination was dominated by nonhost volatiles. (**3**) Comparable GC-EAD work was not obtained for CLB, and ALB GC-EAD may not be an appropriate proxy. (**4**) All ALB hosts included in the study were CLB hosts. I hypothesized that a CLB host blend would be include an ALB host blend, thus an ALB blend may be of dual use in the field as an ALB and a CLB lure.

All three statistical methods indicated that (*E*)-nerolidol, (*E*)-4,8-dimethyl-1,3,7-nonatriene, 6-methyl-5-hepten-2-ol, isoamyl benzoate, benzyl acetate, α-humulene, (*E*)-caryophyllene, and δ-cadinene were among the most indicative ALB host compounds within an arbitrary cutoff. All of these compounds have been noted as insect attractants (Dethier 1947, El-Sayed 2003). Although additional compounds were also host indicative, complex odorant mixtures appear to have redundant compounds, and are reducible to a handful of important odorants (Riffell et al. 2009, Bruce and Pickett 2011, Gregg et al. 2018, Haverkamp et al. 2018). Thus, these compounds, minus the commercially unavailable (*E*)-4,8-dimethyl-1,3,7-nonatriene and δ-cadinene, were selected for future field bioassays. (*E*)-Nerolidol is an herbivore-induced sesquiterpenoid with two enantiomers biosynthesized from farnesyl diphosphate and an intermediate in the production of the sesquiterpenoid (*E*)-4,8-dimethyl-1,3,7-nonatriene (Chan et al. 2016). 6-Methyl-5-hepten-2-ol is a carotenoid-related compound also known as sulcatol, the aggregation pheromone of the scolytid *Gnathotrichus sulcatus* (Curculionidae) (Byrne et al. 1974). Isoamyl benzoate and benzyl acetate are esters of a class characteristic of fruit and floral

odors (Beekwilder et al. 2004). Encouragingly, benzyl acetate containing floral volatile blends have been noted to attract multiple species of cerambycids including Lamiinae (Sakakibara et al. 1996, 1997, 1998, Shibata et al. 1996, Wang 2017). Finally, α-humulene and (*E*)-caryophyllene are sesquiterpene stereoisomers also biosynthesized from farnesyl diphosphate (Dehal, 1988). Lure blends containing α-humulene, other *Citrus* bark extract terpenes, and CLB cuticular hydrocarbons were noted to attract CLB (Yasui et al. 2007, 2008), while (*E*)-caryophyllene was noted in an ALB host vs. nonhost comparison ALB host versus nonhost comparison (Wickham 2009) and multiple lure blends containing the compound have been shown to be attractive to ALB (Nehme et al. 2009, 2010, Wickham et al. 2012, Meng et al. 2014, Yu et al. 2017, Zhu et al. 2017) or CLB (Zhu et al. 2017). This previous host vs. nonhost comparison identified ten antennally active compounds and determined that camphene, *cis*-3-hexen-1-ol, nonanal, linalool, and δ-3-carene discriminated ALB hosts from nonhosts (Wickham 2009). In the current analysis, ALB antennally active responses to δ-3-carene analytical standards were not observed and ALB antennally active responses to camphene at the dosage found in samples were not observed.

## Conclusion

These results, which showed a subset of host volatiles that was characteristic of ALB hosts, supports the ratio hypothesis of host attraction and the existence of a host percept. PCA, DA, and RF all returned similar sets of ALB host indicative compounds. Benzyl acetate, α-humulene, (*E*)-nerolidol, (*E*)-caryophyllene, isoamyl benzoate, and 6-methyl-5-hepten-2-ol were selected for further study. In addition, our current knowledge of ALB antennally active compounds was greatly expanded.

**TABLE 2-1:** Host and Non-Host Species Selected for Analysis

| **Species** | **Order** | **ALB Host** | **CLB Host** |
|---|---|---|---|
| *Ailanthus altissima* | Sapindales | No | No |
| *Citrus microcarpa* | Sapindales | No | Yes |
| *Liriodendron tulipiferus x chinensis* | Magnoliaceae | No | No |
| *Melia azedarach* | Sapindales | No | Yes |
| *Morus alba* | Rosales | No | Yes |
| *Salix babylionica* | Malpighiales | Yes | Yes |
| *Ulmus parvifolia* | Rosales | Yes | Yes |

**TABLE 2-2:** The 20 Most Abundant Compounds in Representative Hardwood Aerations.

| *Ulmus parvifolis* | | | |
|---|---|---|---|
| NIST Library Match | RI | Integration | % Abundance |
| 1,3,6-Octatriene, 3,7-dimethyl- | 1046.4 | 25229564 | 32.38 |
| α-Farnesene | 1502.5 | 10638877 | 13.65 |
| Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene- | 1113.5 | 10160247 | 13.04 |
| 2-Hexanol, (R)- | 809.8 | 9592144 | 12.31 |
| Decanoic acid, decyl ester | 1628.2 | 5991607 | 7.69 |
| 1,1-Dimethyl-3-chloropropanol | 806.8 | 1903646 | 2.44 |
| 2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E- | 1206.3 | 1545459 | 1.98 |
| Morpholine, 4-octadecyl- | 1881.6 | 1413688 | 1.81 |
| 3-Hexen-1-ol, acetate, (E)- | 1005.5 | 1346252 | 1.73 |
| 5-Hepten-2-one, 6-methyl- | 984.2 | 706021 | 0.91 |
| Methyl salicylate | 1188.8 | 617346 | 0.79 |
| β-Myrcene | 989.3 | 607330 | 0.78 |
| 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- | 1128.5 | 606394 | 0.78 |
| Furan, 3-(4,8-dimethyl-3,7-nondienyl)-, (E)- | 1571.6 | 549479 | 0.71 |
| 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- | 1559.1 | 420688 | 0.54 |
| 3-Hexen-1-ol, benzoate, (Z)- | 1568.9 | 338827 | 0.43 |
| 1,3,6-10-Dodecatetraene 3,7,11-trimethyl-, (Z,E)- | 1488.1 | 326591 | 0.42 |
| 2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E- | 1204.8 | 317299 | 0.41 |
| 1,6-Octadien-3-ol, 3,7-dimethyl- | 1098.9 | 277036 | 0.41 |
| 5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol | 1203.2 | 266480 | 0.36 |
| *Ailanthus* | | | |
| NIST Library Match | RI | Integration | % Abundance |
| Undecane | 1100.3 | 16616408 | 20.69 |
| 2-Hexanol, (R)- | 809.8 | 15856166 | 19.74 |
| Decanoic acid, decyl ester | 1628.2 | 10332540 | 12.86 |
| 3-Hexen-1-ol, acetate, (E)- | 1005.5 | 7931555 | 9.88 |
| α-Farnesene | 1502.5 | 7307662 | 9.10 |
| Morpholine, 4-octadecyl- | 1881.6 | 5268118 | 6.56 |
| 1,1-Dimethyl-3-chloropropanol | 806.8 | 3449555 | 4.29 |
| 1,3,6-Octatriene, 3,7-dimethyl- | 1046.4 | 3175233 | 3.95 |
| Morpholine, 4-octadecyl- | 2085.5 | 1439392 | 1.79 |
| 1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-morpholinopropan-1-one | 1674.5 | 1155927 | 1.44 |
| Methyl salicylate | 1188.8 | 759514 | 0.95 |
| o-Xylene | 867.4 | 641200 | 0.80 |
| Ethylbenzene | 858.3 | 506502 | 0.63 |
| 1-Propene, 1-chloro-2-methyl- | 810.8 | 380461 | 0.47 |
| 3-Hexen-1-ol, (Z)- | 852.8 | 378790 | 0.47 |
| 2,2-Bis(chloromethyl)-1-propanol | 886.6 | 308363 | 0.38 |
| p-Xylene | 868.9 | 247966 | 0.31 |
| Benzene, 1,3-dimethyl- | 891.7 | 196609 | 0.24 |
| Propionic acid, 2-isopropoxy-, methyl ester | 816.7 | 195153 | 0.24 |
| Hexanal | 802.1 | 175253 | 0.22 |
| 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 1458.7 | 143416 | 0.18 |
| *Liriodendron* | | | |
| NIST Library Match | RI | Integration | % Abundance |
| 1,3,6-Octatriene, 3,7-dimethyl- | 1046.4 | 1.01E+08 | 54.60 |
| 2-Hexanol, (R)- | 809.8 | 15286268 | 8.24 |
| 1,3,6-Octatriene, 3,7-dimethyl-, (E)- | 1035.6 | 8787862 | 4.74 |
| 2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E- | 1206.3 | 8150987 | 4.40 |
| Decanoic acid, decyl ester | 1628.2 | 7844398 | 4.23 |
| 2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E- | 1204.8 | 6570737 | 3.54 |
| β-Myrcene | 989.3 | 4403132 | 2.37 |
| 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- | 1128.5 | 4301040 | 2.32 |
| 1,1-Dimethyl-3-chloropropanol | 806.8 | 3290757 | 1.77 |
| Morpholine, 4-octadecyl- | 1881.6 | 3059737 | 1.65 |
| Decanal | 1205.7 | 1357769 | 0.73 |
| 5-Hepten-2-one, 6-methyl- | 984.2 | 1313261 | 0.71 |

| | | | |
|---|---|---|---|
| 3-Hexen-1-ol, 2-ethyl- | 1266.4 | 926942 | 0.50 |
| 1R-α-Pinene | 932.6 | 861332 | 0.46 |
| 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 1057.0 | 802434 | 0.43 |
| Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)- | 1422.2 | 769244 | 0.41 |
| β-Pinene | 976.5 | 767421 | 0.41 |
| Morpholine, 4-octadecyl- | 2085.5 | 733058 | 0.40 |
| 1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-morpholinopropan-1-one | 1674.5 | 690323 | 0.37 |
| 3-Hexen-1-ol, (Z)- | 852.8 | 670233 | 0.36 |
| Benzene, 1-methyl-4-(1-methylethyl)- | 1023.2 | 640499 | 0.35 |

*Melia*

| NIST Library Match | RI | Integration | % Abundance |
|---|---|---|---|
| Decanoic acid, decyl ester | 1628.2 | 17200643 | 28.99 |
| 3-Hexen-1-ol, acetate, (E)- | 1005.5 | 16667464 | 28.09 |
| Morpholine, 4-octadecyl- | 1881.6 | 7923463 | 13.35 |
| Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene- | 1113.5 | 5346863 | 9.01 |
| 3-Hexen-1-ol, (Z)- | 852.8 | 2863608 | 4.83 |
| 1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-Worpholinopropan-1-one | 1674.5 | 1579023 | 2.66 |
| Morpholine, 4-octadecyl- | 2085.5 | 1462838 | 2.47 |
| Heptane, 2,2,4,6,6-pentamethyl- | 990.3 | 890102 | 1.50 |
| Limonene | 1028.5 | 684081 | 1.15 |
| Butanoic acid, 3-hexenyl ester, (Z)- | 1185.3 | 448671 | 0.76 |
| 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 1458.7 | 345452 | 0.58 |
| 1R-α-Pinene | 932.6 | 344615 | 0.58 |
| Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl- | 923.6 | 342559 | 0.58 |
| β-Myrcene | 989.3 | 287136 | 0.48 |
| o-Xylene | 867.4 | 266997 | 0.45 |
| Ethylbenzene | 858.3 | 189868 | 0.32 |
| Camphene | 948.8 | 158421 | 0.27 |
| Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- | 971.8 | 133673 | 0.23 |
| p-Xylene | 868.9 | 118349 | 0.20 |
| Butanoic acid, 3-hexenyl ester, (Z)- | 1142.8 | 107939 | 0.18 |

*Morus alba*

| NIST Library Match | RI | Integration | % Abundance |
|---|---|---|---|
| 3-Hexen-1-ol, acetate, (E)- | 1005.5 | 62839121 | 56.53 |
| Decanoic acid, decyl ester | 1628.2 | 8551907 | 7.69 |
| 3-Hexen-1-ol, (Z)- | 852.8 | 7149176 | 6.43 |
| 1,3,6-Octatriene, 3,7-dimethyl-, (E)- | 1035.6 | 5045997 | 4.54 |
| Acetic acid, hexyl ester | 1013.2 | 3925320 | 3.53 |
| Morpholine, 4-octadecyl- | 1881.6 | 2961258 | 2.66 |
| Caryophyllene | 1418.2 | 2760013 | 2.48 |
| 1,3,6-Octatriene, 3,7-dimethyl- | 1046.4 | 1897405 | 1.71 |
| Butanoic acid, 3-hexenyl ester, (Z)- | 1185.3 | 1703047 | 1.53 |
| 2-Hexenal | 850.3 | 1162155 | 1.05 |
| 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)- | 1127.7 | 1131363 | 1.02 |
| 1-Hexanol | 866.2 | 1129848 | 1.02 |
| Heptane, 2,2,4,6,6-pentamethyl- | 990.3 | 904142 | 0.81 |
| 2-Hexen-1-ol, (E)- | 864.3 | 764609 | 0.69 |
| 1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-morpholinopropan-1-one | 1674.5 | 688982 | 0.62 |
| Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-, (1S)- | 1146.7 | 677511 | 0.61 |
| 2-Hexen-1-ol, acetate, (Z)- | 1015.4 | 639199 | 0.58 |
| α-Caryophyllene | 1455.6 | 536757 | 0.48 |
| Morpholine, 4-octadecyl- | 2085.5 | 462987 | 0.42 |
| 2,2-Dimethylpropanoic anhydride | 1059.1 | 412208 | 0.37 |

*Salix babylonica*

| NIST Library Match | RI | Integration | % Abundance |
|---|---|---|---|
| Decanoic acid, decyl ester | 1628.2 | 2079108 | 25.53 |
| Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene- | 1113.5 | 2055062 | 25.23 |
| 3-Hexen-1-ol, acetate, (E)- | 1005.5 | 916976 | 11.26 |
| Heptane, 2,2,4,6,6-pentamethyl- | 990.3 | 444492 | 5.46 |
| Caryophyllene | 1418.2 | 401180 | 4.93 |
| Morpholine, 4-octadecyl- | 1881.6 | 379691 | 4.66 |
| 1,3,6-Octatriene, 3,7-dimethyl- | 1046.4 | 195506 | 2.40 |

| | | | |
|---|---|---|---|
| o-Xylene | 867.4 | 163145 | 2.00 |
| Ethylbenzene | 858.3 | 128529 | 1.58 |
| 1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-morpholinopropan-1-one | 1674.5 | 105354 | 1.29 |
| p-Xylene | 868.9 | 72870 | 0.89 |
| 3-Hexen-1-ol, (Z)- | 852.8 | 66580 | 0.82 |
| Octane | 803.3 | 64284 | 0.79 |
| Acetic acid, phenylmethyl ester | 1161.4 | 57264 | 0.70 |
| α-Farnesene | 1502.5 | 45476 | 0.56 |
| Benzene, 1,3-dimethyl- | 891.8 | 40945 | 0.50 |
| Acetic acid, butyl ester | 813.9 | 31269 | 0.38 |
| Styrene | 890.7 | 28288 | 0.35 |
| Benzene, (1-methylethyl)- | 960.9 | 25105 | 0.31 |
| Decanal | 1171.1 | 23924 | 0.29 |

*Citrus microcarpa*

| NIST Library Match | RI | Integration | % Abundance |
|---|---|---|---|
| α-Farnesene | 1502.5 | 10778780 | 22.80 |
| Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene- | 1113.5 | 7392043 | 15.64 |
| Decanoic acid, decyl ester | 1628.2 | 6026936 | 12.75 |
| β-Pinene | 976.5 | 4694051 | 9.93 |
| 1,3,6-Octatriene, 3,7-dimethyl- | 1046.4 | 4531157 | 9.59 |
| N-Morpholinomethyl-isopropyl-sulfide | 1866.4 | 1883281 | 3.98 |
| 3-Hexen-1-ol, acetate, (E)- | 1005.5 | 1465931 | 3.10 |
| Furan, 3-(4,8-dimethyl-3,7-nondienyl)-, (E)- | 1571.6 | 1182889 | 2.50 |
| Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- | 971.8 | 1047287 | 2.22 |
| Limonene | 1028.5 | 1035189 | 2.19 |
| 2-Hexanol, (R)- | 809.8 | 859747 | 1.82 |
| Homosalate | 1872.0 | 854761 | 1.81 |
| 1,6-Octadien-3-ol, 3,7-dimethyl- | 1098.9 | 590209 | 1.25 |
| 5-Hepten-2-one, 6-methyl- | 984.2 | 399606 | 0.85 |
| 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- | 1478.4 | 387878 | 0.82 |
| 1R-α-Pinene | 932.6 | 362764 | 0.77 |
| 4-Penten-2-ol | 867.9 | 317181 | 0.67 |
| 6-Undecylamine | 1662.5 | 310885 | 0.66 |
| Caryophyllene | 1418.2 | 302960 | 0.64 |
| 3-Pentanol | 856.2 | 262931 | 0.56 |

**TABLE 2-3:** Compounds Identified as ALB Antennally Active Using Synthetic Standards Only

| | CAS # | GC-MS RT | GC-MS RI |
|---|---|---|---|
| Benzaldehyde | 100-52-7 | 5.741 | 819.1 |
| Camphene | 79-92-5 | 8.910* | 928.0* |
| Hexanal | 66-25-1 | 5.290 | 802.9 |
| *cis*-Linalool Oxide | 5989-33-3 | 13.354* | 1053.4* |
| 1-Octen-3-ol | 3391-86-4 | 10.670 | 979.0 |
| Pentyl hexanoate | 540-07-8 | 12.849* | 1040* |
| α-Phellandrene | 99-83-2 | 10.827* | 983.6* |
| α-Pinene | 80-56-8 | 8.425* | 913.9* |
| *trans*-Pinocarveol | 1674-08-4 | 15.626* | 1115.4* |
| Propyl hexanoate | 626-77-7 | 9.535* | 940* |

*****Identified as antennally active during preliminary analysis in 2014.

**TABLE 2-4:** ALB Antennally Active Compounds Selected for Statistical Analysis.

| GC-EAD Resp. #[a] | Compound ID | CAS # | Class | # of Samples | GC-MS RT | GC-MS RI | Ref. AI[b] | Ref. KI[b] |
|---|---|---|---|---|---|---|---|---|
| 9 | (*E*)-2-Hexenal | 6728-26-3 | Green Leaf Aldehyde | 35 | 6.57 | 849.6 | 846 | 855 |
| 10 | (*3Z*)-Hexenol | 928-96-1 | Green Leaf Alcohol | 50 | 6.65 | 852.3 | 850 | 859 |
| 12 | (*2E*)-Hexenol | 928-95-0 | Green Leaf Alcohol | 25 | 6.93 | 862.5 | 854 | 862 |
| 13 | *n*-Hexanol | 111-27-3 | Green Leaf Alcohol | 35 | 7.03 | 866.2 | 863 | 870 |
| 18 | Sabinene | 3387-41-5 | Monoterpene | 22 | 10.4 | 971.3 | 969 | 975 |
| 19 | β-Pinene | 127-91-3 | Monoterpene | 34 | 10.53 | 975.0 | 974 | 979 |
| 20 | Unknown monoterpene | | Monoterpene | 17 | 10.62 | 977.7 | | |
| 22 | Myrcene | 123-35-3 | Monoterpene | 46 | 11.01 | 989.0 | 988 | 990 |
| 23 | 6-Methyl-5-hepten-2-ol | 1569-60-4 | Carotenoid-related | 9 | 11.13 | 992.4 | 989 | 991 |
| 26 | Unknown monoterpene | | Monoterpene | 12 | 11.57 | 1004.8 | | |
| 27 | (*3Z*)-Hexenyl acetate | 3681-71-8 | Green Leaf Ester | 49 | 11.57 | 1004.9 | 1004 | 1005 |
| 28 | Hexyl acetate | 142-92-7 | Green Leaf Ester | 29 | 11.83 | 1012.0 | 1007 | 1009 |
| 29 | (*2E*)-Hexenyl acetate | 2497-18-9 | Green Leaf Ester | 11 | 11.93 | 1014.7 | 1010 | 1013 |
| 30 | Limonene | 138-86-3 | Monoterpene | 44 | 12.42 | 1027.9 | 1024 | 1029 |
| 31 | Benzyl alcohol | 100-51-6 | Benzenoid | 25 | 12.55 | 1031.6 | 1026 | 1031 |
| 32 | (*Z*)-β-Ocimene | 3338-55-4 | Monoterpene | 46 | 12.69 | 1035.4 | 1032 | 1037 |
| 34 | (*E*)-β-Ocimene | 3779-61-1 | Monoterpene | 54 | 13.08 | 1046 | 1044 | 1050 |
| 36 | γ-Terpinene | 99-85-4 | Monoterpene | 19 | 13.47 | 1056.6 | 1054 | 1059 |
| 40 | Fenchone | 1195-79-5 | Monoterpenoid | 1 | 14.47 | 1083.8 | 1083 | 1086 |
| 41 | *p*-Mentha-2,4(8)-diene | 586-63-0 | Monoterpene | 12 | 14.48 | 1083.9 | 1085 | 1088 |
| 42 | *trans*-Linalool oxide | 34995-77-2 | Monoterpenoid | 11 | 14.55 | 1086.1 | 1084 | 1086 |
| 43 | Methyl benzoate | 93-58-3 | Benzenoid | 15 | 14.78 | 1092.2 | 1088 | 1090 |
| 44 | (*3Z*)-Hexenyl propionate | 33467-74-2 | Green Leaf Ester | 9 | 14.97 | 1097.5 | 1095 | 1096 |
| 45 | Linalool | 78-70-6 | Monoterpenoid | 37 | 15.01 | 1098.4 | 1095 | 1096 |
| 47 | (*E*)-4,8-Dimethyl-1,3,7-nonatriene | 19945-61-0 | Monoterpenoid | 45 | 15.53 | 1112.8 | | |
| 48 | Unknown oxygenated monoterpenoid | | Monoterpenoid | 13 | 15.69 | 1117.1 | | |
| 49 | Unknown monoterpene | | Monoterpene | 18 | 15.80 | 1120.1 | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50 | *allo*-Ocimene | 7216-56-0 | Monoterpenoid | 27 | 16.07 | 1127.7 | 1128 | 1132 |
| 51 | Unknown monoterpene | | Monoterpene | 26 | 16.10 | 1128.5 | | |
| 53 | Unknown oxygenated monoterpenoid | | Monoterpenoid | 11 | 16.95 | 1151.8 | | |
| 54 | Benzyl Acetate | 140-11-4 | Benzenoid | 11 | 17.28 | 1161.1 | 1157 | 1162 |
| 55 | *p*-Mentha-1,5-dien-8-ol | 1686-20-0 | Monoterpenoid | 21 | 17.61 | 1170.1 | 1166 | 1170 |
| 56 | (*3Z)*-Hexenyl butanoate | 16491-36-4 | Green Leaf Ester | 24 | 18.14 | 1184.5 | 1184 | 1186 |
| 57 | Methyl salicylate | 119-36-8 | Lignin-related | 27 | 18.32 | 1189.6 | 1190 | 1191 |
| 58 | Unknown oxygenated terpenoid | | Terpenoid | 16 | 18.74 | 1201.3 | | |
| 60 | Unknown oxygenated terpenoid | | Terpenoid | 22 | 18.92 | 1206.4 | | |
| 62 | Unknown | | Unknown | 13 | 21.0 | 1266.2 | | |
| 65 | α-Cubebene | 17699-14-8 | Sesquiterpene | 5 | 23.7 | 1346.2 | 1348 | 1351 |
| 66 | β-Cubebene | 13744-15-5 | Sesquiterpene | 13 | 25.03 | 1386.6 | 1387 | 1388 |
| 67 | γ-Elemene | 29873-88-2 | Sesquiterpene | 11 | 25.06 | 1387.6 | 1389 | 1390 |
| 68 | Unknown | | Unknown | 2 | 25.39 | 1397.5 | | |
| 70 | Unknown oxygenated terpenoid | | Terpenoid | 13 | 25.94 | 1414.9 | | |
| 71 | Bergamotene | 18252-46-5 | Sesquiterpene | 7 | 25.76 | 1409.3 | 1411 | 1412 |
| 72 | (*E*)-Caryophyllene | 87-44-5 | Sesquiterpene | 32 | 26.02 | 1417.7 | 1417 | 1419 |
| 73 | Unknown oxygenated terpenoid | | Terpenoid | 14 | 26.17 | 1422.5 | | |
| 75 | Isoamyl Benzoate | 94-46-2 | Green Leaf Ester | 6 | 26.60 | 1436.2 | 1433 | 1435 |
| 77 | α-Humulene | 6753-98-6 | Sesquiterpene | 20 | 27.19 | 1454.9 | 1452 | 1454 |
| 78 | Germacrene D | 23986-74-5 | Sesquiterpene | 23 | 27.92 | 1478.3 | 1480 | 1481 |
| 79 | (*Z,E*)-α-Farnesene | 26560-14-5 | Sesquiterpene | 28 | 28.25 | 1488.8 | | |
| 80 | Unknown sesquiterpene | | Sesquiterpene | 10 | 28.38 | 1493.0 | | |
| 81 | (*E,E*)-α-Farnesene | 502-61-4 | Sesquiterpene | 49 | 28.67 | 1502.4 | 1505 | 1505 |
| 82 | δ-Cadinene | 483-76-1 | Sesquiterpene | 15 | 29.10 | 1516.9 | 1522 | 1523 |
| 83 | (*E)*-Nerolidol | 40716-6-3 | Sesquiterpenoid | 15 | 30.36 | 1559.6 | 1561 | 1563 |
| 84 | (*3Z*)-Hexenyl benzoate | 25152-85-6 | Green Leaf Ester | 17 | 30.63 | 1568.7 | 1565 | 1566 |

[a]See Figure 2-3 [b](Adams 2007)

**TABLE 2-5:** T-Test of PCA PC 1-3 Significance by Host vs. Nonhost Groups

| By ALB Host / Non-Hosts | | By CLB Host / Non-Hosts | |
|---|---|---|---|
| PC | p-Value | PC | p-Value |
| PC 1 | 0.593 | PC 1 | **0.023** |
| PC 2 | 0.788 | PC 2 | **0.017** |
| PC 3 | **0.000** | PC 3 | **0.000** |

**TABLE 2-6** Influential ALB Host and Nonhost Indicative Compounds from PCA, DA, and RF.

Principle Component Analysis (PCA)

| Host Indicative Compounds | | | Nonhost Indicative Compounds | | |
|---|---|---|---|---|---|
| Resp #[a] | Compound ID | PC3 Eig. | Resp # | Compound ID | PC3 Eig. |
| **23[b]** | **6-Methyl-5-hepten-2-ol** | **0.289** | 50 | *allo*-Ocimene | -0.147 |
| **75** | **Isoamyl benzoate** | **0.282** | 80 | Unknown Sesquiterpene | -0.148 |
| **83** | **(*E*)-Nerolidol** | **0.250** | 32 | (*Z*)-β-Ocimene | -0.154 |
| **54** | **Benzyl acetate** | **0.225** | 10 | (3*Z*)-Hexenol | -0.169 |
| **82** | **δ-Cadinene** | **0.212** | 13 | *n*-Hexenol | -0.171 |
| **77** | **α-Humulene** | **0.202** | 18 | Sabinene | -0.188 |
| **43** | **Methyl Benzoate** | **0.188** | 19 | β-Pinene | -0.198 |
| **72** | **(*E*)-Caryophyllene** | **0.149** | 39 | (*E*)-2-Hexenal | -0.209 |
| **47** | **(*E*)-4,8-Dimethyl-1,3,7-nonatriene** | **0.142** | 12 | (*2E*)-Hexenol | -0.223 |

Discriminate Analysis (DA)

| Host Indicative Compounds | | | | Nonhost Indicative Compounds | | | |
|---|---|---|---|---|---|---|---|
| Resp #[a] | Compound ID | Can1 | p-value | Resp # | Compound ID | Can1 | p-value |
| **54[b]** | **Benzyl acetate** | **$2.93 \times 10^{-2}$** | <.0001 | 19 | β-Pinene | $-2.58 \times 10^{-2}$ | <.0001 |
| **77** | **α-Humulene** | **$2.78 \times 10^{-2}$** | <.0001 | 41 | *p*-Mentha-2,4(8)-diene | $-1.81 \times 10^{-2}$ | 0.0004 |
| **75** | **Isoamyl benzoate** | **$1.86 \times 10^{-2}$** | 0.0001 | 18 | Sabinene | $-1.51 \times 10^{-2}$ | 0.0015 |
| **82** | **δ-Cadinene** | **$1.76 \times 10^{-2}$** | 0.0003 | 9 | (*E*)-2-Hexenal | $-1.26 \times 10^{-2}$ | 0.0070 |
| **23** | **6-Methyl-5-hepten-2-ol** | **$1.73 \times 10^{-2}$** | 0.0003 | 10 | (*3E*)-Hexenol | $-1.26 \times 10^{-2}$ | 0.0077 |
| **83** | **(*E*)-Nerolidol** | **$1.71 \times 10^{-2}$** | 0.0004 | 30 | Limonene | $-1.23 \times 10^{-2}$ | 0.0084 |
| **72** | **(*E*)-Caryophyllene** | **$1.60 \times 10^{-2}$** | 0.0008 | 80 | Unknown sesquiterpene | $-1.21 \times 10^{-2}$ | 0.0094 |
| **47** | **(*E*)-4,8-Dimethyl-1,3,7-nonatriene** | **$1.27 \times 10^{-2}$** | 0.0067 | 67 | γ-Elemene | $-1.12 \times 10^{-2}$ | 0.0164 |
| **31** | **Benzyl Alcohol** | **$9.4 \times 10^{-3}$** | 0.0415 | 36 | γ-Terpinene | $-9.7 \times 10^{-3}$ | 0.0353 |

Random Forests (RF)

| Influential Predictor Variables | | |
|---|---|---|
| Resp #[a] | Compound ID | %IncMSE |
| **54[b]** | **Benzyl Acetate** | **49.96** |
| **77** | **α-Humulene** | **42.25** |
| **83** | **(*E*)-Nerolidol** | **35.40** |
| **72** | **(*E*)-Caryophyllene** | **32.06** |
| 41 | *p*-Mentha-2,4(8)-diene | 31.10 |
| 19 | β-Pinene | 30.85 |
| **75** | **Isoamyl Benzoate** | **26.14** |
| **47** | **(*E*)-4,8-Dimethyl-1,3,7-nonatriene** | **23.78** |
| **23** | **6-Methyl-5-hepten-2-ol** | **21.82** |
| **82** | **δ-Cadinene** | **16.10** |

[a]See Figure 2-3.

[b]Bold font indicates host indicative compounds.

**TABLE 2-7:** Influential CLB Host vs. Nonhost Compounds from DA and RF.

Discriminate Analysis (DA)

| Host Indicative Compounds | | | | Nonhost Indicative Compounds | | | |
|---|---|---|---|---|---|---|---|
| Resp #[a] | Compound ID | Can1 | | Resp # | Compound ID | Can1 | |
| **47**[b] | **(*E*)-4,8-Dimethyl-1,3,7-nonatriene** | **$1.6 \times 10^{-3}$** | 0.0001 | 41 | *p*-Mentha-2,4(8)-diene | $-8.25 \times 10^{-4}$ | 0.0001 |
| **56** | **(*3Z*)-Hexenyl butanoate** | **$6.3 \times 10^{-4}$** | 0.0003 | 50 | *allo*-Ocimene | $-6.22 \times 10^{-4}$ | 0.0004 |
| **42** | ***trans*-Linalool Oxide** | **$5.2 \times 10^{-4}$** | 0.0025 | 13 | *n*-Hexenol | $-5.79 \times 10^{-4}$ | 0.008 |
| **23** | **6-Methyl-5-hepten-2-ol** | **$4.6 \times 10^{-4}$** | 0.0067 | 62 | Unknown | $-5.24 \times 10^{-4}$ | 0.0022 |
| **29** | **(*2E*)-Hexenyl acetate** | **$4.3 \times 10^{-4}$** | 0.0195 | 9 | (*E*)-2-Hexenal | $-5.14 \times 10^{-4}$ | 0.0026 |
| **27** | **(*3Z*)-Hexenyl acetate** | **$3.9 \times 10^{-4}$** | 0.0101 | 55 | *p*-Mentha-1,5-dien-8-ol | $-4.31 \times 10^{-4}$ | 0.0751 |
| **84** | **(*3Z*)-Hexenyl benzoate** | **$3.9 \times 10^{-4}$** | 0.0210 | 18 | Sabinene | $-4.16 \times 10^{-4}$ | 0.0135 |
| **54** | **Benzyl Acetate** | **$3.9 \times 10^{-4}$** | 0.0216 | 70 | Unknown oxygenated terpenoid | $-4.08 \times 10^{-4}$ | 0.0468 |
| **82** | **δ-Cadinene** | **$3.5 \times 10^{-4}$** | 0.0369 | 32 | (*Z*)-β-Ocimene | $-4.07 \times 10^{-4}$ | 0.0154 |

Random Forests (RF)

| Resp # | Compound ID | %IncMSE |
|---|---|---|
| **47** | **(*E*)-4,8-Dimethyl-1,3,7-nonatriene** | **61.74** |
| 41 | *p*-Mentha-2,4(8)-diene | 38.70 |
| 13 | *n*-Hexenol | 28.96 |
| 50 | *allo*-Ocimene | 28.86 |
| 28 | Hexyl Acetate | 27.46 |
| **56** | **(*3Z*)-Hexenyl butanoate** | **26.50** |
| 62 | Unknown | 24.15 |
| **27** | **(*3Z*)-Hexenyl acetate** | **21.65** |
| 32 | (Z)-β-Ocimene | 20.58 |
| 70 | Unknown oxygenated terpenoid | 18.89 |

[a]See Figure 2-3.

[b]Bold font indicates host indicative compounds.

**TABLE 2-8:** Heat Map of Average GC-MS Integration Values of Select Host or Non-Host Indicative Compounds by Tree Species

| **Host Indicative Compound for:** | | | ALB | ALB | ALB | ALB | ALB | ALB | ALB | ALB & CLB |
|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **ALB Host** | **CLB Host** | Benzyl acetate | α-Humulene | (*E*)-Nerolidol | (*E*)-Caryophyllene | Isoamyl benzoate | 6-Methyl-5-hepten-2-ol | δ-Cadinene | (*E*)-4,8-Dimethyl-1,3,7-nonatriene |
| *Ailanthus altissima* | No | No | 0 ± 0 | 0 ± 0 | 0 ± 0 | 5010 ± 13400 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 6320 ± 12400 |
| *Citrus microcarpa* | No | **Yes** | 0 ± 0 | 26600 ± 84300 | 0 ± 0 | 589000 ± 1440000 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1450000 ± 2250000 |
| *Liriodendron tulipiferus x chinensis* | No | No | 0 ± 0 | 55300 ± 92600 | 89400 ± 103000 | 449000 ± 518000 | 0 ± 0 | 0 ± 0 | 3370 ± 5540 | 1090000 ± 2660000 |
| *Melia azedarach* | No | **Yes** | 0 ± 0 | 0 ± 0 | 0 ± 0 | 5790 ± 14200 | 0 ± 0 | 229 ± 561 | 0 ± 0 | 991000 ± 1870000 |
| *Morus alba* | No | **Yes** | 0 ± 0 | 107000 ± 189000 | 3170 ± 8370 | 629000 ± 1090000 | 0 ± 0 | 1560 ± 4130 | 8740 ± 14900 | 179000 ± 232000 |
| *Salix babylionica* | **Yes** | **Yes** | 253000 ± 284000 | 115000 ± 109000 | 1610 ± 2970 | 1050000 ± 968000 | 0 ± 0 | 377 ± 1070 | 4600 ± 7840 | 974000 ± 866000 |
| *Ulmus parvifolia* | **Yes** | **Yes** | 1470 ± 1870 | 63800 ± 63700 | 411000 ± 419000 | 407000 ± 350000 | 162000 ± 143000 | 50400 ± 42300 | 9280 ± 11200 | 7750000 ± 8210000 |

| **Non-Host Indicative Compound for:** | | | ALB & CLB | ALB & CLB | ALB & CLB | ALB |
|---|---|---|---|---|---|---|
| **Species** | **ALB Host** | **CLB Host** | *n*-Hexenol | allo-Ocimene | p-Mentha-2,4(8)-diene | β-Pinene |
| *Ailanthus altissima* | **No** | **No** | 62800 ± 98600 | 94100 ± 265000 | 7080 ± 23000 | 11100 ± 11600 |
| *Citrus microcarpa* | **No** | Yes | 28500 ± 90000 | 1290000 ± 4090000 | 198000 ± 625000 | 1600000 ± 1770000 |
| *Liriodendron tulipiferus x chinensis* | **No** | **No** | 46100 ± 29700 | 1690000 ± 1770000 | 261000 ± 309000 | 677000 ± 736000 |
| *Melia azedarach* | **No** | Yes | 19300 ± 35100 | 325000 ± 797000 | 23900 ± 58400 | 86300 ± 191000 |
| *Morus alba* | **No** | Yes | 1440000 ± 3170000 | 281000 ± 341000 | 16100 ± 30400 | 17400 ± 31600 |
| *Salix babylionica* | Yes | Yes | 1430 ± 2660 | 6520 ± 12900 | 1050 ± 1940 | 1130000 ± 3190000 |
| *Ulmus parvifolia* | Yes | Yes | 241000 ± 509000 | 15000 ± 13100 | 16600 ± 16800 | 1540 ± 3090 |


**FIGURE 2-1:** Static headspace aeration method of *Morus alba* foliage and a blank control (both in 2L Erlenmeyer flasks) (Nanjing, China, 2017). Photo taken by author.

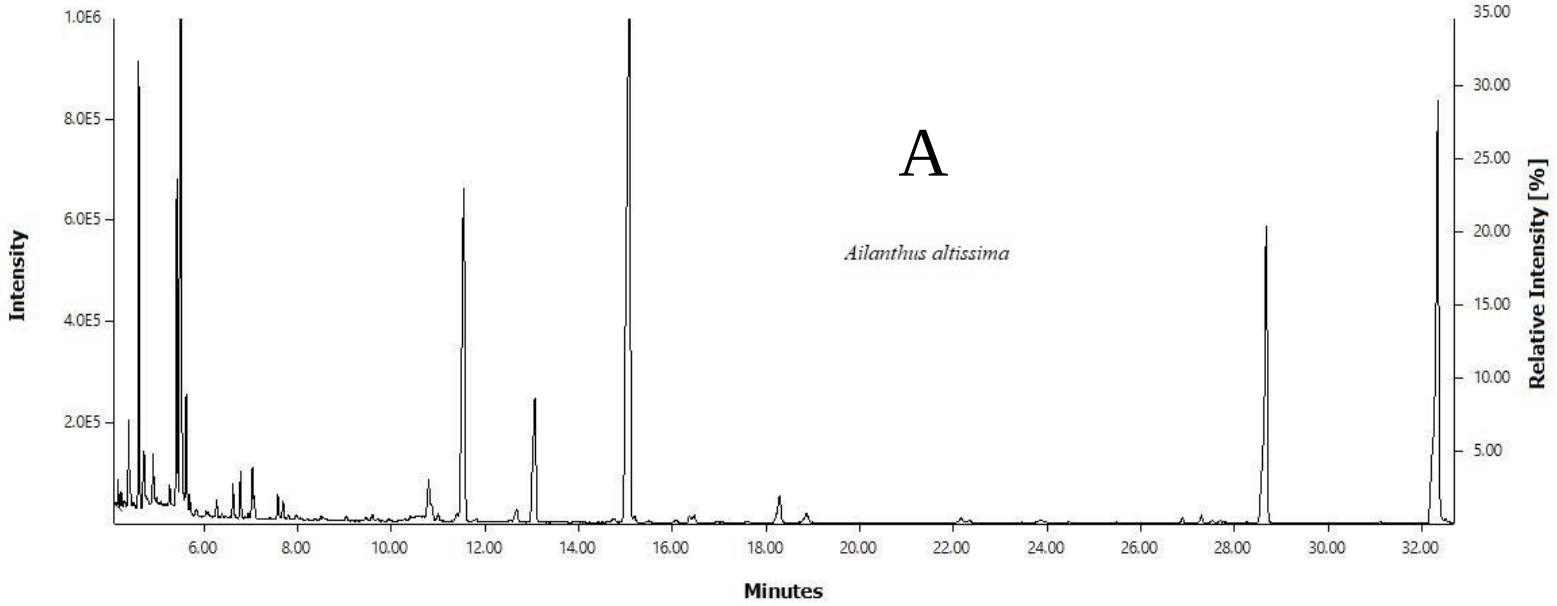
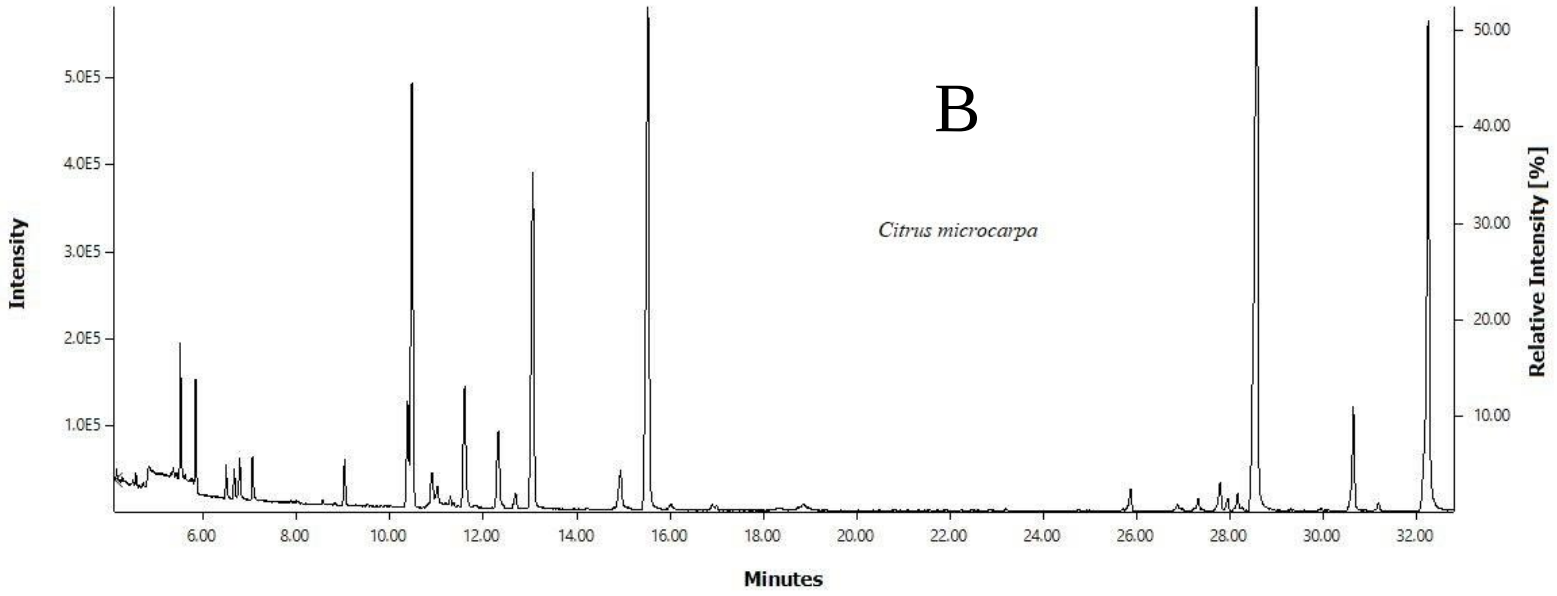
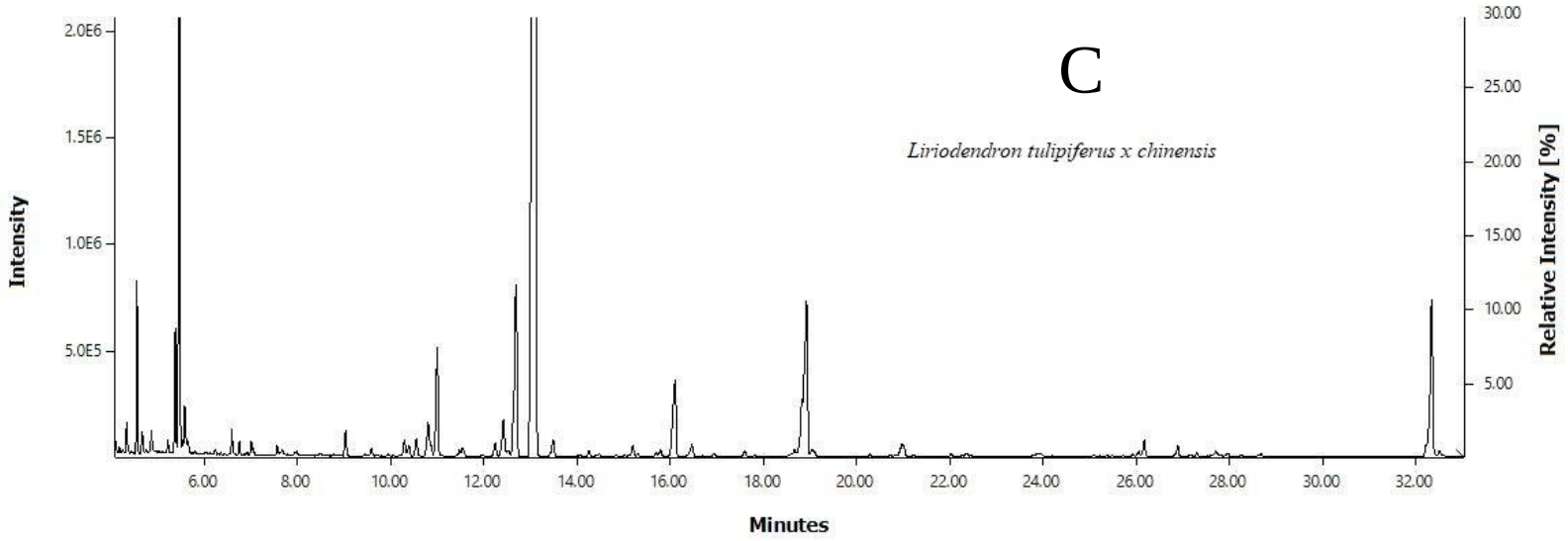
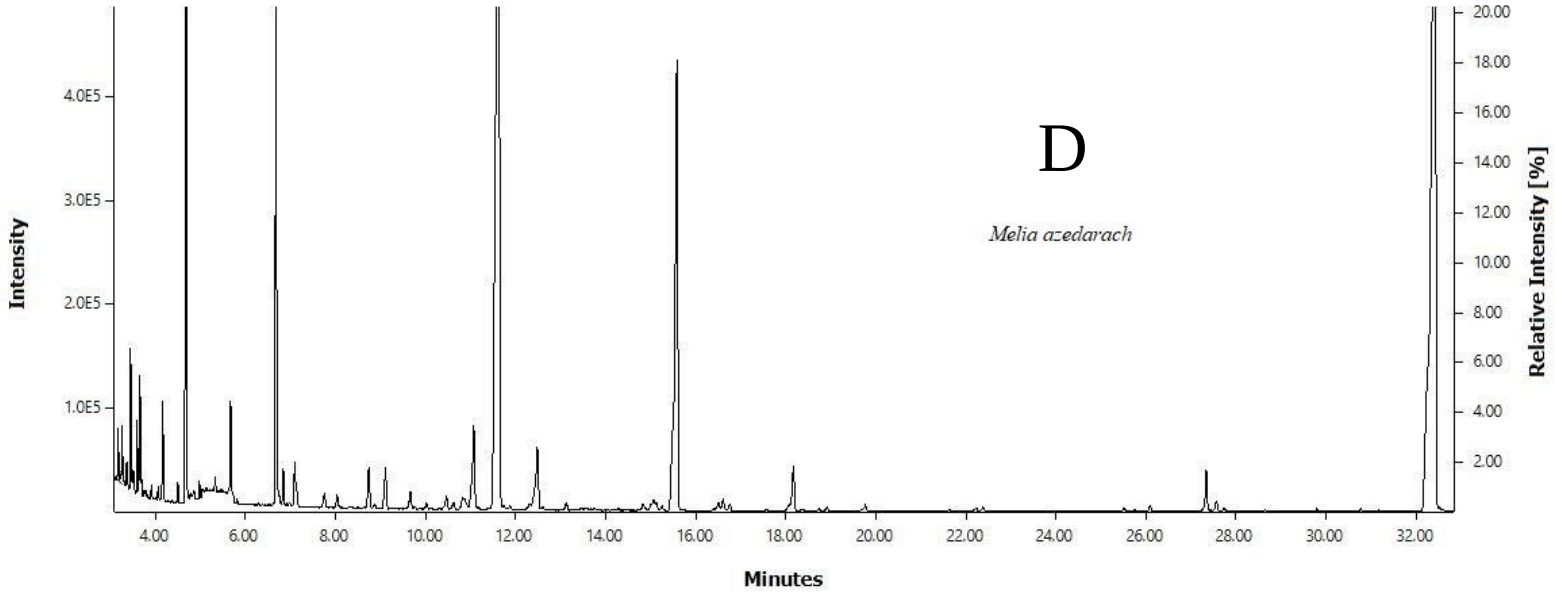
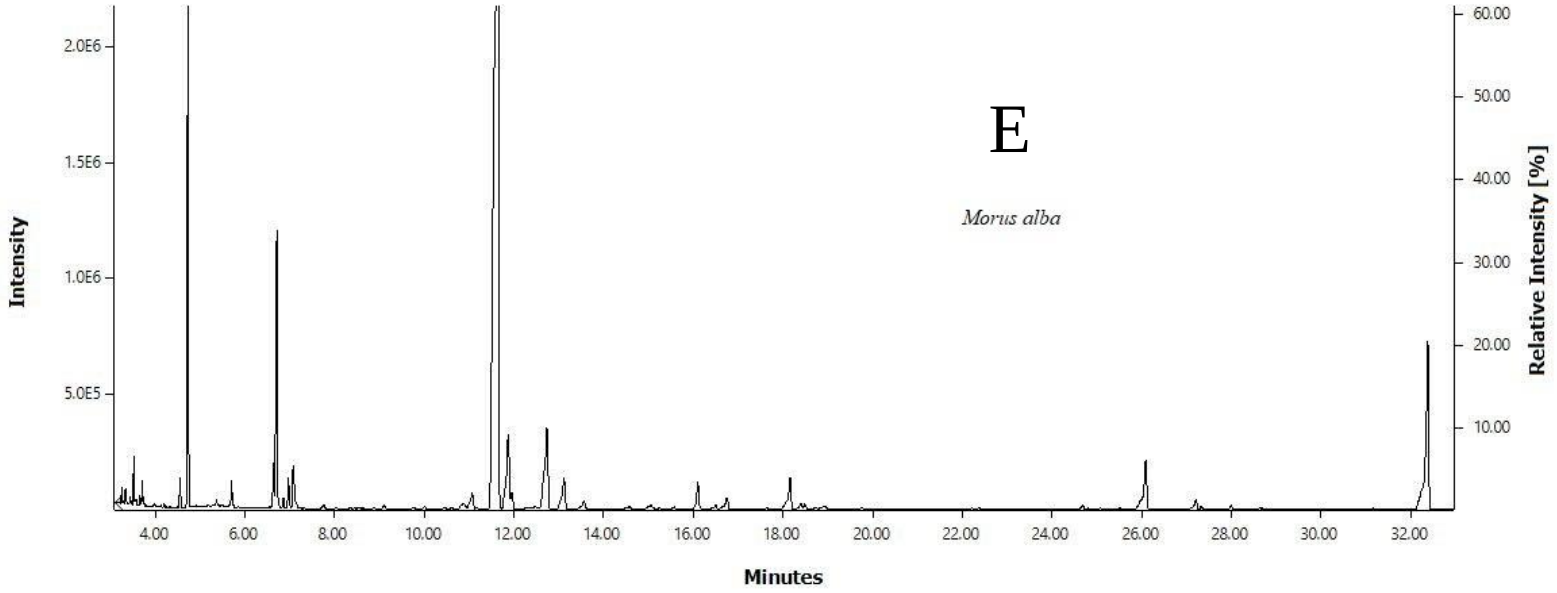


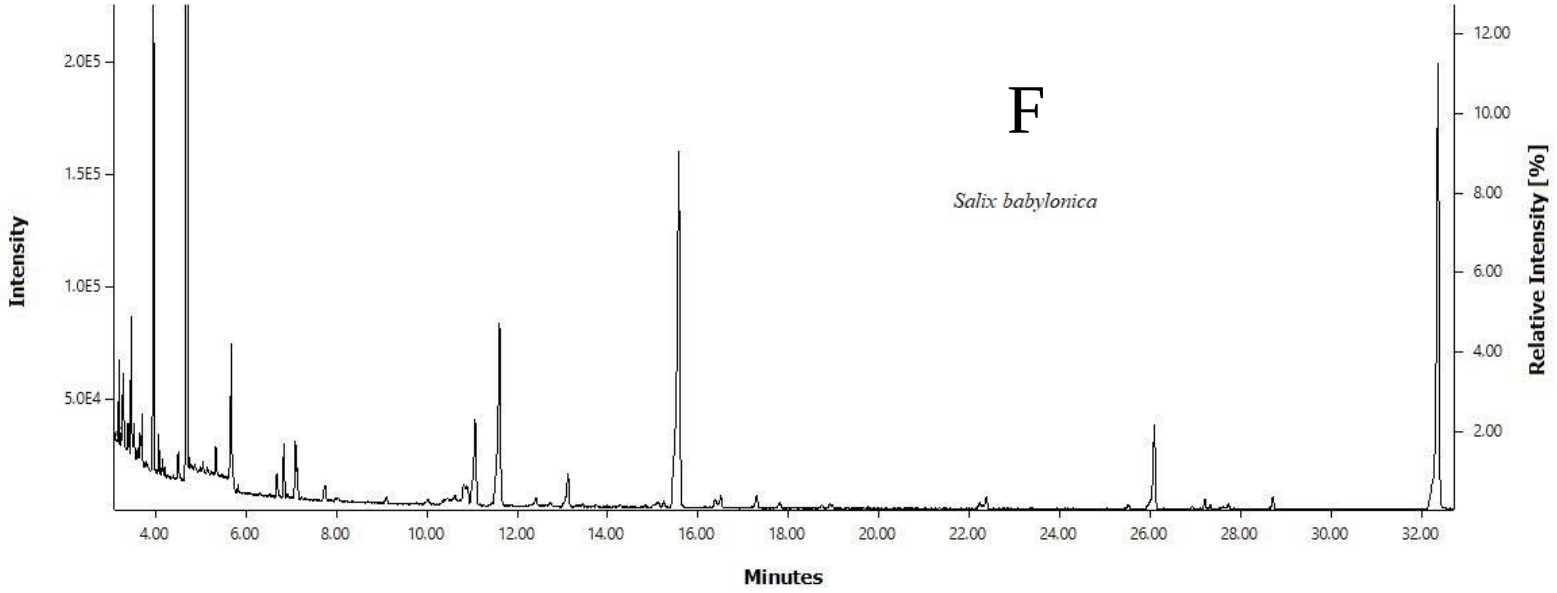

**FIGURE 2-2**. Representative GC-MS Chromatograms (TIC) of each nonhost species. *Ailanthus altissima* aeration (**A**)**,** *Citrus microcarpa* aeration (**B**), *Liriodendron tulipiferus x chinensis* aeration (**C**), *Melia azedarach* aeration (**D**), *Morus alba* aeration (**E**), *Salix babylonica* aeration (**F**), and *Ulmus parvifolia* aeration (**G**) and its blank control (**G**). Y-axis are scaled according to the height of the most abundant peak. (Continued on the next pages)

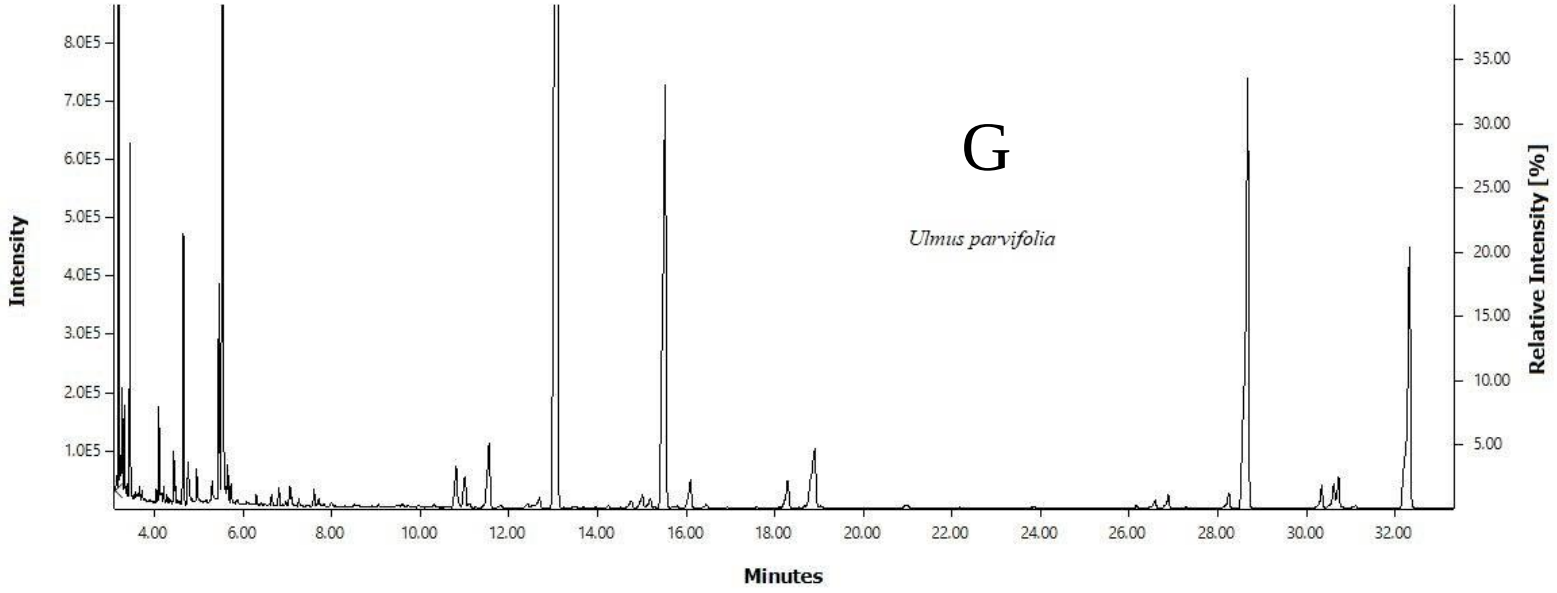

B
Citrus microcarpa
Intensity
Relative Intensity [%]
Minutes
5.0E5
4.0E5
3.0E5
2.0E5
1.0E5
50.00
40.00
30.00
20.00
10.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00


C
Liriodendron tulipiferus x chinensis
Intensity
Relative Intensity [%]
Minutes
2.0E6
1.5E6
1.0E6
5.0E5
30.00
25.00
20.00
15.00
10.00
5.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00


D
Melia azedarach
Intensity
Relative Intensity [%]
Minutes
4.0E5
3.0E5
2.0E5
1.0E5
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00


E
Morus alba
Intensity
Relative Intensity [%]
Minutes
2.0E6
1.5E6
1.0E6
5.0E5
60.00
50.00
40.00
30.00
20.00
10.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00


F
Salix babylonica
Intensity
Relative Intensity [%]
Minutes
2.0E5
1.5E5
1.0E5
5.0E4
12.00
10.00
8.00
6.00
4.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00


G
Ulmus parvifolia
Intensity
Relative Intensity [%]
Minutes
8.0E5
7.0E5
6.0E5
5.0E5
4.0E5
3.0E5
2.0E5
1.0E5
35.00
30.00
25.00
20.00
15.00
10.00
5.00
4.00
6.00
8.00
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22.00
24.00
26.00
28.00
30.00
32.00

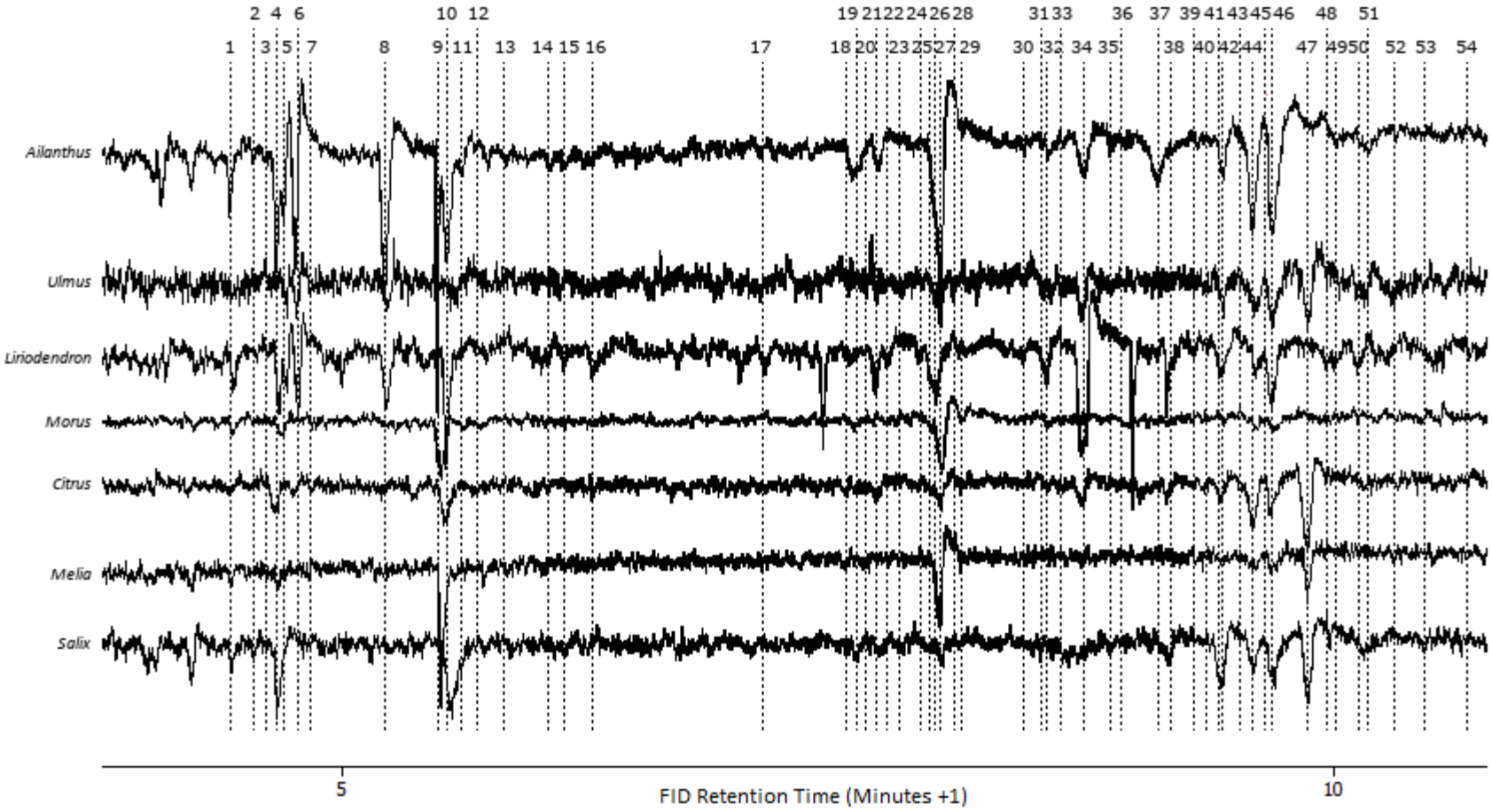
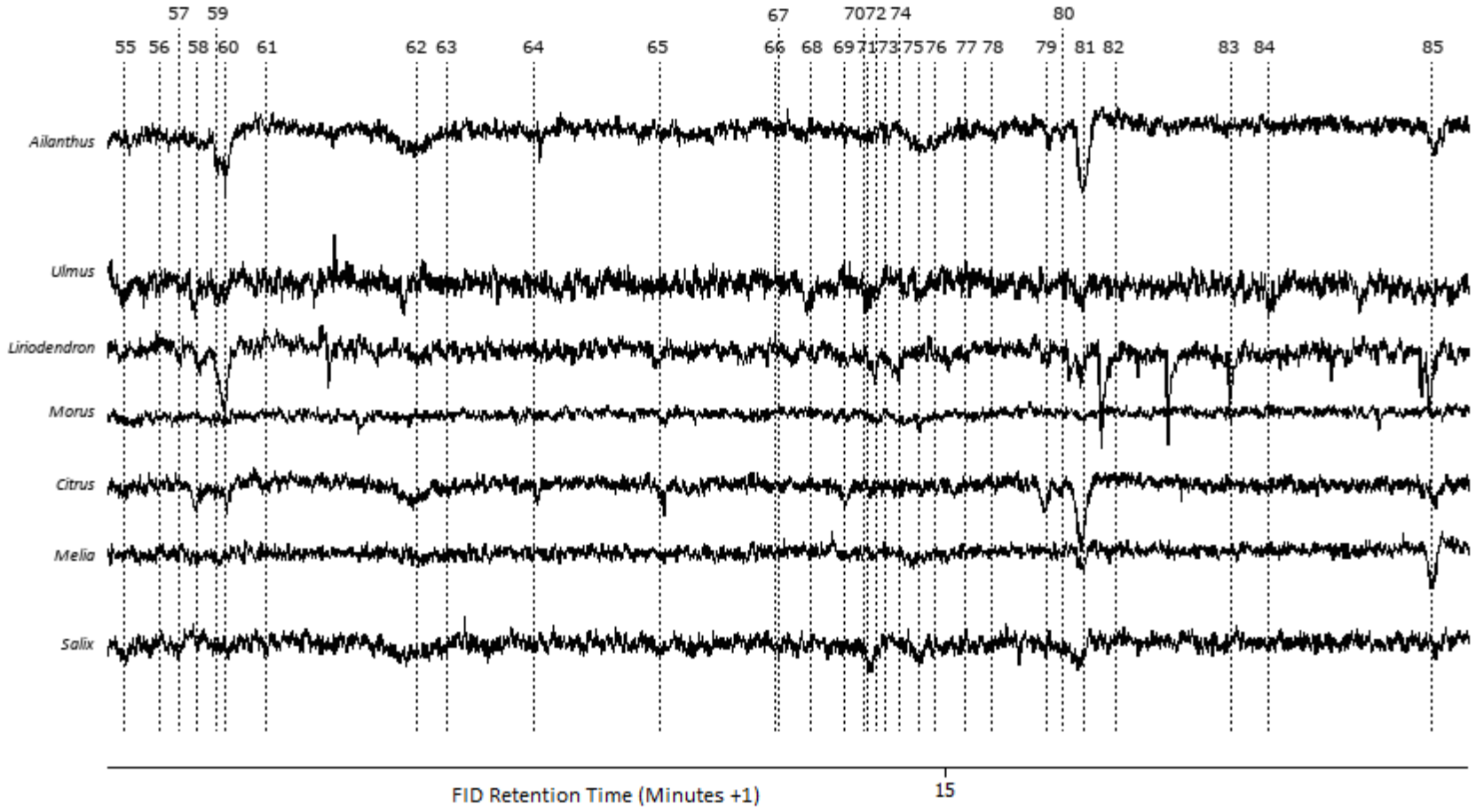



**FIGURE 2-3:** ALB GC-EAD Responses to representative hardwood aerations. Antennally active compound retention times are indicated by dashed lines and the response #'s (Table 10). Traces are best examples obtained using each sample type and were individually vertically scaled to improve response visibility from the baseline (Continued on the next page).


55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
Ailanthus
Ulmus
Liriodendron
Morus
Citrus
Melia
Salix
FID Retention Time (Minutes +1)
15

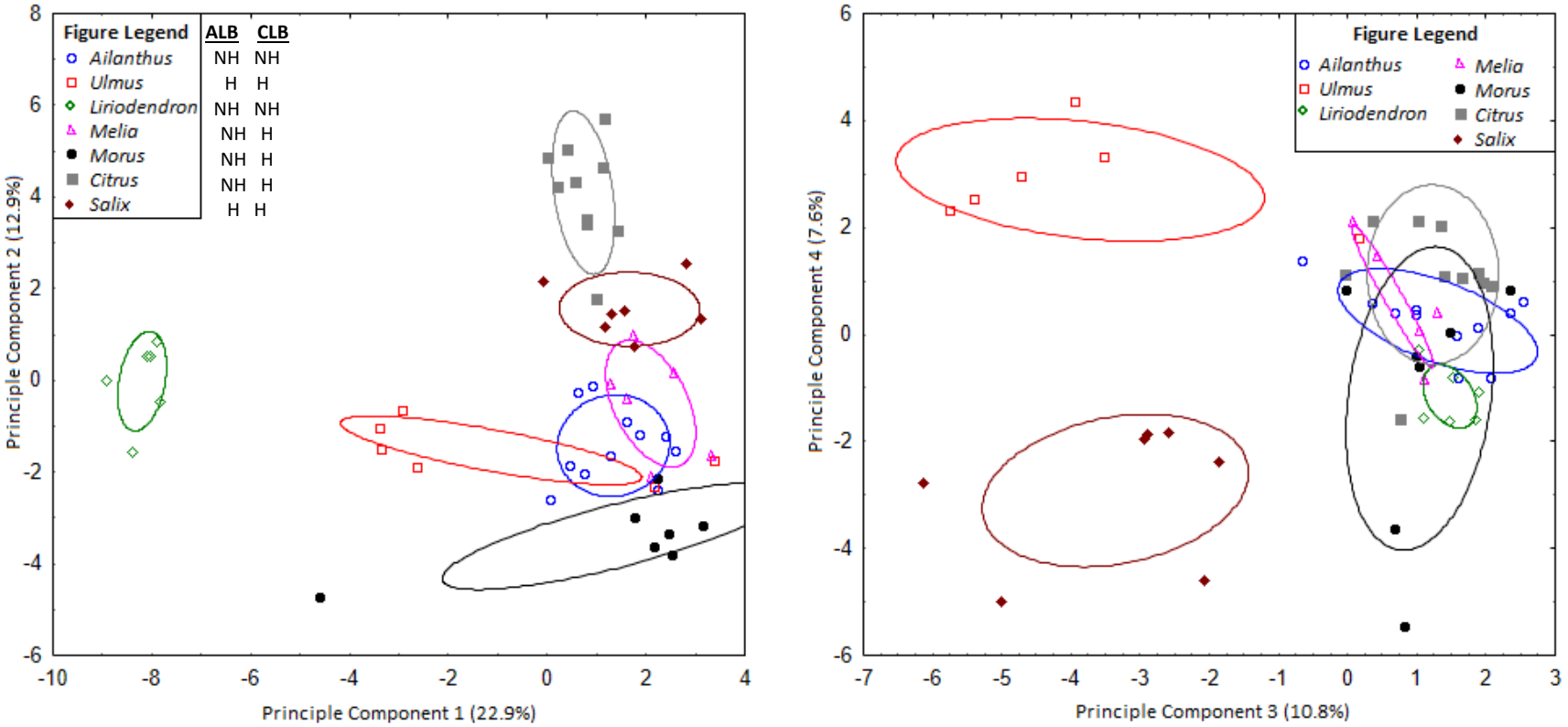



**FIGURE 2-4:** PCA of hardwood aeration samples by quantities of ALB antennally active volatiles. Range ellipses are for visual purposes and are not test of significance.

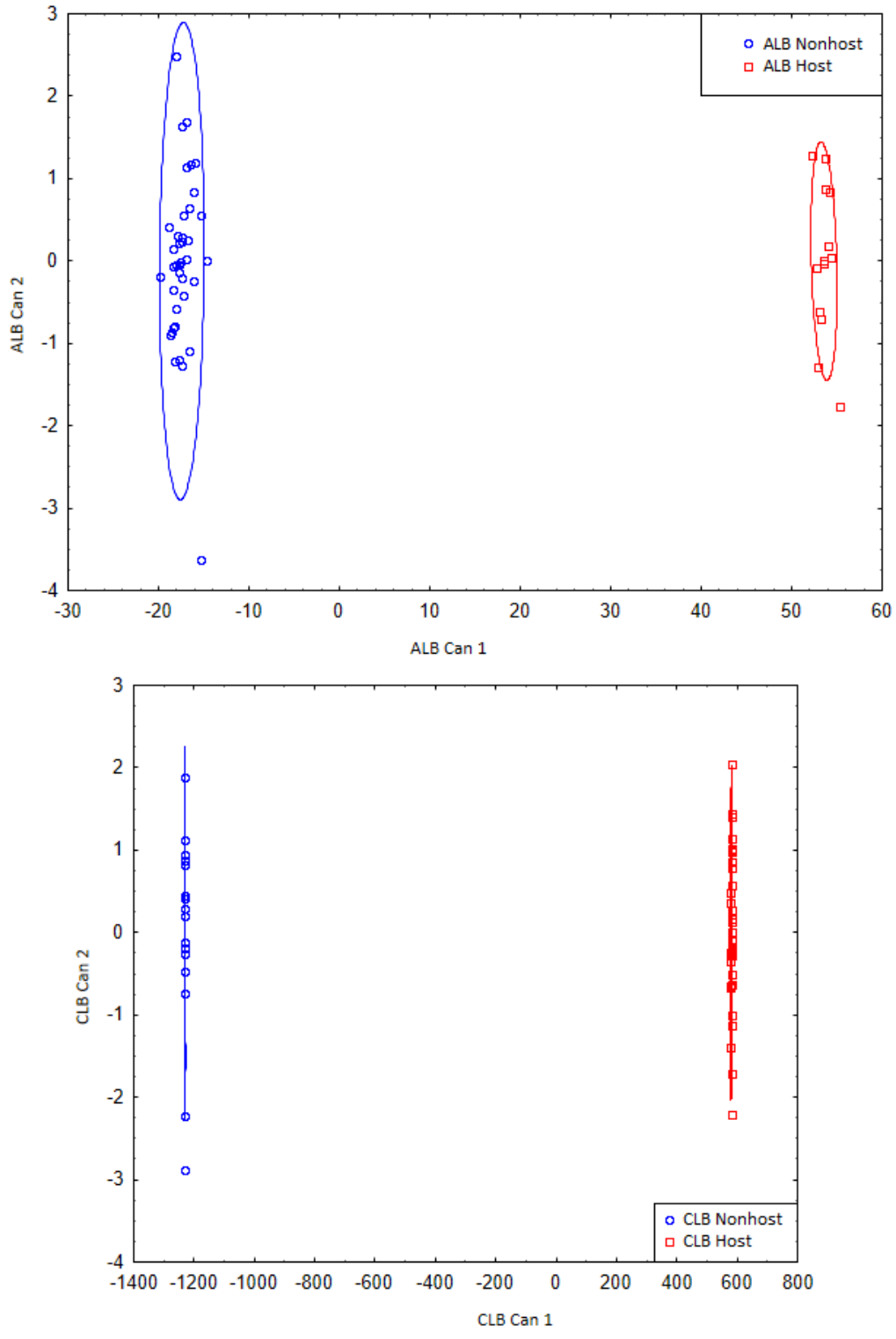



**FIGURE 2-5:** DA by ALB host and nonhost groups. Range ellipses are for visual purposes only and are not a test of significance.

# CHAPTER 3: FIELD TRAPPING EVALUATION

## Introduction

Asian Longhorned Beetle (ALB, *Anoplophora glabripennis*) and Citrus Longhorned Beetle (CLB, *Anoplophora chinensis*) (Subfamily: Lamiinae, Tribe: Lamiini) are sympatric cerambycids native to East Asia. ALB is native to both mainland China and the Korean Peninsula (CABI 2019) with the yellow-spotted form found in Northern China historically recognized as *Anoplophora nobilis* currently junior to *A. glabripennis* (Lingafelter and Hoebeke 2002). CLB is native to mainland China, Japan, Korea, the Philippines, Myanmar, and Taiwan, with isolated populations in Malaysia, Vietnam, and Indonesia (CABI 2019) and with the Japanese CLB historically recognized as *Anoplophora malasiaca* currently synonymized under *A. chinensis* (Lingafelter and Hoebeke 2002). Both species are polyphagous pests of woody trees with ALB infesting a wide range of hardwood trees from at least 15 families including maple, elm, willow, and poplar species (Haack et al. 2010) and the even more broadly polyphagous CLB infesting trees from at least 36 families including maple, elm, willow, and poplar, and agriculturally important trees such as citrus, fig, pecan, and plum (Haack et al. 2010).Host damage is two-fold, with larval xylophagous feeding damaging woody tissues and adult feeding damaging host leaves and twigs. Within their native ranges, both species are serious pests and outbreaks have caused massive amounts of damage (Ji et al. 2011). In China, ALB is estimated to cause at least $1.5 billion USD annual damage and has contributed to the death of millions of non-native poplars planted as part of the Three-Norths shelterbelt region afforestation project (Cao 2008, Hu et al. 2009), while CLB infestation has complicated similar attempts to halt dune erosion using *Casuarina* monocultures (Ge et al. 2014). In Japan, CLB is a major agricultural pest of citrus (Adachi and others 1990). Both ALB and CLB are readily invasive. North

American and European trees lack evolved resistance to either species and may prove to be suitable hosts. In addition, larval presence within woody tissues hides their presence and facilitates accidental transport. (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018)

Field traps with attractive lures are an essential monitoring tool for many pest species. In native ranges or within established invasive populations, field monitoring traps can provide information on presence/absence, emergence period, and population size necessary to inform IPM activities without the need for labor intensive visual monitoring. Highly attractive lures can also be used in tactics such as mating disruption, which decreases population numbers by interfering with mating communication, and mass trapping, which decreases pest numbers by trapping and killing massive numbers of individual insects (Suckling et al. 2014). An additional, less emphasized application of monitoring traps includes detecting the presence of endangered, protected species in locations with low population numbers (Kosi et al. 2017). Within the Cerambycidae, several practical uses of monitoring traps include the monitoring the spread of invasive *Tetropium fuscum* in Canada (Rhainds et al. 2011) and a nationwide survey for pine-feeding wood-borers in New Zealand (Brockerhoff et al. 2006).

Specifically, highly attractive pheromone and/or host volatile lures for ALB are needed in non-native areas for use as monitoring traps at ports-of-entry, within and around the borders of locations with established populations, and as part of general pest surveys in order to identify new pest locations. ALB and CLB larvae, due to their subcortical habits, are difficult to detect so monitoring traps for adults would quickly signal when follow-up inspection is needed. For the same reason, delimitation of infested areas is difficult as is the determination of successful eradication. Reliance on visual inspection to identify all established populations across a large geographic area is time consuming, expensive, and ineffective (Haack et al. 2010). Current

monitoring traps are in use and in development for the above applications. Specifically, hundreds of monitoring traps were placed in Worchester, Massachusetts. Despite their low attractiveness, these traps were able to detect ALB outside the known infestation area, advancing eradication efforts (Meng et al. 2014).

In this study, I test the ALB host compounds identified as part of a host percept in Chapter 2 at a trapping location with both ALB and CLB to evaluate their effectiveness for both species simultaneously. The site was in Bengbu, Anhui Province, China, which is within the native range of both ALB and CLB and a field collaborator at the trapping location confirmed the presence of both species. Prior to the beginning of the trapping period, adult CLB were observed feeding on *Lagerstroemia* sp. and signs of beetle infestation including exit holes and larval frass were observed on *Salix* sp.. I have previously observed ALB and CLB co-infestation on *Salix babylonica* on the Nanjing Forestry University campus, suggesting that both ALB and CLB may often co-infest this species. Host volatiles were tested alone and in combination with a 1:1 mixture of 4-(*n*-heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol, the two compounds previously identified as the male ALB pheromone by Zhang et al. (2002), to examine possible synergy and compare the attractiveness of the host volatile blend to a previously reported lure. Although the male CLB pheromone was identified as 4-(*n*-heptyloxy)butan-1-ol only, 4-(*n*-heptyloxy)butanal did not show an inhibitory effect, meaning that the two component pheromone lure can be also used to attract CLB (Hansen et al. 2015).

**Methods**

The application of ALB host compounds as attractive field lures was tested in Bengbu, China during the summer of 2018 alongside the Huaihe River in an urban *Salix* sp. windbreak

forest (32°56'55.9"N 117°21'09.0"E) (Figure 3-1). Additional hosts of ALB and/or CLB such as *Albizia* sp., *Lagerstroemia* sp., *Populus* sp., and *Morus* sp., were present in the trapping area and surroundings.

The four lure treatments tested (N=10) were host volatiles alone (HV), host volatiles in combination with male pheromone (HV+P), pheromone alone (P), and isopropanol control (Control). Both host volatile treatments contained ALB host compounds in the ratio present in ALB host samples: 254 mg benzyl acetate, 200 mg α-humulene, 416 mg nerolidol, 256 mg (*E*)-caryophyllene, 176 mg isoamyl benzoate, and 298 mg 6-methyl-5-hepten-2-ol. α-Humulene (≥96.0% purity) and 6-methyl-5-hepten-2-ol (≥99% purity) were purchased from Sigma-Aldrich, cis/trans nerolidol (≥97% purity) and benzyl acetate (99% purity) were purchased from Alfa Aesar, and isoamyl benzoate (98.0% purity) and (*E*)-caryophyllene (≥90.0% purity) were purchased from TCI America. Pheromone treatments contained equal amounts of 4-(*n*-heptyloxy)butanal and 4-(*n*-heptyloxy)butan-1-ol (400mg (June 5$^{th}$ to July 17$^{th}$) or 200mg (July 17$^{th}$ to August 21$^{st}$) each). Pheromone was supplied by the Millar lab at the University of California, Riverside.

Semiochemicals were diluted to 1 mL per lure with chromatography grade isopropanol place in heat-sealed packets made from polyethylene tubing, then placed in Fluon-coated (DuPont Chemical Co, Wilmington, DE, USA), black panel intercept traps (IPM Technologies, Portland, OR, USA) in a block arrangement by treatment. Nine blocks were hung in *Salix* sp. windbreak trees and one block was hung in *Albizia* sp. trees, for a total of ten spatial replicates. Panel trap buckets were filled with a 1:1 mixture of water and automobile antifreeze. Traps were set from June 5$^{th}$ to August 21$^{st}$, lures were replaced biweekly, and ALB and CLB trap catch was recorded weekly, for a total of 10 temporal replicates. The trapping site flooded the week of

August 14th to August 21st and 8 traps could not be reached for collection. Results from this week are reported but not included in statistical analysis.

Differences in trap catch by sex were analyzed with chi-square then pooled for analysis. Normality violations were tested with the Anderson-Darling test followed by analysis with the Kruskal-Wallis nonparametric analysis test and a post-hoc Mann Whitney pairwise tests (Minitab 17). As no CLB were captured in any control treatment, 0.001 substituted for one 0 value.

**Results**

A total of six female ALB, eleven male ALB, twenty-five female CLB, and twenty-eight male CLB were collected from June 12th to August 14th across all treatments. CLB were collected throughout the entire trapping period while ALB were collected from June 26th to August 14th , 2018 (Table 3-1). On August 21st, during which 8 traps could not be monitored due to flooding, one female CLB was collected in a pheromone only treatment. August 21st catch results were not included in the statistical analysis. Chi-square did not show significant treatment effects in trap catch by beetle sex for either ALB or CLB in any treatment, so trap catch was pooled for subsequent analysis. The Anderson-Darling univariate normality test indicated multiple treatments were in violation of normality ($p<0.005$). Non-parametric Kruskal-Wallis tests indicated a significant treatment effect for CLB ($p<0.000$, $\alpha=0.05$), but not for ALB ($p=0.374$, $\alpha=0.05$). Subsequent Mann-Whitney pairwise tests indicated that the CLB trap catch in the HV+P or P treatments were significantly greater than HV or Control treatments (Table 3-2). P and HV+P treatments were not significantly different from each other ($p=0.866$) and HV and Control treatments were not significantly different from each other ($p=0.310$).

**Discussion**

Although host volatile lures were not significantly more attractive in the field than solvent controls, these results do provide valuable information. Although non-significant, traps with the HV lures did captured more ALB and CLB individuals than the control treatment. In addition, traps with the HV+P lures captured more CLB than the P treatment alone, and it is possible that additional trapping would reveal significant differences. The attractiveness of lures can be difficult to assess in the field because trapping location, population size, weather, and other unforeseen factors can influence trap catches results to the extent that a real biological effect may not be detected in a single experiment. For example, in one round of trapping, *Tetropium fuscum* pheromone lures were only significant in one trapping location and (Sweeney et al. 2010), while testing of HV + pheromone lures found significance during only one field test but not others (Nehme et al. 2010).

HV traps captured only three CLB while pheromone containing lures captured a total of 50 individuals. This reaffirms that 4-(*n*-heptyloxy)butan-1-ol is attractive lure in the field, and the addition of HVs to 4-(*n*-heptyloxy)butan-1-ol is not an improvement over the previously reported pheromone (Hansen et al. 2015). This may have been due to the omission of (*E*)-4,8-dimethyl-1,3,7-nonatriene, the major CLB host volatile, which was not available for testing. It is also possible that ALB GC-EAD is not an appropriate proxy for the identification of CLB antennally active compounds, i.e., at the peripheral receptor level, the species may differ. Finally, it is possible that if only HV and control traps were tested, CLB would have chosen the HV traps over the control treatment, but with competition from the pheromone traps, this effect was obscured.

The ALB trap catch results are less clear. Although HV, HV+P, and P treatments all captured more beetles than the control treatment, the differences were not significant. Based on visual assessment, and communication with on-site collaborators, in this trapping location the CLB population was much higher and emerged sooner than the ALB population. It is possible that the low trap catches were due to low population numbers. However, due to the visual observation of beetles and larval exit holes in the study area, I do not think this is the case. I suggest weak attraction to male pheromones is a more likely explanation as to why ALB were not captured in significant numbers in the treatments that included the pheromones. It remains possible that further testing in a trapping location with a larger ALB population would have revealed a significant treatment difference. Previous research suggests that trap catch in control treatments is common with ALB, CLB, and other large species of cerambycids when using black intercept traps. The traps are designed to be inherently attractive to cerambycids (Graham and Poland 2012) and were hung in host trees. Trap catch of non-target cerambycids is common in both treatments and controls in these kinds of field trapping experiments during a caged beetle experiment with both target and non-target species were caught in black controls (unpublished data). I suggest that ALB, at best, were only weakly attracted to the pheromone and/or host volatile lures, such that the attractiveness of black intercept traps as landing sites in general was a factor. In contrast, intercept traps with pheromone containing lures were much more attractive to CLB, causing CLB to choose those landing sites over those offered by blank controls.

## Conclusion

4-(*n*-heptyloxy)butan-1-ol lures captured significantly more CLB than host volatile or control lures and remain the best known lures for trapping CLB. No lure captured significantly

more ALB than the controls. This study did not detect a significant difference between the ALB male pheromones and controls.

**TABLE 3-1** Beetle Trap Catch in Bengbu, 2018, by Species, Beetle Sex, and Collection Date

| | CLB | | | ALB | | |
|---|---|---|---|---|---|---|
| Collection Date | Females | Males | Total | Females | Males | Total |
| June 12th | 3 | 6 | 9 | 0 | 0 | 0 |
| June 19th | 9 | 4 | 13 | 0 | 0 | 0 |
| June 26th | 2 | 6 | 8 | 2 | 1 | 3 |
| July 3rd | 1 | 1 | 2 | 0 | 0 | 0 |
| July 10th | 2 | 0 | 2 | 0 | 2 | 2 |
| July 17th | 3 | 4 | 7 | 0 | 3 | 3 |
| July 24th | 2 | 2 | 4 | 0 | 3 | 3 |
| July 31st | 0 | 3 | 3 | 0 | 0 | 0 |
| August 7th | 1 | 2 | 3 | 2 | 2 | 4 |
| August 14th | 2 | 0 | 2 | 2 | 0 | 2 |
| Totals | 25 | 28 | 53 | 6 | 11 | 17 |

**TABLE 3-2** Average Trap Catch per Collection and Treatment Effects of CLB and ALB Trap Catch in Bengbu, 2018 (significant differences indicated by superscripts).

| | CLB | | | ALB | | |
|---|---|---|---|---|---|---|
| Treatment | Females | Males | Total | Females | Males | Total |
| HV + P | 0.1091±0.3397 | 0.1364±0.3926 | 0.2455±0.5750[a] | 0.0091±0.0949 | 0.0273±0.1629 | 0.0364±0.1872 |
| HV | 0.0091±0.0949 | 0.0182±0.1336 | 0.0273±0.1629[b] | 0 | 0.0364±0.1872 | 0.0364±0.1872 |
| P | 0.1091±0.3895 | 0.1000±0.3289 | 0.2091±0.0636[a] | 0.0455±0.2083 | 0.0182±0.1336 | 0.0636±0.2441 |
| Control | 0 | 0 | 0[b] | 0 | 0.0182±0.1336 | 0.0182±0.1336 |
| Totals (Count) | 25 | 28 | 53 | 6 | 11 | 17 |

**FIGURE 3-1**: Bengbu Trapping Location Vistas and hung Black Intercept Panel Trap. (Photos taken by author, Summer 2018).

# CHAPTER 4: COMPARISON OF ALB AND CLB CUTICULAR HYDROCARBONS

## Introduction

The insect integument has an outer layer that is composed of cuticular hydrocarbons (CHCs) and lipids. The CHCs consist primarily of 21 to 50 carbon straight-chain or methyl-branched alkanes and alkenes. These compounds are secreted by epidermal cells from fatty acid precursors and host-sequestered compounds (Blomquist and Bagnères 2010). Although CHC-based non-volatile pheromones have not received as much attention as volatile pheromones in chemical ecology due to their limited practical use in monitoring and control, their functions as contact, short-range, and trail pheromones have equally critical roles in mate location and selection (Blomquist and Bagnères 2010). In eusocial insects, they are also critical nestmate recognition (i.e., kinship) signals mediating a wide range of behaviors (Blomquist and Bagnères 2010). Within the Cerambycidae, the role of CHCs as contact mate-recognition pheromones is well documented and synthetic, female-imitating blends of CHCs are capable of inducing male mate-recognition responses, including attempted mounting and copulation of hydrocarbon coated models (Wang 2017). This response is inducible even in the absence of other female stimuli, highlighting the importance of CHCs in Cerambycid mate recognition (Ginzel 2010).

Genes related to CHC production have been implicated as multi-effect speciation genes, or drivers of speciation with multiple physiological roles (Chung et al. 2014, Chung and Carroll 2015, Blackman 2016, Finck et al. 2016). In addition to their role as semiochemicals, CHCs protect insects against desiccation and UV light damage (Otte et al. 2018). CHC genes are also associated with membrane phospholipid biosynthesis, indirectly connecting CHC gene expression profiles and cold tolerance. Thus, CHCs are both essential sex pheromones and contribute to an insect's environmental fitness, providing a potential evolutionary path for

species divergence. CHCs evolutionarily selected for optimum performance in different environmental conditions, such as drier or wetter locations, may enable changes in mate preference leading to speciation even in sympatric populations. Furthermore, because CHCs and their precursors may be sequestered from hosts, host choice can also alter CHC profiles, subsequently influencing CHC expression. It is hypothesized that host choice may lead to divergent CHC profiles, leading to partial reproductive isolation and subsequent speciation (Chung and Carroll 2015, Xue et al. 2016, Otte et al. 2018). For example, in two *Drosophila* species, mutations in a *cis*-regulatory sequence controlling fatty acid synthase (*mFAS*) were found to alter both CHC profiles and desiccation-sensitivity (Chung et al. 2014). However, research on this topic is incomplete. More studies on speciation genes in closely related species are needed in insects and other animals (Blackman 2016, Haynes 2017).

Asian Longhorned Beetle (ALB, *Anoplophora glabripennis*) and Citrus Longhorned Beetle (CLB, *Anoplophora chinensis*) (Subfamily: Lamiinae, Tribe: Lamiini) are sympatric cerambycids native to East Asia. ALB is native to both mainland China and the Korean Peninsula (CABI 2019) with the yellow-spotted form found in Northern China historically recognized as *Anoplophora nobilis* currently junior to *A. glabripennis* (Lingafelter and Hoebeke 2002). CLB is native to mainland China, Japan, Korea, the Philippines, Myanmar, and Taiwan, with isolated populations in Malaysia, Vietnam, and Indonesia (CABI 2019) and with the Japanese CLB historically recognized as *Anoplophora malasiaca* currently synonymized under *A. chinensis* (Lingafelter and Hoebeke 2002). Both species are polyphagous pests of woody trees with ALB infesting a wide range of hardwood trees from at least 15 families including maple, elm, willow, and poplar species (Haack et al. 2010) and the even more broadly polyphagous CLB infesting trees from at least 36 families including maple, elm, willow, and poplar, and

agriculturally important trees such as citrus, fig, pecan, and plum (Haack et al. 2010).Host damage is two-fold, with larval xylophagous feeding damaging woody tissues and adult feeding damaging host leaves and twigs. Within their native ranges, both species are serious pests and outbreaks have caused massive amounts of damage (Ji et al. 2011). In China, ALB is estimated to cause at least $1.5 billion USD annual damage and has contributed to the death of millions of non-native poplars planted as part of the Three-Norths shelterbelt region afforestation project (Cao 2008, Hu et al. 2009), while CLB infestation has complicated similar attempts to halt dune erosion using *Casuarina* monocultures (Ge et al. 2014). In Japan, CLB is a major agricultural pest of citrus (Adachi and others 1990). Both ALB and CLB are readily invasive. North American and European trees lack evolved resistance to either species and may prove to be suitable hosts. In addition, larval presence within woody tissues hides their presence and facilitates accidental transport. (Hu et al. 2009, Haack et al. 2010, Hérard and Maspero 2018)

CHC based contact pheromones have been identified in both ALB and CLB. Although the two species share a volatile pheromone component (Zhang et al. 2002, Hansen et al. 2015), their CHCs diverge dramatically. A female-produced contact pheromone was reported in CLB by demonstrating male attempts to copulate with female body-wash coated paper rolls and glass dummies (Wang 1998, Fukaya et al. 1999). Further research detected eight female body wash alkanes, $C_{27}$, $C_{29}$, $4MeC_{26}$, $4MeC_{28}$, $9MeC_{27}$, $9MeC_{29}$, $15MeC_{31}$, and $15MeC_{33}$, whose synthetic blend was able to induce the male mating response towards coated glass rods in combination with a polar body wash fraction (Fukaya et al. 2000). The required polar compounds were later identified as five ketones and a mixture of all thirteen compounds was sufficient to induce the male mating response (Yasui et al. 2003). Dietary sesquiterpenes have also been implicated in intraspecific recognition. Specifically, β-elemene found on the elytra of female CLB fed on

mandarin orange was suggested to repeal *Salix*-fed male CLB (Yasui et al. 2008, Yasui and Fujiwara-Tsujii 2016).

Contact pheromones were also demonstrated in ALB by male attempts to copulate with female body wash coated objects. In addition, males were repelled by male body wash, and females were repelled by both male and female body wash (Li, Tokoro, et al. 1999). Later work identified a 1:2:2:8:1 mixture of five alkenes, $Z9C_{23}$, $Z9C_{25}$, $Z7C_{25}$, $Z9C_{27}$, and $Z7C_{27}$, all of which were more prevalent in female body washes than male body washes, and induced the male ALB mating responses towards coated microcentrifuge tubes (Zhang et al. 2003). A complete characterization of both virgin and mated male and female ALB cuticular hydrocarbons was later reported and significant field attraction to lures composed of three CHC oxidation products, heptanal, nonanal, and hexadecanal, was demonstrated (Wickham 2009, Wickham et al. 2012). ALB males orient towards a trail pheromone consisting of the three contact pheromone compounds, $Z9C_{23}$, $Z9C_{25}$, and $Z7C_{25}$, in combination with $2MeC_{22}$ (Hoover et al. 2014).

Despite the close phylogenetic relationship between the species, the reported ALB and CLB contact pheromones do not share a single compound and the CLB contact pheromone appears to require an additional class of compounds (ketones). However, it is important to note that CHC blends are a diverse mixture of many compounds with signal redundancy and identification of a blend that induces mate-response does not necessarily preclude the existence of other active blends. The level of acceptable redundancy is unclear and multiple mate-response inducing blends may exist. It is possible the identified mixtures are not "the" contact pheromones of either species, and alternate, more similar, mixtures of CHCs are capable of inducing male mating responses. Of the male ALB contact pheromone compounds, only $9C_{25}$ and $9C_{27}$ were reported in CLB body wash hexane fraction (Fukaya et al., 2000). Although the CLB contact

pheromone compounds $C_{27}$ and $C_{29}$ were detected in the ALB, presence/absence of the other compounds apart from $15MeC_{33}$ (undetected) cannot be confirmed because methyl-branch locations of many alkanes were not specified. ALB and CLB are sister species who share a volatile pheromone component. Is the divergence of their CHCs to such an extent that compound identity, rather than ratios of important compounds, is altered? Currently, there are large methodological differences in hydrocarbon collection by different research groups, with Zhang et al. (2003) using hexane full-body extracts after an ethanol dip, Wickham et al. (2009) using the solvent-free method solid phase microextraction, and Fukaya et al (2000) using ether extraction of the elytra followed by fractionation. We performed hydrocarbon extraction and characterization from both species using an identical method in order to directly compare differences in ALB and CLB and explore potential implications of CHC divergence.

## Methods

### Beetle Collection

The source, collection date, and CHC extraction date of ALB and CLB adults are summarized in Table 4-1. Specimens were collected from the Nanjing Forestry University Campus (32°04'45.0"N 118°48'44.5"E), the Nanjing Forestry University Xiashu Forest Research Station (32°07'18.3"N 119°12'45.3"E), the Nanjing Small Peach Garden in Jiangsu (32°04'33.9"N 118°44'48.8"E) and Hunchun, Jilin (42°51'45.5"N 130°21'58.3"E) in 2017. Additional Jiangsu specimens were donated by Nanjing Forestry University students. CHCs were extracted at the Nanjing Forestry University campus. Beetles were kept in plastic food storage containers and fed host twigs until use.

CHC Extraction

Beetles were freeze killed prior to CHC extraction. Elytra were removed, briefly dipped in 2 mL chromatography grade ethanol to remove contaminates. The remaining ethanol was allowed to evaporate, and the elytra were placed in a Teflon capped glass amber vial (Thermo Scientific, Waltham, Massachusetts, USA) with 2 mL chromatography grade hexane and sonicated for 2 minutes. Elytra were then removed and the hexane CHC extracts were stored in a freezer prior to transportation to Syracuse, NY, USA.

GC-MS Analysis

A 3 µL aliquot of each crude CHC sample was manually injected onto a GC-MS system (7890A-5976C VL EI MSD with triple-axis detector) with an HP-5MS non-polar chromatography column (L 30m, ID 0.250mm, F 0.25µm) (Agilent Technologies, Santa Clara, California). The GC oven temperature program was 60°C for 1 minute, then 5°C/min to 300°C, then 300°C for 20 minutes. A library of CHCs found in all samples was created using open-source AMDIS Version 2.71 software (available at: https://chemdata.nist.gov/mass-spc/amdis/downloads/). Retention times were calculated according to Kovats Standard C7-C30 (Sigma-Aldrich, St. Louis, Missouri). Putative compound identity was attempted according to the MS spectra and previously reported CHC compounds (Fukaya et al. 2000, Wickham 2009, Hoover et al. 2014). The proportion of each compound in a sample was calculated by AMDIS integration and used in the statistical analyses.

Statistical Analysis

Data were transformed via the Aitchison (1986) transformation for compositional data. A stepwise discriminate analysis (stepwise DA) with four separate groups (ALB females, ALB males, CLB females, and CLB males) followed by additional four stepwise DA comparing subgroups (ALB males verses ALB females, CLB males versus CLB females, ALB males versus CLB males, and ALB females versus CLB females). DA were conducted with SAS™ statistical software using the STEPDISC procedure followed by the CANDISC procedure with the distance option (SAS Institute Inc. 2013). Two principle component analyses (PCA) were performed with Minitab™ statistical software to explore differences within the female ALB samples and within the male ALB samples (Minitab Inc. 2018). DA and PCA were plotted in R 3.6.0 (R Core Team 2014).

**Results**

A total of 70 potential CHC compounds were detected from retention index 2100 to retention index 3200 including straight chain alkanes *n*-$C_{23}$ to *n*-$C_{29}$ and $C_{31}$ as well as a variety of Z7- and Z9- odd-chain length alkenes and 2Me-, 3Me-, 7Me-, 11Me-, 13-Me, 14-Me, and 15-Me methylated alkanes. Chromatographs different by species and sex, which each group showing a characteristic pattern (Figure 4-1). Many trace compounds were also detected that were unidentifiable from the mass spectra. Compound identity, retention index, retention time, average CHC proportion by species and sex, and sample detection number by species and sex are reported in Table 4-2.

Stepwise DA showed clear separation between all four subgroups by species and sex (Figure 4-2, Mahalanobis Distance all $p<.0001$, Wilks' Lambda, Pillai's Trace, Hotelling-Lawley

Trace, and Roy's Greatest Root all p<.0001). Fifteen variables were retained by the stepwise model, of which 11Me$C_{24}$, an unidentified alkyne, *n*-$C_{26}$, Z9$C_{27}$, 13Me$C_{27}$, 9Me$C_{27}$, an unidentified alkene, 13Me$C_{29}$, 3Me$C_{29}$, and four additional unknown compounds were significantly different between groups (Table 4-3). Stepwise DA for all additional subgroup comparisons showed clear separation between groups (Mahalanobis Distances all p<.0001, Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace, and Roy's Greatest Root all p<.0001). Nine variables were retained by the ALB males versus ALB females stepwise model, of which 11Me$C_{24}$, Z9$C_{25}$, Z7$C_{25}$, 3Me$C_{25}$, Z7$C_{27}$, an unidentified alkene, and two additional identified compounds were significantly different between groups (Table 4-3). Six variables were retained by the CLB males versus CLB females stepwise model, of which 9Me$C_{27}$ and Z$C_{28}$ were significantly different between groups (Table 4-3). Four variables were retained by the ALB males versus CLB males stepwise model, of which 11Me$C_{24}$ and an unidentified alkene were significantly different between groups (Table 4-3). Finally, ten variables were retained by the ALB females versus CLB females stepwise model, of which 10Me$C_{22}$, Z9$C_{25}$, Z7$C_{25}$, 9Me$C_{27}$, and four additional unidentified compounds were significantly different between groups (Table 4-3).

The Table 4-3 shaded matrix illustrates that although many compounds were detected in small amounts, samples were dominated by relatively few compounds. The compounds *n*-$C_{25}$, *n*-$C_{27}$, *n*-$C_{29}$ and 2Me$C_{28}$ were respectively 11%, 33%, 7%, and 6% of ALB female CHC extract, 4%, 17%, 8%, and 20% of ALB male extract, 5%, 19%, 8%, and 40% of CLB female extract, and 17%, 22%, 6%, and 26% of CLB male extract. Additional abundant compounds in ALB female body washes were *n*-$C_{26}$ (3%), Z9$C_{27}$ (12%), 13Me$C_{27}$ (6%), and *n*-$C_{28}$ (3%). Another abundant compound in ALB male body wash samples was 11Me$C_{25}$ (7%), other abundant

compounds in CLB female body wash were *n*-$C_{28}$ (3%) and 3Me$C_{29}$ (5%), and other abundant compounds in CLB male body washes were 11Me$C_{25}$ (7%) and 2Me$C_{26}$ (3%), Z9$C_{27}$ (3%), and 2Me$C_{30}$ (3%).

The two PCAs performed to examine possible in-group separation of ALB females or ALB males by adult host choice, collection area, or collection date (Table 4-1) showed clear visual separation between *Acer*-collected beetles from Hunchun, Jilin (54F-59F and 48M-52M) versus beetles collected from Jiangsu (Figure 4-3 and Figure 4-4). Hunchun female samples visually separated along PC1 and PC2 combined, while Hunchun male samples separated along PC2. Unknown (RI=2713.1), Z$C_{28}$, *n*-$C_{28}$, Z9$C_{29}$, *n*-$C_{29}$ and unknown (RI=2913.3) were characteristic of Hunchun female samples, while unknown (RI=2194.5), *n*-$C_{24}$, Z9$C_{25}$, Z7$C_{25}$, *n*-$C_{25}$, and 3Me$C_{25}$ were characteristic of Jiangsu female samples. Unknown (RI=2741.7), unknown (RI=2641.5), unknown (RI=2675.1), Z7$C_{27}$, unknown (RI=2768.4), and unknown (RI=2965.7) were characteristic of Hunchun male samples, while unknown (RI=2766.7), 3Me$C_{29}$, unknown (RI=2194.5), 2Me$C_{26}$, unknown (RI=2167), and 11Me$C_{24}$ were characteristic of non-Hunchun samples. These data are visualized in Table 22, which presents the average proportion of CHC by location and sex along with the PC2 coefficients of the eigenvector from the ALB male only PCA.

**Discussion**

Comparison to Previous CHC Reports

In this study, ALB CHCs were generally the same as those described by Zhang et al. (2003), Wickham et al. (2009), and Hoover et al. (2014). The five ALB female contact pheromones Z9$C_{23}$, Z9$C_{25}$, Z7$C_{25}$, Z9$C_{27}$, and Z7$C_{27}$ (1:2:2:8:1) (Zhang et al. 2003) were detected in my ALB female samples at a somewhat different ratio (1:2:2:31:4), and all of these

compounds were either absent or detected only in trace amounts in male samples. However, 2MeC$_{22}$, a compound of the ALB trail pheromone (Hoover et al. 2014), was not found in any ALB sample in this study. This RI of this compound was reported as 2261 on a DB-5MS column (Hoover et al. 2014), indicating it should have eluted immediately prior to Z9C$_{23}$ (RI 2277). No compounds were detected at RI 2261 in this analysis. Although most pheromone components were detected in our elytra wash, 2MeC$_{22}$ may occur in the trail pheromone only.

Although our study did not differentiate between beetle age or mating status and used elytra washes rather than SPME samples, the most abundant CHCs in our ALB samples were also detected by Wickham (2009). The dominant ALB female compounds *n*-C$_{27}$ (33%), Z9C$_{27}$ (12%), *n*-C$_{25}$ (11%), *n*-C$_{29}$ (7%), 2MeC$_{28}$ (6%), and 13MeC$_{27}$ (6%) were respectively 5%, 18%, 5%, 0.3%, 3%, and 10% in Wickham's (2009) mated 14-20 day old female samples. The dominant ALB male compounds 11MeC$_{23}$ (30%), 2MeC$_{28}$ (20%), *n*-C$_{27}$ (17%), *n*-C$_{29}$ (8%), and 11MeC$_{25}$ (7%) were respectively 44%, multiple MeC$_{28}$ possibilities, 2.6%, 0.56%, and 13.4% in Wickham's (2009) mated male 14-20 day old ALB samples. Many additional compounds detected by Wickham et al. (2009) and Hoover et al. (2014) were also detected in our samples.

Despite the similarities seen in ALB CHCs, several of the CLB CHCs found in this study different from those reported by Fukaya (2000). The female contact pheromones C$_{27}$, C$_{29}$, 4MeC$_{26}$, 4MeC$_{28}$, 9MeC$_{27}$, 9MeC$_{29}$, 15MeC$_{31}$, and 15MeC$_{33}$ (Fukaya et al. 2000) were detected in our samples at 19%, 10%, 0%, 0%, 2%, and 0% in female samples, respectively. Fukaya et al (2000) reported 4MeC$_{26}$ as eluting between Z/E9C$_{27}$ and *n*-C$_{27}$. We did not detect branched alkanes in this range, although this compound may have coeluted with the large *n*-C$_{27}$ peak and thus was not detected. Similarly, 4MeC$_{28}$ was reported as eluting between a C$_{28}$ diene and Z/E9C$_{29}$. In this area, 2MeC$_{28}$ was detected in relatively high quantities in female samples

(40%), but not 4MeC$_{28}$. Finally, in this study, all compounds eluting after 15MeC$_{31}$ were found in small amounts and were not identified. It is possible one of these compounds is 15MeC$_{33}$. Highly abundant female CLB CHCs were also different from those reported by Fukaya (2000). We found 2MeC$_{28}$ (40%), *n*-C$_{27}$ (19%), *n*-C$_{29}$ Fukaya (2000) 0 µg, 88 µg, 26 µg, 0 µg, and 6.7 µg of these compound, respectively, per female. In the Fukaya (2008) study, the most abundant compounds were 9Z/EC$_{27}$ (280 µg per female), 4MeC$_{28}$ (139 µg per female), *n*-C$_{27}$ (88 µg per female), 13Me/15MeC$_{32}$ (80 µg per female), and 9Me/11Me/13MeC$_{27}$ (77 µg per female). In our study, these compounds were respectively 1.5% (Z9), 0%, 19%, 0%, and 1% (9Me/13Me).

Many factors may explain differences in our results and those from previous reports. It has been demonstrated that CHC composition changes based on age and mating status in ALB (Wickham, 2009). As the specimens used in this study were wild caught, mating status and age was unknown, and different proportions of each status or age may have influenced results. Additionally, methodological differences in sample preparation can influence CHC composition in longhorned beetles (Ginzel 2010). Although Fukaya et al. (2000) used elytra CHC extracts, their solvent of choice was ether rather than hexane, while Zhang et al. (2003) used whole body wash and Wickham performed solvent-free solid-phase microextraction (2009). In addition, geographic variation of CLB and ALB has not been studied. In our study we used wild caught CLB and ALB from China. Fukaya et al. sampled CLB CHCs in Japan as the junior synonym *Anoplophora malasiaca* (2000). Wickham et al. (2009) sampled from a laboratory colony of ALB in the United States.

Sex Differences in ALB CHCs

Three of the ALB female contact pheromone compounds (Zhang et al. 2003) were retained in the stepwise model and showed a significant difference between male and female ALB samples. Although the other two compounds were also found at greater values in female samples, they were not retained in the model. Which was also seen by Zhang et al. (2003), several of these compounds were relatively minor components, and in many of our female sample they were not above the detection limit.

The two most abundant male CHCs were methylated alkanes (a combined 50% of the total CHC blend), while the two most abundant female CHCs were straight chain alkanes (a combined 43% of the total CHC blend). In female and male samples, respectively, the two methylated alkanes were only 7% and straight chains only 21% of the total CHC blend. Since female ALB are heavier and larger than male ALB (Keena 2006) the two species are under different physical constraints. In addition, adaptive trade-offs between CHC production and other biological functions vary between these sexes due to behavioral and biological differences such as mate-seeking, gamete production, egg niche chewing and oviposition. The melting point of an alkane mixture is believed to related to desiccation rate, with increased methyl-branching or desaturation correlated with less desiccation. However, the interaction of major CHC compounds, minor compounds, and other variables such as melanization is poorly understood (Gibbs and Rajpurohit 2010). In addition, CHCs mediate intrasex communication as well as parasitoid-host communication and other types of interspecies communication (Ginzel and Blomquist 2016).

Sex Differences in CLB CHCs

CLB males and females were visually separated by the stepwise DA. Only one of the previously known female CLB contact pheromone compounds (Fukaya et al. 2000) was retained in the stepwise model. This compound, 15Me$C_{31}$, was found almost exclusively in female samples, while the other two detected compounds, *n*-$C_{27}$ and *n*-$C_{29}$, were not consistent female indicators. CLB male samples contained many short-chain minor compounds not found in CLB female samples, while CLB female samples contained many long-chain minor compounds not found in CLB male samples. In contrast with ALB, the identity of major compounds in CLB was similar in both male and female samples. 2Me$C_{28}$, a methylated alkane, and the same three straight-chain alkanes were relatively abundant in both sexes.

Species Differences in ALB and CLB CHCs

Due to methodical differences among studies, it has previously not been possible to directly compare ALB and CLB CHCs. CHC pheromones and composition have been given comparably less attention despite their important critical role in insect communication, and both ALB and CLB are of interest not only due to their pest status but also their status as sympatric sister species. More information is needed on CHCs in closely related species to better understand their evolution and role in speciation.

DA of all samples showed clear visual separation of samples by sex and species subgroups, which illustrates the expected CHC sex differences were not overshadowed by differences due to mating status, or age. Two of the previously reported ALB female CHC contact pheromones, Z9$C_{25}$, and Z7$C_{25}$ were included in the stepwise model and found in significantly higher proportions in ALB female samples. Five of the six previously reported

female ALB contact pheromones (Zhang et al. 2003) were completely undetected in CLB female samples, suggesting that ALB females and CLB females can be differentiated by these compounds. None of the three previously reported female CLB contact pheromones (Fukaya et al. 2003) were included in the ALB females versus CLB females stepwise model, although the proportion of $15MeC_{31}$ was approximately ten times greater in female CLB samples.

ALB and CLB geographic ranges overlap in China with CLB populations extending to Japan and Southern China and ALB populations extending to Northern China and Korea (CABI, 2019). Theoretically, tolerance of different climates should be reflected in the CHC profiles of both species. However, the shaded matrix in table 4-3 illustrates that major CHCs in both species are the same odd-chain *n*-alkanes and $2MeC_{28}$. One major compound in male ALB samples only, $11MeC_{23}$, is an exception. ALB were collected from both Jiangsu (central China) and Jilin (northern China), while CLB were collected in Jiangsu. It is possible that CHC profiles in these species show strong phenotypic variation and/or life stage differences (Wickham 2009, Ginzel and Blomquist 2016), or that the other functions of CHCs have overshadowed these differences.

Collection Location and Host Differences in ALB CHCs

The original goal of this analysis was to detect sequestered terpenes in the cuticular hydrocarbon layer, according to the analysis performed by Yasui et al. (2007), which found different sequestration of sesquiterpenes based on host. However, only trace amounts of sesquiterpenes were detected, and no effect of host species CHC was apparent effect besides that contained within the Hunchun collection group. The host, collection date, and geographic collection location of Hunchun samples and others differed. Much of the PCA discrimination between the two collection groups appears to be due to minor compounds that were not

identified and an interpretation of why Hunchun samples were different from others is not apparent. However, the overall conclusion that there is a difference between sampling groups in ALB, whatever its reason or basis, remains clear. These differences may be due to phenotypic plasticity or inherent genetic population differences.

As the ALB emergence period in Hunchun, Jilin (Nothern China) is later in the season than their emergence period in Jiangsu (Southern China), Hunchun samples may be from younger beetles despite their later collection date. If discrimination was based on age or mating status, I would expect the two sample groups to show the compound differences indicative of the differences between young verses old, virgin, or both, versus mated as described by Wickham et al. (2009). Wickham found CHC proportions of $11MeC_{23}$ were greater in mated females and sexually mature males, while $Z7C_{27}$ proportions were greater in virgin females. Hunchun female samples had higher proportions of both $11MeC_{23}$ and $Z7C_{27}$, suggesting that their PCA separation is not due to mating status. According to PC2 coefficients of the eigenvector, Hunchun male samples did not separate from the others based on $11MeC_{23}$, suggesting the difference was not due to sexual maturity. Wickham did not fully identify the chemical structure of other age and mating status indicative compounds so they cannot be directly compared and his results do not provide evidence supporting the hypothesis that the Hunchun samples differed from the others due to age or mating status.

## Conclusion

CHC profiles of ALB and CLB showed distinct species and sex differences. ALB CHC profiles were comparable to previous results. All five of the Zhang et al. (2003) female contact pheromone components were detected in greater amounts in female ALB samples than male

samples and were comparable to those collected by Wickham (2009) and Hoover et al. (2014). Male and female ALB showed differences in major compound proportions, with male profiles expressing higher proportions of methylated alkanes and female profiles expressing higher proportions of straight-chain alkanes. The Fukaya et al. CLB female contact pheromone components (2000) were not consistently detected in CLB samples. Within-species PCA by sex showed *Acer*-fed ALB samples collected in Hunchun, Jilin were a distinct sample group. More research is needed on both host effect and the geographic variation of CHCs in both ALB and CLB.

**TABLE 4-1:** ALB and CLB Collection Date, Location, Extraction Date and Host Information

| Sample # | Sex | Collection Date (2017) | Extraction Date (2017) | Collected From | Host Twigs Fed | Collection Location |
|---|---|---|---|---|---|---|
| **Species: CLB** | | | | | | |
| 1 | Female | 6/25 | 6/27 | *Koelreuteria* | Not fed | Nanjing, Jiangsu |
| 8 | Female | 6/20 | 6/27 | *Salix* | *Salix* | -* |
| 41 | Female | 6/28 | 7/12 | *Pterocarya* | *Pterocarya* | Nanjing, Jiangsu |
| 43 | Female | 6/28 | 7/12 | *Platanus* | *Platanus* | Nanjing, Jiangsu |
| 44 | Female | 6/27 | 7/12 | *Koelreuteria* | *Koelreuteria* | Xiashu, Jiangsu |
| 46 | Female | 6/27 | 7/12 | *Koelreuteria* | *Koelreuteria* | Xiashu, Jiangsu |
| 47 | Female | 6/29 | 7/12 | *Platanus* | *Platanus* | Nanjing, Jiangsu |
| 2 | Male | 6/25 | 6/27 | *Koelreuteria* | Not fed | Nanjing, Jiangsu |
| 4 | Male | - | 6/27 | *Castanea* | *Salix* | - |
| 5 | Male | - | 6/27 | *Castanea* | *Salix* | - |
| 6 | Male | - | 6/27 | *Castanea* | *Salix* | - |
| 7 | Male | - | 6/27 | *Castanea* | *Salix* | - |
| 9 | Male | - | 6/27 | - | *Salix* | - |
| 45 | Male | 6/29 | 7/12 | *Platanus* | *Platanus* | Nanjing, Jiangsu |
| **Species: ALB** | | | | | | |
| 14 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 15 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 16 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 18 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 21 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 22 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 23 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 25 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 26 | Female | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 28 | Female | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 29 | Female | 6/28 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 30 | Female | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 31 | Female | 6/28 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 33 | Female | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 34 | Female | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 36 | Female | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 37 | Female | 6/28 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 38 | Female | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 54 | Female | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 55 | Female | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 56 | Female | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 57 | Female | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 58 | Female | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |

| | | | | | | |
|---|---|---|---|---|---|---|
| 59 | Female | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 10 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 11 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 12 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 13 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 17 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 19 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 20 | Male | 6/29 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 24 | Male | 6/28 | 7/10 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 27 | Male | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 32 | Male | 6/28 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 35 | Male | 6/28 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 39 | Male | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 40 | Male | 6/29 | 7/12 | *Salix* | *Salix* | Nanjing, Jiangsu |
| 48 | Male | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 49 | Male | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 50 | Male | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 51 | Male | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |
| 52 | Male | - | 7/28 | *Acer* | *Acer* | Hunchun, Jilin |

*Not provided.

**TABLE 4-2:** CHC Average Proportions in ALB Females, ALB Males, CLB Females, and CLB Males

| | | | | ALB Females (N=24) | | ALB Males (N=18) | | CLB Females (N=7) | | CLB Males (N=7) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound ID | RI | RT | Ref. RI* | Average | Count | Average | Count | Average | Count | Average | Count |
| Unknown | 2160 | 33.8985 | | 0.095 ± 0.185 | 11 | 0.068 ± 0.13 | 5 | 0.242 ± 0.362 | 4 | 0.292 ± 0.309 | 5 |
| Unknown | 2167 | 34.02527 | | 0.571 ± 1.077 | 14 | 0.442 ± 0.781 | 8 | 0.78 ± 0.964 | 6 | 0.563 ± 0.478 | 6 |
| Unknown | 2194.5 | 34.51311 | | 0.08 ± 0.135 | 14 | 0.09 ± 0.095 | 10 | 0.229 ± 0.263 | 6 | 0.121 ± 0.132 | 5 |
| 10Me$C_{22}$ | 2239.1 | 35.28632 | | | | 0.032 ± 0.049 | 6 | | | | |
| Z9$C_{23}$ | 2277 | 35.92267 | 2277 | 0.39 ± 1.331 | 3 | | | | | | |
| *n*-$C_{23}$ | 2303.2 | 36.37452 | 2300 | 2.118 ± 1.203 | 24 | 1.324 ± 1.128 | 18 | 0.03 ± 0.066 | 2 | 1.247 ± 0.971 | 6 |
| 11Me$C_{23}$ | 2340.1 | 36.96108 | 2325 | 0.545 ± 0.527 | 20 | 29.509 ± 8.014 | 18 | 0.011 ± 0.029 | 1 | 1.234 ± 1.097 | 6 |
| 9Me$C_{23}$ | 2340.2 | 36.98319 | | | | 0.166 ± 0.237 | 10 | | | 0.204 ± 0.539 | 1 |
| *n*-$C_{24}$ | 2403.5 | 38.01933 | 2400 | 0.637 ± 0.492 | 21 | 0.072 ± 0.094 | 9 | 0.046 ± 0.072 | 3 | 0.385 ± 0.291 | 6 |
| 11Me$C_{24}$ | 2437.5 | 38.56456 | 2430 | | | 0.696 ± 0.384 | 15 | | | 0.032 ± 0.083 | 1 |
| 2Me$C_{24}$ | 2466.6 | 39.02144 | 2460 | 0.172 ± 0.176 | 17 | 0.469 ± 0.318 | 15 | 0.005 ± 0.014 | 1 | 0.243 ± 0.32 | 4 |
| Z9$C_{25}$ | 2477.6 | 39.18795 | 2478 | 0.703 ± 1.125 | 15 | 0.005 ± 0.022 | 1 | | | 1.386 ± 1.282 | 6 |
| Z7$C_{25}$ | 2484.4 | 39.30585 | 2485 | 0.699 ± 0.984 | 19 | 0.014 ± 0.06 | 1 | | | 0.036 ± 0.096 | 1 |
| *n*-$C_{25}$ | 2504.1 | 39.60443 | 2500 | 10.646 ± 5.69 | 24 | 3.793 ± 1.358 | 18 | 5.146 ± 5.247 | 7 | 16.821 ± 8.707 | 7 |
| Alkyne | 2512.2 | 39.7275 | | 0.006 ± 0.027 | 1 | 0.012 ± 0.05 | 1 | | | 0.07 ± 0.099 | 3 |
| 11Me$C_{25}$ | 2536.9 | 40.11344 | 2530 | 0.509 ± 0.345 | 22 | 7.126 ± 2.507 | 18 | 0.103 ± 0.236 | 2 | 7.165 ± 6.443 | 6 |
| Unknown | 2557.7 | 40.43337 | | | | | | | | 0.091 ± 0.153 | 3 |
| 13Me$C_{25}$ | 2566 | 40.56075 | | 0.003 ± 0.013 | 1 | 0.135 ± 0.17 | 9 | | | 0.028 ± 0.05 | 2 |
| 3Me$C_{25}$ | 2576.7 | 40.71421 | 2571 | 0.369 ± 0.336 | 19 | 0.013 ± 0.053 | 1 | 0.008 ± 0.021 | 1 | 0.041 ± 0.074 | 2 |
| *n*-$C_{26}$ | 2603.6 | 41.12649 | 2600 | 2.943 ± 1.044 | 24 | 0.439 ± 0.274 | 16 | 1.5 ± 1.436 | 7 | 2.112 ± 0.485 | 7 |
| 13Me$C_{26}$ | 2635.5 | 41.59903 | 2627 | 0.084 ± 0.095 | 12 | 0.08 ± 0.12 | 7 | | | 0.054 ± 0.105 | 2 |
| Unknown | 2641.5 | 41.67935 | | | | 0.014 ± 0.043 | 2 | | | | |
| 2Me$C_{26}$ | 2666.8 | 42.05564 | 2660 | 2.456 ± 1.301 | 24 | 2.609 ± 1.643 | 16 | 1.158 ± 0.567 | 7 | 3.494 ± 2.628 | 7 |
| Unknown | 2674.8 | 42.1724 | | 0.107 ± 0.525 | 1 | | | | | | |
| Unknown | 2675.1 | 42.16783 | | 0.239 ± 0.4 | 11 | 0.021 ± 0.061 | 2 | 0.05 ± 0.132 | 1 | 0.01 ± 0.027 | 1 |
| Z9$C_{27}$ | 2679 | 42.22929 | 2677 | 12.417 ± 7.5 | 24 | 1.097 ± 2.097 | 11 | 1.515 ± 2.819 | 3 | 2.611 ± 1.736 | 7 |
| Z7$C_{27}$ | 2686.2 | 42.33918 | 2686 | 1.578 ± 1.818 | 19 | 0.204 ± 0.618 | 2 | | | | |
| *n*-$C_{27}$ | 2704.6 | 42.59982 | 2700 | 32.667 ± 6.327 | 24 | 16.954 ± 7.663 | 18 | 18.758 ± 7.006 | 7 | 22.471 ± 4.331 | 7 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Unknown | 2713.1 | 42.71765 | | 0.043 ± 0.166 | 3 | 0.065 ± 0.166 | 4 | | | 0.096 ± 0.112 | 4 |
| Alkene | 2715.8 | 42.76768 | | | | 0.191 ± 0.238 | 9 | | | | |
| 13Me$C_{27}$ | 2734.8 | 43.03936 | 2729 | 5.834 ± 3.185 | 24 | 0.686 ± 0.409 | 15 | 0.002 ± 0.005 | 1 | 0.093 ± 0.195 | 2 |
| 9Me$C_{27}$ | 2739.1 | 43.09489 | | | | | | 1.223 ± 0.819 | 7 | 0.189 ± 0.358 | 2 |
| Unknown | 2741.7 | 43.1316 | | | | 0.015 ± 0.049 | 2 | | | | |
| Unknown | 2753.1 | 43.2928 | | | | | | 0.061 ± 0.104 | 2 | | |
| Alkene | 2760.0 | 43.37794 | | 0.224 ± 0.24 | 13 | 1.221 ± 0.87 | 17 | | | | |
| Unknown | 2762.0 | 43.4252 | | | | 0.224 ± 0.413 | 8 | | | | |
| Unknown | 2766.7 | 43.48684 | | 0.025 ± 0.045 | 6 | 0.236 ± 0.163 | 13 | 0.361 ± 0.197 | 6 | 0.187 ± 0.11 | 6 |
| Unknown | 2768.4 | 43.50935 | | | | 0.038 ± 0.123 | 2 | | | | |
| 3Me$C_{27}$ | 2777.3 | 43.63303 | 2722 | 1.824 ± 0.785 | 23 | 0.154 ± 0.238 | 6 | 0.745 ± 0.347 | 7 | 0.589 ± 0.625 | 4 |
| Z$C_{28}$ | 2779.9 | 43.66682 | | 0.009 ± 0.031 | 2 | 0.168 ± 0.219 | 9 | | | 0.367 ± 0.445 | 4 |
| *n*-$C_{28}$ | 2803.8 | 44.01096 | 2800 | 3.089 ± 1.616 | 24 | 1.371 ± 0.586 | 18 | 2.766 ± 1.578 | 7 | 1.266 ± 0.787 | 7 |
| Alkene | 2809.9 | 44.09007 | | | | 0.139 ± 0.243 | 6 | | | | |
| Unknown | 2814.1 | 44.14361 | | 0.025 ± 0.046 | 8 | 0.017 ± 0.048 | 4 | 0.052 ± 0.073 | 3 | 0.076 ± 0.2 | 1 |
| 14Me$C_{28}$ | 2833.8 | 44.42454 | 2828 | 0.202 ± 0.186 | 16 | 0.079 ± 0.101 | 8 | 0.029 ± 0.076 | 1 | | |
| 2Me$C_{28}$ | 2868.4 | 44.88131 | 2860 | 6.465 ± 2.79 | 24 | 20.254 ± 8.776 | 18 | 39.695 ± 10.96 | 7 | 25.806 ± 11.007 | 7 |
| Z9$C_{29}$ | 2880.0 | 45.05885 | 2877 | 1.697 ± 2.037 | 23 | 0.23 ± 0.491 | 4 | 0.86 ± 1.473 | 2 | | |
| *n*-$C_{29}$ | 2903.5 | 45.38209 | 2900 | 7.324 ± 2.627 | 24 | 7.863 ± 4.783 | 18 | 10.034 ± 3.332 | 7 | 5.644 ± 3.09 | 7 |
| Unknown | 2913.3 | 45.5072 | | 0.008 ± 0.038 | 2 | 0.033 ± 0.081 | 3 | | | | |
| Unknown | 2924.8 | 45.6599 | | | | | | | | 0.032 ± 0.084 | 1 |
| 13Me$C_{29}$ | 2933.8 | 45.77577 | | 2.176 ± 1.403 | 24 | 0.277 ± 0.187 | 15 | 2.487 ± 2.79 | 6 | 0.075 ± 0.198 | 1 |
| Unknown | 2937.9 | 45.83525 | | | | | | 0.193 ± 0.4 | 2 | | |
| Unknown | 2944.1 | 45.9073 | | | | | | 0.268 ± 0.455 | 3 | | |
| Unknown | 2952.7 | 46.0272 | | | | | | 0.046 ± 0.121 | 1 | | |
| Unknown | 2958.7 | 46.1081 | | | | | | 0.097 ± 0.216 | 2 | | |
| Unknown | 2965.7 | 46.19835 | | | | 0.205 ± 0.659 | 2 | | | | |
| 3Me$C_{29}$ | 2977.8 | 46.35511 | | 0.531 ± 0.456 | 20 | 0.14 ± 0.168 | 9 | 5.413 ± 2.358 | 7 | 1.338 ± 1.539 | 6 |
| Unknown | 3024.3 | 46.96291 | | 0.004 ± 0.015 | 2 | 0.153 ± 0.273 | 11 | | | 0.042 ± 0.111 | 1 |
| Unknown | 3031.8 | 47.0709 | | | | | | 0.032 ± 0.084 | 1 | | |
| Unknown | 3049.8 | 47.3077 | | | | | | 0.074 ± 0.196 | 1 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Unknown | 3057.5 | 47.4098 | | | | | 0.076 ± 0.202 | 1 | | |
| 2MeC$_{30}$ | 3065.3 | 47.51101 | 0.169 ± 0.139 | 16 | 0.719 ± 0.373 | 17 | 1.247 ± 0.85 | 7 | 2.782 ± 1.594 | 7 |
| ZC$_{31}$ | 3079.4 | 47.6976 | | | | | 0.239 ± 0.417 | 2 | | |
| *n*-C$_{31}$ | 3101.6 | 47.98277 | 0.095 ± 0.185 | 8 | 0.107 ± 0.327 | 5 | 0.413 ± 0.261 | 7 | 0.647 ± 0.522 | 7 |
| 15MeC$_{31}$ | 3128.3 | 48.34201 | 0.187 ± 0.165 | 16 | | | 1.799 ± 1.485 | 7 | 0.04 ± 0.107 | 1 |
| Unknown | 3150.9 | 48.64123 | | | | | 1.472 ± 1.748 | 4 | | |
| Unknown | 3159.7 | 48.75945 | 0.028 ± 0.055 | 6 | 0.035 ± 0.105 | 2 | | | | |
| Unknown | 3163.9 | 48.8105 | 0.003 ± 0.012 | 1 | | | 0.119 ± 0.258 | 2 | | |
| Unknown | 3170.6 | 48.90515 | 0.005 ± 0.026 | 1 | | | 0.061 ± 0.106 | 2 | 0.022 ± 0.057 | 1 |
| Unknown | 3174.0 | 48.94365 | | | | | 0.401 ± 0.805 | 2 | | |
| Unknown | 3197.7 | 49.2576 | | | | | 0.155 ± 0.41 | 1 | | |

*Retention index values from Hoover et al. (2014)

**TABLE 4-3:** Average CHC Proportion and Pairwise Comparison Significance

| | Average CHC Proportion | | | | Significance of Variables included in Stepwise DA Models | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | ALB F | ALB M | CLB F | CLB M | All Four Groups | ALB F–ALB M | CLB F-CLB M | ALB M-CLB M | ALB F-CLB F |
| Unknown | 0.095 | 0.068 | 0.242 | 0.292 | | | | | |
| Unknown | 0.571 | 0.442 | 0.780 | 0.563 | 0.1103 | | | | |
| Unknown | 0.080 | 0.090 | 0.229 | 0.121 | | | | | |
| 10MeC$_{22}$ | 0 | 0.032 | 0 | 0 | | | | | 0.0331 |
| **Z9C$_{23}$**[b] | 0.390 | 0 | 0 | 0 | | | | | |
| *n*-C$_{23}$ | 2.118 | 1.324 | 0.030 | 1.247 | | | | | |
| 11MeC$_{23}$ | 0.545 | 29.509 | 0.011 | 1.234 | | | | | |
| 9MeC$_{23}$ | 0 | 0.166 | 0 | 0.204 | | | | | |
| *n*-C$_{24}$ | 0.637 | 0.072 | 0.046 | 0.385 | | | | | |
| 11MeC$_{24}$ | 0 | 0.696 | 0 | 0.032 | <.0001 | <.0001 | | 0.0002 | |
| 2MeC$_{24}$ | 0.172 | 0.469 | 0.005 | 0.243 | | | | | |
| **Z9C$_{25}$** | 0.703 | 0.005 | 0 | 1.386 | | <.0001 | | | <.0001 |
| **Z7C$_{25}$** | 0.699 | 0.014 | 0 | 0.036 | | <.0001 | | | <.0001 |
| *n*-C$_{25}$ | 10.646 | 3.793 | 5.146 | 16.821 | | | | | |
| Alkyne | 0.006 | 0.012 | 0 | 0.070 | 0.0014 | | | | |
| 11MeC$_{25}$ | 0.509 | 7.126 | 0.103 | 7.165 | | | | | |
| Unknown | 0 | 0 | 0 | 0.091 | <.0001 | | | | |
| 13MeC$_{25}$ | 0.003 | 0.135 | 0 | 0.028 | | | | 0.1202 | |
| 3MeC$_{25}$ | 0.369 | 0.013 | 0.008 | 0.041 | | <.0001 | | | |
| *n*-C$_{26}$ | 2.943 | 0.439 | 1.500 | 2.112 | <.0001 | | | | |
| 13MeC$_{26}$ | 0.084 | 0.080 | 0 | 0.054 | | | | | |
| Unknown | 0 | 0.014 | 0 | 0 | | | | | |
| 2MeC$_{26}$ | 2.456 | 2.609 | 1.158 | 3.494 | | 0.2303 | | | |
| Unknown | 0.107 | 0 | 0 | 0 | | | | | |
| Unknown | 0.239 | 0.021 | 0.050 | 0.010 | | | | | |
| **Z9C$_{27}$** | 12.417 | 1.097 | 1.515 | 2.611 | | | | | |
| **Z7C$_{27}$** | 1.578 | 0.204 | 0 | 0 | <.0001 | <.0001 | | | |
| *n-C$_{27}$* | 32.667 | 16.954 | 18.758 | 22.471 | | | | | 0.7713 |
| Unknown | 0.043 | 0.065 | 0 | 0.096 | 0.0199 | | | | |
| Alkene | 0 | 0.191 | 0 | 0 | | | | | |
| 13MeC$_{27}$ | 5.834 | 0.686 | 0.002 | 0.093 | <.0001 | | | | |
| 9MeC$_{27}$ | 0 | 0 | 1.223 | 0.189 | <.0001 | | 0.0017 | | <.0001 |
| Unknown | 0 | 0.015 | 0 | 0 | | | | | |
| Unknown | 0 | 0 | 0.061 | 0 | | | | | |
| Alkene | 0.224 | 1.221 | 0 | 0 | <.0001 | 0.0001 | | <.0001 | |
| Unknown | 0 | 0.224 | 0 | 0 | | | | 0.0587 | |
| Unknown | 0.025 | 0.236 | 0.361 | 0.187 | | | | | <.0001 |
| Unknown | 0 | 0.038 | 0 | 0 | | 0.0219 | | | |
| 3MeC27 | 1.824 | 0.154 | 0.745 | 0.589 | | | 0.0613 | | |
| ZC$_{28}$ | 0.009 | 0.168 | 0 | 0.367 | | | 0.0072 | | |
| *n*-C$_{28}$ | 3.089 | 1.371 | 2.766 | 1.266 | | | | | |
| Alkene | 0 | 0.139 | 0 | 0 | | | | | |
| Unknown | 0.025 | 0.017 | 0.052 | 0.076 | | | | | |
| 14MeC$_{28}$ | 0.202 | 0.079 | 0.029 | 0.000 | | | | | |
| 2MeC$_{28}$ | 6.465 | 20.254 | 39.695 | 25.806 | | | 0.2647 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Z9C$_{29}$ | 1.697 | 0.230 | 0.860 | 0 | | | | | |
| *n-C$_{29}$* | 7.324 | 7.863 | 10.034 | 5.644 | 0.3283 | | | | |
| Unknown | 0.008 | 0.033 | 0 | 0 | | 0.1715 | | | |
| Unknown | 0 | 0 | 0 | 0.032 | | | | | |
| 13MeC$_{29}$ | 2.176 | 0.277 | 2.487 | 0.075 | <.0001 | | | | |
| Unknown | 0 | 0 | 0.193 | 0 | | | | | 0.0011 |
| Unknown | 0 | 0 | 0.268 | 0 | | | | | |
| Unknown | 0 | 0 | 0.046 | 0 | 0.0335 | | | | 0.0161 |
| Unknown | 0 | 0 | 0.097 | 0 | | | | | 0.0015 |
| Unknown | 0 | 0.205 | 0 | 0 | | | | | |
| 3MeC$_{29}$ | 0.531 | 0.140 | 5.413 | 1.338 | 0.0001 | | | | |
| Unknown | 0.004 | 0.153 | 0.000 | 0.042 | | | | | |
| Unknown | 0 | 0 | 0.032 | 0 | 0.0296 | | | | 0.0135 |
| Unknown | 0 | 0 | 0.074 | 0 | | | | | |
| Unknown | 0 | 0 | 0.076 | 0 | | | | | |
| 2MeC$_{30}$ | 0.169 | 0.719 | 1.247 | 2.782 | | | | | |
| ZC$_{31}$ | 0 | 0 | 0.239 | 0 | | | | | |
| *n*-C$_{31}$ | 0.095 | 0.107 | 0.413 | 0.647 | | | | | |
| *15MeC$_{31}$* | 0.187 | 0 | 1.799 | 0.040 | | | <.0001 | | |
| Unknown | 0 | 0 | 1.472 | 0 | | | 0.0146 | | |
| Unknown | 0.028 | 0.035 | 0 | 0 | | | | | |
| Unknown | 0.003 | 0 | 0.119 | 0 | | | | | |
| Unknown | 0.005 | 0 | 0.061 | 0.022 | | | | | |
| Unknown | 0 | 0 | 0.401 | 0 | | | | | |
| Unknown | 0 | 0 | 0.155 | 0 | | | | | |

[a]Post-hoc Krustal Wallis pairwise tests corrected with Dunn's test for multiple comparisons. Data were transformed by the Aitchison transformation for conformation data. (*=p<0.05,**=p<0.001, ***=p<0.0001)

[b]ALB female contact pheromone compounds in bold. CLB female contact pheromone components in italic.

**TABLE 4-5** CHC Compound Proportion of "Hunchun" and "Others" Collection Groups with Male ALB only PC2 coefficients of the eigenvector

| | | Male Beetles | | | | | Female Beetles | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hunchun (N=5) | | Other (N=13) | | | Hunchun (N=6) | | Other (N=18) | |
| Compound | RI | Average | Count | Average | Count | PC2 | Average | Count | Average | Count |
| Unknown | 2160 | | | 0.094 ± 0.14 | 5 | -0.113 | | | 0.127 ± 0.199 | 11 |
| Unknown | 2167 | | | 0.612 ± 0.833 | 8 | -0.141 | | | 0.762 ± 1.157 | 14 |
| Unknown | 2194.5 | | | 0.124 ± 0.086 | 10 | -0.175 | | | 0.107 ± 0.142 | 14 |
| 10Me$_{22}$ | 2239.1 | 0.026 ± 0.053 | 1 | 0.034 ± 0.045 | 5 | -0.041 | | | | |
| Z9C$_{23}$ | 2277 | | | | | 0 | 1.551 ± 2.235 | 2 | 0.003 ± 0.014 | 1 |
| *n*-C$_{23}$ | 2303.2 | 2.024 ± 1.309 | 5 | 1.055 ± 0.863 | 13 | 0.084 | 0.95 ± 0.335 | 6 | 2.507 ± 1.098 | 18 |
| 11MeC$_{23}$ | 2340.1 | 30.189 ± 10.357 | 5 | 29.248 ± 6.519 | 13 | 0.02 | 0.82 ± 0.888 | 3 | 0.453 ± 0.241 | 17 |
| 9MeC$_{23}$ | 2340.2 | 0.301 ± 0.337 | 3 | 0.114 ± 0.141 | 7 | 0.053 | | | | |
| *n*-C$_{24}$ | 2403.5 | 0.042 ± 0.084 | 1 | 0.083 ± 0.091 | 8 | -0.092 | 0.107 ± 0.137 | 3 | 0.814 ± 0.422 | 18 |
| 11MeC$_{24}$ | 2437.5 | 0.396 ± 0.486 | 2 | 0.811 ± 0.232 | 13 | -0.136 | | | | |
| 2MeC$_{24}$ | 2466.6 | 0.279 ± 0.392 | 2 | 0.542 ± 0.231 | 13 | -0.134 | 0.026 ± 0.038 | 2 | 0.221 ± 0.173 | 15 |
| Z9C$_{25}$ | 2477.6 | 0.019 ± 0.038 | 1 | | | 0.187 | | | 0.938 ± 1.182 | 15 |
| Z7C$_{25}$ | 2484.4 | 0.051 ± 0.101 | 1 | | | 0.186 | 0.066 ± 0.124 | 2 | 0.91 ± 1.027 | 17 |
| *n*-C$_{25}$ | 2504.1 | 4.125 ± 1.047 | 5 | 3.666 ± 1.389 | 13 | 0.002 | 3.956 ± 2.044 | 6 | 12.876 ± 4.481 | 18 |
| Alkyne | 2512.2 | 0.042 ± 0.084 | 1 | | | 0.187 | 0.022 ± 0.05 | 1 | | |
| 11MeC$_{25}$ | 2536.9 | 7.196 ± 2.404 | 5 | 7.098 ± 2.447 | 13 | 0.006 | 0.41 ± 0.391 | 4 | 0.542 ± 0.311 | 18 |
| Unknown | 2557.7 | | | | | 0 | | | | |
| 13MeC$_{25}$ | 2566 | 0.024 ± 0.048 | 1 | 0.178 ± 0.174 | 8 | -0.115 | | | 0.003 ± 0.014 | 1 |
| 3MeC$_{25}$ | 2576.7 | 0.045 ± 0.09 | 1 | | | 0.187 | 0.055 ± 0.082 | 2 | 0.474 ± 0.313 | 17 |
| *n*-C$_{26}$ | 2603.6 | 0.292 ± 0.324 | 3 | 0.496 ± 0.215 | 13 | -0.132 | 1.829 ± 0.71 | 6 | 3.314 ± 0.821 | 18 |
| 13MeC$_{26}$ | 2635.5 | 0.031 ± 0.062 | 1 | 0.099 ± 0.127 | 6 | -0.072 | | | 0.112 ± 0.091 | 12 |
| Unknown | 2641.5 | 0.052 ± 0.066 | 2 | | | 0.24 | | | | |
| 2MeC$_{26}$ | 2666.8 | 0.524 ± 0.467 | 3 | 3.41 ± 1.065 | 13 | -0.152 | 1.246 ± 0.634 | 6 | 2.859 ± 1.174 | 18 |
| Unknown | 2674.8 | | | | | 0 | 0.428 ± 0.958 | 1 | | |
| Unknown | 2675.1 | 0.075 ± 0.092 | 2 | | | 0.238 | 0.632 ± 0.541 | 4 | 0.108 ± 0.195 | 7 |
| Z9C$_{27}$ | 2679 | 3.525 ± 2.575 | 5 | 0.163 ± 0.243 | 6 | 0.161 | 17.004 ± 7.174 | 6 | 10.888 ± 6.735 | 18 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Z7C$_{27}$ | 2686.2 | 0.736 ± 0.954 | 2 | | | 0.234 | 3.908 ± 2.075 | 6 | 0.801 ± 0.61 | 13 |
| *n*-C$_{27}$ | 2704.6 | 22.319 ± 11.645 | 5 | 14.891 ± 3.052 | 13 | -0.004 | 34.784 ± 5.558 | 6 | 31.961 ± 6.234 | 18 |
| Unknown | 2713.1 | 0.233 ± 0.235 | 3 | 0.001 ± 0.003 | 1 | 0.206 | 0.173 ± 0.288 | 3 | | |
| Alkene | 2715.8 | 0.156 ± 0.199 | 2 | 0.204 ± 0.242 | 7 | 0.009 | | | | |
| 13MeC$_{27}$ | 2734.8 | 0.658 ± 0.569 | 3 | 0.697 ± 0.307 | 12 | -0.079 | 4.535 ± 2.39 | 6 | 6.267 ± 3.211 | 18 |
| 9MeC$_{27}$ | 2739.1 | | | | | 0 | | | | |
| Unknown | 2741.7 | 0.054 ± 0.079 | 2 | | | 0.242 | | | | |
| Unknown | 2753.1 | | | | | 0 | | | | |
| Alkene | 2760.0 | 2.256 ± 0.659 | 5 | 0.823 ± 0.503 | 12 | 0.061 | 0.101 ± 0.227 | 1 | 0.265 ± 0.223 | 12 |
| Unknown | 2762.0 | 0.001 ± 0.001 | 1 | 0.31 ± 0.444 | 7 | -0.105 | | | | |
| Unknown | 2766.7 | | | 0.327 ± 0.071 | 13 | -0.246 | | | 0.033 ± 0.049 | 6 |
| Unknown | 2768.4 | 0.136 ± 0.195 | 2 | | | 0.233 | | | | |
| 3MeC27 | 2777.3 | | | 0.213 ± 0.247 | 6 | -0.136 | 1.006 ± 0.476 | 5 | 2.097 ± 0.644 | 18 |
| ZC$_{28}$ | 2779.9 | 0.084 ± 0.168 | 1 | 0.2 ± 0.219 | 8 | -0.087 | 0.036 ± 0.052 | 2 | | |
| *n*-C$_{28}$ | 2803.8 | 1.472 ± 0.926 | 5 | 1.332 ± 0.336 | 13 | -0.038 | 4.463 ± 1.734 | 6 | 2.631 ± 1.223 | 18 |
| Alkene | 2809.9 | 0.242 ± 0.302 | 2 | 0.099 ± 0.191 | 4 | 0.091 | | | | |
| Unknown | 2814.1 | 0.04 ± 0.08 | 1 | 0.008 ± 0.017 | 3 | 0.037 | 0.046 ± 0.063 | 3 | 0.018 ± 0.034 | 5 |
| 14MeC$_{28}$ | 2833.8 | 0.046 ± 0.092 | 1 | 0.092 ± 0.097 | 7 | -0.069 | 0.126 ± 0.185 | 2 | 0.227 ± 0.174 | 14 |
| 2MeC$_{28}$ | 2868.4 | 7.681 ± 2.087 | 5 | 25.089 ± 3.855 | 13 | -0.12 | 4.653 ± 1.361 | 6 | 7.069 ± 2.805 | 18 |
| Z9C$_{29}$ | 2880.0 | 0.755 ± 0.642 | 3 | 0.028 ± 0.097 | 1 | 0.188 | 4.23 ± 2.533 | 6 | 0.852 ± 0.557 | 17 |
| *n*-C$_{29}$ | 2903.5 | 11.634 ± 7.169 | 5 | 6.413 ± 1.607 | 13 | -0.003 | 9.101 ± 2.168 | 6 | 6.731 ± 2.418 | 18 |
| Unknown | 2913.3 | 0.118 ± 0.111 | 3 | | | 0.213 | 0.033 ± 0.069 | 2 | | |
| Unknown | 2924.8 | | | | | 0 | | | | |
| 13MeC$_{29}$ | 2933.8 | 0.22 ± 0.191 | 3 | 0.299 ± 0.173 | 12 | -0.084 | 2.643 ± 1.498 | 6 | 2.021 ± 1.292 | 18 |
| Unknown | 2937.9 | | | | | 0 | | | | |
| Unknown | 2944.1 | | | | | 0 | | | | |
| Unknown | 2952.7 | | | | | 0 | | | | |
| Unknown | 2958.7 | | | | | 0 | | | | |
| Unknown | 2965.7 | 0.738 ± 1.042 | 2 | | | 0.23 | | | | |
| 3MeC$_{29}$ | 2977.8 | | | 0.194 ± 0.162 | 9 | -0.176 | 0.274 ± 0.206 | 4 | 0.616 ± 0.472 | 16 |
| Unknown | 3024.3 | 0.379 ± 0.412 | 3 | 0.066 ± 0.07 | 8 | 0.055 | 0.011 ± 0.024 | 1 | 0.002 ± 0.009 | 1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Unknown | 3031.8 | | | | | 0 | | | | |
| Unknown | 3049.8 | | | | | 0 | | | | |
| Unknown | 3057.5 | | | | | 0 | | | | |
| $2MeC_{30}$ | 3065.3 | 0.396 ± 0.296 | 4 | 0.843 ± 0.304 | 13 | -0.123 | 0.097 ± 0.139 | 2 | 0.193 ± 0.127 | 14 |
| $ZC_{31}$ | 3079.4 | | | | | 0 | | | | |
| *n*-$C_{31}$ | 3101.6 | 0.342 ± 0.534 | 2 | 0.016 ± 0.032 | 3 | -0.015 | 0.348 ± 0.209 | 5 | 0.01 ± 0.025 | 3 |
| *15MeC$_{31}$* | 3128.3 | | | | | 0 | 0.298 ± 0.168 | 5 | 0.15 ± 0.141 | 11 |
| Unknown | 3150.9 | | | | | 0 | | | | |
| Unknown | 3159.7 | 0.047 ± 0.095 | 1 | 0.03 ± 0.105 | 1 | 0.064 | 0.032 ± 0.059 | 2 | 0.026 ± 0.052 | 4 |
| Unknown | 3163.9 | | | | | 0 | | | 0.003 ± 0.014 | 1 |
| Unknown | 3170.6 | | | | | 0 | | | 0.007 ± 0.03 | 1 |
| Unknown | 3174.0 | | | | | 0 | | | | |
| Unknown | 3197.7 | | | | | 0 | | | | |

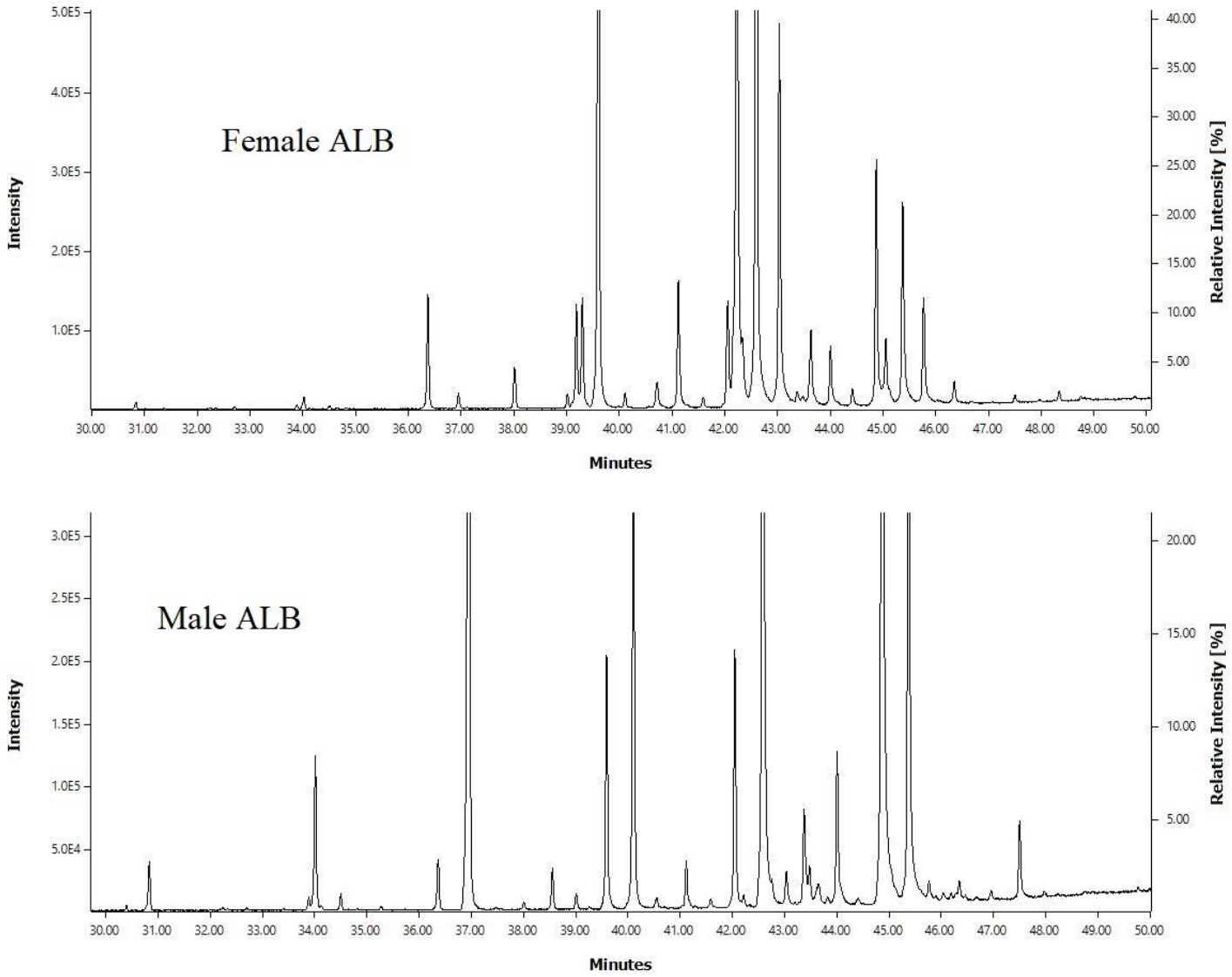
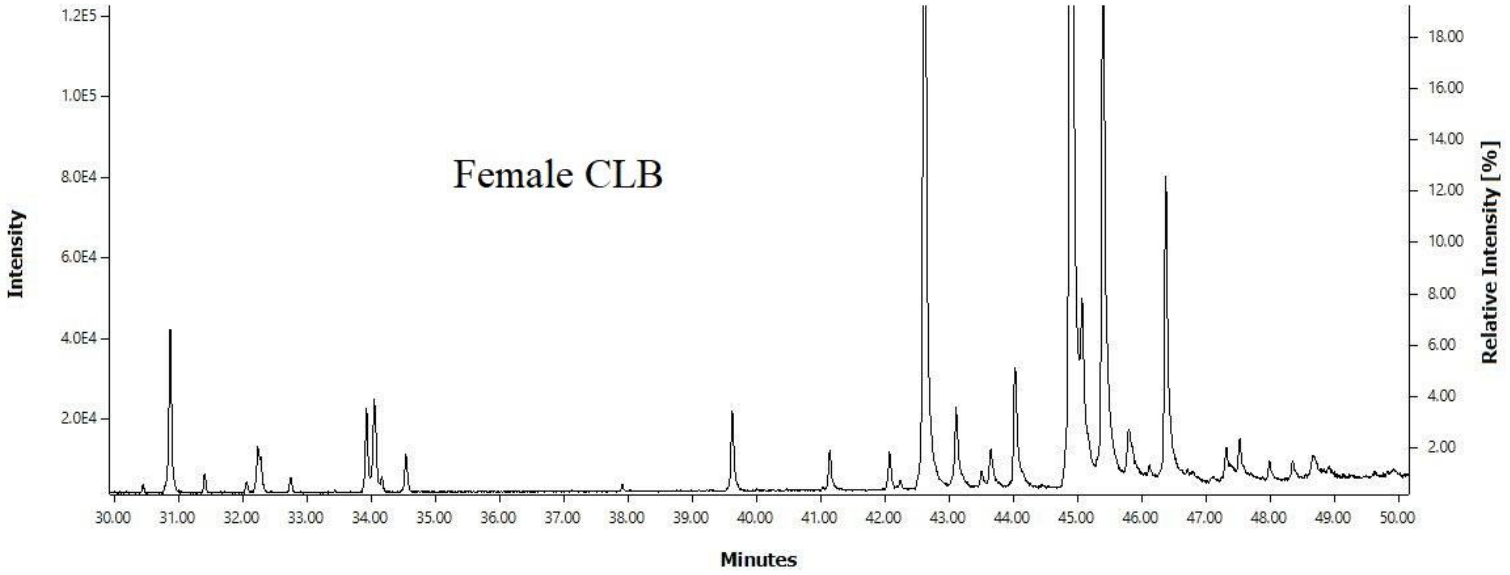
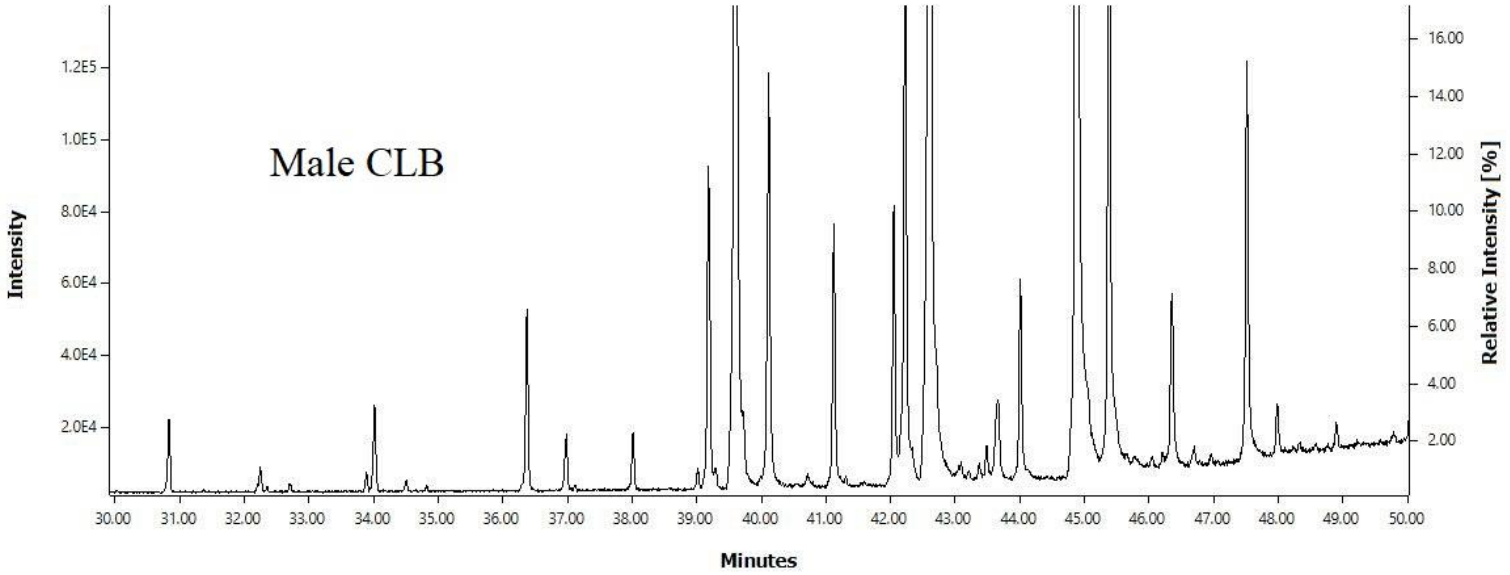



**Figure 4-1**: Representative GC-MS chromatograms (TIC) of CHC samples. Female ALB (**A**), male ALB (**B**), female CLB (**C**), and male CLB (**D)**. (Continued on the next page.)


Female CLB
Intensity
Relative Intensity [%]
Minutes
1.2E5
1.0E5
8.0E4
6.0E4
4.0E4
2.0E4
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
30.00 31.00 32.00 33.00 34.00 35.00 36.00 37.00 38.00 39.00 40.00 41.00 42.00 43.00 44.00 45.00 46.00 47.00 48.00 49.00 50.00


Male CLB
Intensity
Relative Intensity [%]
Minutes
1.2E5
1.0E5
8.0E4
6.0E4
4.0E4
2.0E4
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
30.00 31.00 32.00 33.00 34.00 35.00 36.00 37.00 38.00 39.00 40.00 41.00 42.00 43.00 44.00 45.00 46.00 47.00 48.00 49.00 50.00

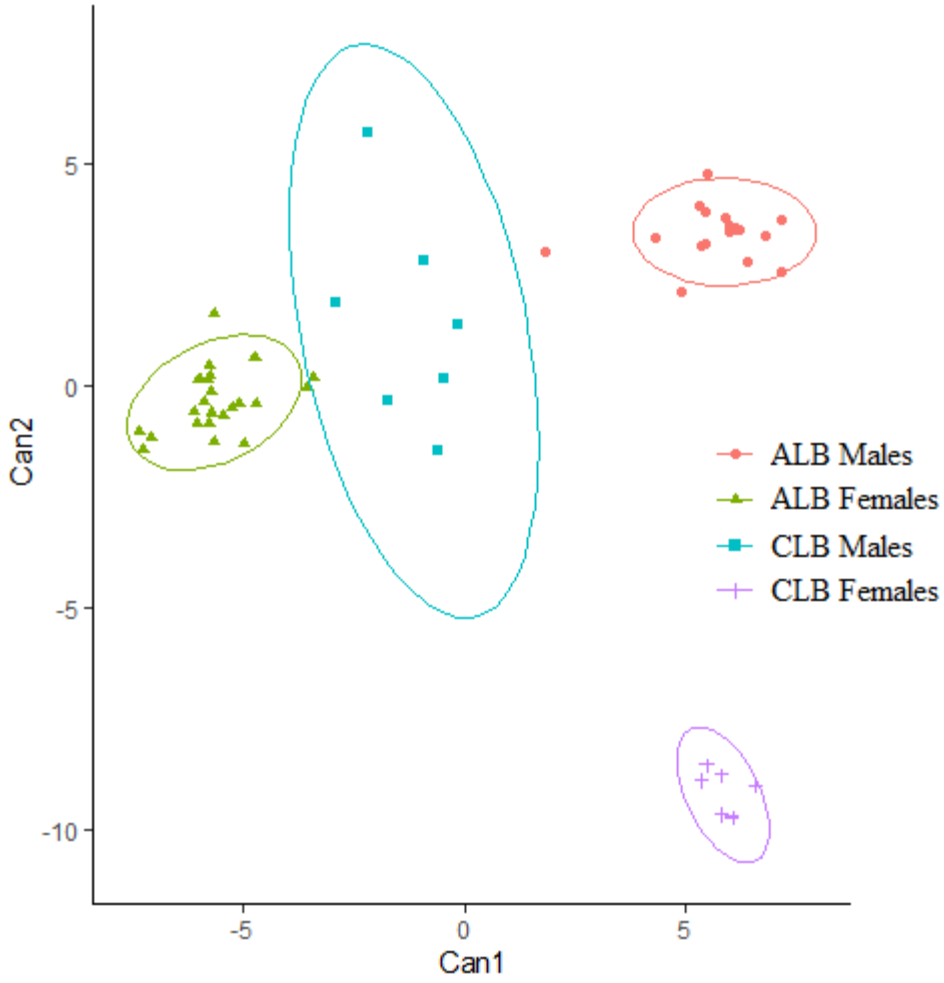



**FIGURE 4-2**: DA separation of ALB female, ALB male, CLB female, and CLB male samples by CHC composition. Normal confidence ellipses are for visual purposes only and are not a significance test.

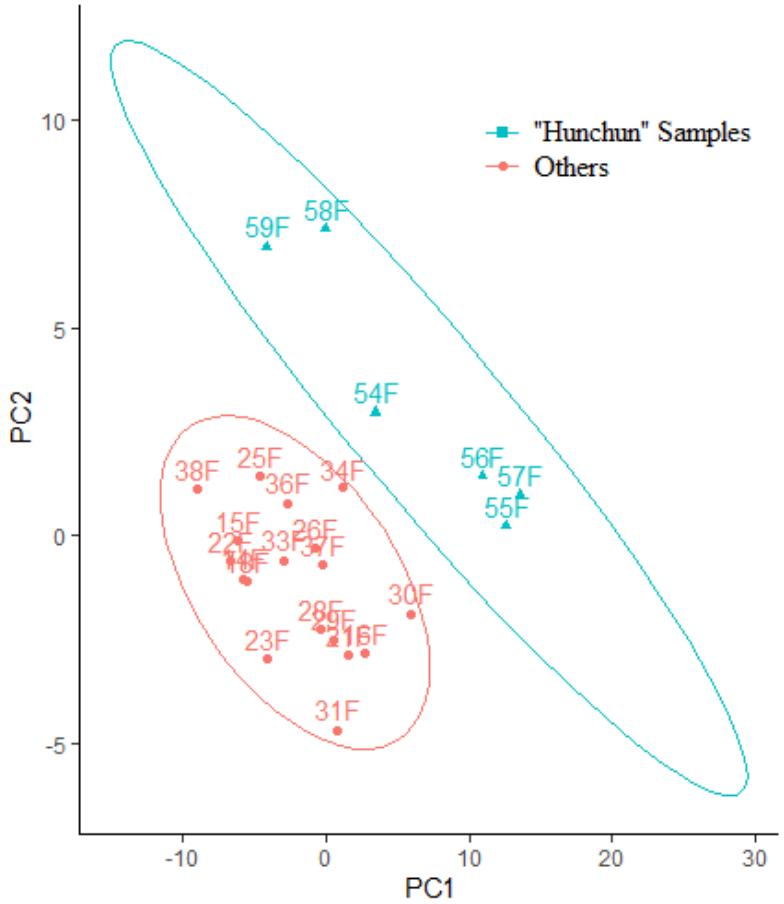



**FIGURE 4-3:** PCA separation of ALB female samples by CHC composition. Normal confidence ellipses are for visual purposes only and are not a significance test.

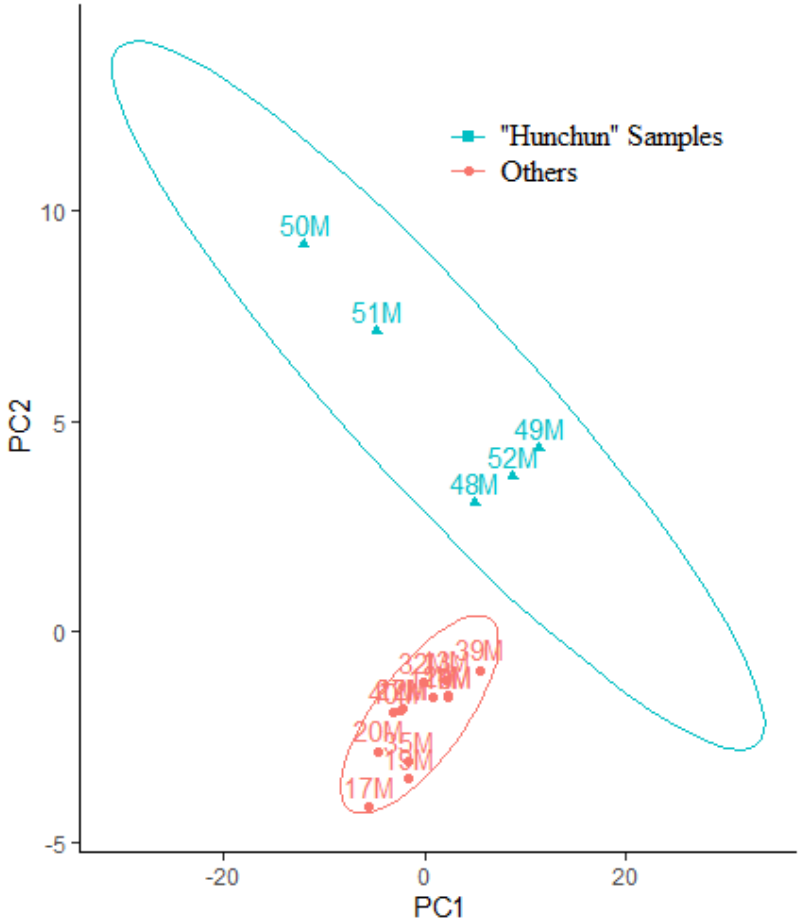



**FIGURE 4-4:** PCA separation of ALB male samples by CHC composition. Normal confidence ellipses are for visual purposes only and are not a significance test.

# CHAPTER 5: INTEGRATED CHEMICAL COMMUNICATION SYSTEMS AND CONCLUSIONS

## Introduction

Wickham (2009) proposed that ALB conspecific attraction is a behavioral sequence based on "host choice strategy", in which, (1) host kairomones attract females (host choice), (2) the combination of female long-range pheromones and host kairomones attract male ALB, (3) the male short-range pheromone attracts females, then (4) the males identify the female CHC contact pheromone. Since his proposal, there have been additional advances in understanding of ALB and CLB chemical communication systems.

## Male "Short-range" Aggregation Pheromones?

ALB and CLB clearly possess a male aggregation pheromone. Initially, this pheromone appeared to be a classic example of an insect pheromone. It is produced only by one sex, was previously undiscovered in the Cerambycidae, and showed significant, dose-dependent, y-tube bioassay attraction. Although field tests initially failed (Fukaya 2003), subsequent studies demonstrated statistically significant field attraction in the field for both ALB (Nehme 2010) and CLB (Hansen 2015). However, results similar to highly attractive pheromones, such as those of *Lymantria dispar dispar* (Beroza and Knipling 1972) or *Ips pini* (Teale et al. 1991) pheromones have not been obtained. Although high trap catch is a management goal, it is evolutionarily unreasonable to assume that an ALB or CLB pheromone would be comparable to those examples, given the different life history strategies of the insects (Hanks 1999). *Lymantria dispar dispar* females are flightless and short lived, necessitating a strong male attractant. ALB and CLB are obligate feeders as adults, both sexes are good fliers, and can live for months. In

this situation, simultaneous responses to a variety of different signals, including intraspecific, interspecific, and cues from abiotic conditions may be important.

Wickham (2009) suggested that the male pheromone is a short-range volatile pheromone. This is supported by the pheromone's attractiveness in "short-range" y-tube olfactometers and wind-tunnels versus its weak attractiveness in "long-range" field bioassays. Murlis et al. (1992) defined "short-range" pheromones as attractive at concentrations and air current dispersal patterns comparable to near an emitter, while a "long-range" pheromone is attractive at concentrations and air current dispersal levels distant from an emitter (Murlis et al. 1992). Although pheromone attractiveness can be dose-dependent and high concentrations of pheromones may indicate potential intraspecific competition for foliage or oviposition sites (Rudinsky et al. 1973), many long-range pheromones are also attractive at short-ranges and may be more accurately termed "detectable-range" pheromones. Synthetic pheromone lures have artificial concentrations and dispersal patterns and may not accurately imitate natural pheromone plumes. Currently, whether the available cerambycid pheromone-based lures function as long-range or short-range attractants is up for debate. Often, researchers are more interested in whether traps capture any beetles at all versus how far those those beetles traveled to reach the attractant lures. Natural population levels of cerambycids are difficult to measure and other competing attraction sources are difficult to detect. Assuming that ALB and CLB chemical communication is influenced by multiple factors, neither of which overrides the others, it is logical that in a y-tube bioassay or wind-tunnel, where charcoal filtered air is supplied and efforts are taken to reduce the amount of extraneous signals, one component of a multi-component communication system would show a more interpretable effect. In natural systems, organisms are potentially influenced by a multitude of signals.

The discovery of a 3rd male pheromone component in ALB, (3*E*,6*E*)-α-farnesene, which synergized attraction to male pheromones in laboratory bioassays (Crook et al. 2017), is also interesting. While they found significant y-tube attraction of the laboratory raised beetles from the same population and fed the same host material as the (3*E*,6*E*)-α-farnesene emitting individuals, field trapping on a wild population was unsuccessful. Yasui et al.'s (2017) reported the sequestration by CLB of host compounds that varied by natal host and influenced adult attraction. If terpenes acquired, modified, or both, from host-feeding are a component of a male pheromone blend, this would explain why the significant olfactometer attraction (Cook et al. 2017) failed to translate to individuals from different populations in the field.

**Female Trail, Contact, and Long-Range Pheromones?**

Due to the initial identification of the volatile male aggregation pheromone and evidence that the long-range volatile pheromone-producing sex in cerambycids is sex conserved, there has been resistance to the acceptance of female volatile pheromones. However, ALB clearly possesses a female contact pheromone, a female trail pheromone, and a female volatile pheromone (Zhang et al. 2003, Wickham 2009, Hoover et al. 2014, Xu 2018). Evidence that male ALB attempt to mate with synthetic female contact pheromone coated objects is convincing, as is male trail-following of synthetic female trail pheromone, and field and laboratory bioassay attraction towards ozone-oxidized products of female CHC components. Although viewed as three different pheromone signals, the pheromones are integrated. The female contact pheromone was identified by Zhang et al. (2003) as $Z9C_{23}$, $Z9C_{25}$, $Z7C_{25}$, $Z9C_{27}$, and $Z7C_{27}$ were supported by my CHC results, which found the five female contact pheromone components in female elytra in a similar proportion. The ozone-oxidized products of the same

compounds were later identified as a volatile pheromone (Wickham et al. 2012), and $Z9C_{23}$, $Z9C_{25}$, $Z7C_{25}$ were then identified as trail pheromone components (Hoover et al. 2014). This suggests that these pheromone signals may be more integrated and multipurpose than previously assumed. CLB clearly possesses a female contact pheromone, and, as with ALB, the male mating response towards synthetic pheromone coated objects is extremely convincing (Wang 1998, Fukaya et al. 1999). However, this pheromone is not an alkene-only mixture (Yasui 2003), no work has been published examining its ozone-oxidized oxidized products, and no work has been published identifying a CLB trail pheromone.

The existence of a female trail pheromone means that a male short-range pheromone cannot be the sole driver of intraspecific attraction at short range. Instead, I suggest that at the short-range, both contact and volatile cues mediate mate location.

**Host Volatiles**

ALB and CLB are clearly attracted to volatiles produced by their hosts, as reviewed in Chapter 1. Yasui et al. (2007, 2016) and Fujiwara-Tsujii et al.'s (2012) experiments on elytra sequestered sesquiterpenes can also be interpreted in terms host volatiles, showing that not only are the female contact, trail, and volatile pheromones linked, this integration extends to host volatiles.

It is unknown whether the majority of adult ALB and CLB reinfest their natal host or migrate to new host resources. This is important because lures based on host-volatiles may not be as effective if adults prefer to reinfest natal hosts rather than migrate. In Jiangsu, ALB and CLB seemed to prefer certain trees over others (pers. obs.). Both ALB and CLB were repeatedly hand-collected from certain *Salix* trees while CLB were repeatedly hand-collected from certain

*Platanus* trees. However, it is unclear whether the collected individuals were attracted to those trees from long distances or if they were recently emerged from a nearby natal host. The *Koelreuteria* plantation used for field trapping to identify the male CLB pheromone (Hansen et al. 2015) and as a source of several CHC samples, had a large CLB population, with beetles frequently observed flying from tree to tree. However, very few exit holes or egg niches were observed on the *Koelreuteria* trees, suggesting that CLB may have been migrating from other locations to an attractive monoculture host resource. In contrast, many Xuanwu Lake *Salix* trees were riddled with exit holes.

Population studies of CLB have found contagious egg distribution, with more eggs laid in certain locations with less larval and egg mortality in these locations (Adachi 1989). In a CLB-infested Japanese citrus grove, population estimates suggested a large portion of beetles immigrated instead of emerging from the citrus grove itself (Komazaki et al. 1989). In a more detailed study, the residence time of adults was estimated at 5.4 days with females staying twice as long as males, the population contained twice as many immigrate beetles, and beetle host choice was confirmed as contagious. In addition, in one trapping year, female dispersal was significantly less than male dispersal although seasonal differences in dispersal by sex were not noted (Adachi 1990). Although mark-recapture tests have been performed with ALB (Smith et al. 2004), without the natal host effect, the results may not be directly comparable to real world situations. It has been suggested that ALB reinfest their natal host if it remains a good resource but disperse if host quality deteriorates (Sawyer 2009, Trotter III and Hull-Sanders 2015).

In Wickham's (2009) proposed "host choice strategy" chemical communication system, females are the pioneer species and select appropriate hosts. This hypothesis was based on evidence for female ALB as the first-arriving sex on sentinel trees (Smith 2008) and was

supported by Wickham's (2009) capture of more females than males in host volatile containing traps and . Although my host volatile lures were not significantly attractive, in all cases, insignificantly greater numbers of males than females were captured in traps with host volatile lures. The sex ratio of the sampled population was unknown.

My results do not necessarily support the conclusion that females are the host-selecting sex in ALB or CLB. Greater dispersal of virgin, immature females early in the ALB or CLB life cycle has not been noted and both sexes are obligate feeders as adults. I suggest that decisions by any individual ALB or CLB to disperse or not disperse, to orient toward a host or to not, is complex, mediated by the quality of its current location, random disturbance, proximity of other host resources and conspecifics, as well as numerous other factors. Field trapping locations for cerambycids are typically chosen for the presence of already existing populations and hosts species. Although this assures beetle presence, it also assures the presence of already existing host and conspecific signals that compete with test lures.

**CHCs and Speciation**

Speciation is commonly understood as occurring due to geographic barriers, with physical constraints isolating two distinct populations until intermating can no longer occur. How speciation occurs in sympatric population is less clearly understood (Dieckmann and Doebeli 1999), although one possible mechanism is the formation of host-races via changes in host selection (Drès and Mallet 2002). In insects, changes in chemosensory systems are believed to be a major contributor to pre-mating isolation (Smadja and Butlin 2009). ALB and CLB are sympatric species with overlapping host and geographic ranges. Here, I demonstrate that the CHC profiles of ALB and CLB show distinct species differences. In addition, I demonstrate that

the CHC profiles of a geographically separated ALB from the rest of the collection samples shows a distinct difference. Evidence in ALB suggests that CHC profile of a female beetle is important at all levels of the communication pathway, from contact pheromone, to trail pheromone, to volatile pheromone, while in CLB this importance is extended to host choice.

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# APPENDIX

**TABLE A-1:** Compounds Present in Representative GC-MS Total Ion Chromatograms of Hardwood Tree Species Headspace Volatiles

| # | NIST Library Match | Major Ions | RT | RI | GC-MS Peak Integrations | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Ulmus* | *Ailanthus* | *Liriodendron* | *Melia* | *Morus* | *Salix* | *Citrus* |
| 1 | Isopropylcyclobutane | 55, 41, 56, 70, 42, 69, 83, 163 | 5.08 | 794.7 | | 17924 | | | | | |
| 2 | Cyclopentanone | 55, 84, 41, 56, 42, 83, 70, 69, 50 | 5.12 | 796.5 | 8539 | | | 12823 | 44452 | 7748 | |
| 3 | Cyclobutanecarboxylic acid, 4-cyanophenyl ester | 83, 55, 59, 119 | 5.14 | 797.1 | | | | | | | |
| 4 | 1-Butanol, 2,3-dimethyl- | 69, 41, 43, 71, 55, 59, 70, 53, 67, 72 | 5.11 | 796.1 | 15352 | | 17429 | | 9576 | | |
| 5 | 2-Hexanone | 43, 58, 57, 85 | 5.18 | 798.6 | 6813 | | | | 23930 | | 22245 |
| 6 | Hexanal | 41, 56, 44, 43, 57, 55, 67, 70, 82, 42 | 5.28 | 802.1 | 153826 | 175253 | 294575 | | 152617 | | 47920 |
| 7 | 3-Penten-2-one, 4-methyl- | 83, 55, 59, 73, 98 | 5.29 | 802.6 | | | | | 12940 | | |
| 8 | Octane | 43, 41, 57, 85, 56, 71, 44, 42, 55, 70 | 5.31 | 803.3 | | | | 62608 | | 64284 | |
| 9 | Tetrachloroethylene | 49, 84, 166, 47, 129, 51, 131, 207, 164 168 | 5.39 | 806.3 | 9488 | 27890 | 39621 | 10990 | | | |
| 10 | 1,1-Dimethyl-3-chloropropanol | 59, 43, 41, 107, 69, 71, 109, 60, 42, 45 | 5.40 | 806.8 | 1903646 | 3449555 | 3290757 | | | | 26622 |
| 11 | 2-Hexanol, (R)- | 45, 43, 41, 69, 87, 71, 42, 44, 77, 56 | 5.49 | 809.8 | 9592144 | 15856166 | 15286268 | | | | 859747 |
| 12 | Tetrachloroethylene | 166, 129, 164, 131, 168, 94, 96, 133, 45, 59 | 5.49 | 809.8 | | | | | 19945 | | |
| 13 | 1-Propene, 1-chloro-2-methyl- | 55, 90, 41, 92, 85, 54, 53, 77, 75, 49 | 5.51 | 810.8 | 208670 | 380461 | 348752 | | | | |
| 14 | Tetrahydrofuran, 2,2-dimethyl | 85. 59 | 5.55 | 812.0 | 13304 | 25961 | | | | | |
| 15 | Acetic acid, butyl ester | 43, 56, 41, 61, 73, 42, 57, 44, 71, 58 | 5.60 | 813.9 | 84949 | 122333 | 169767 | 25133 | 48534 | 31269 | 22650 |
| 16 | 2,2-Dimethyl-3-hydroxypropionaldehyde | 56, 57, 55, 72, 54 | 5.64 | 815.5 | 14655 | 24548 | 19174 | | | | |
| 17 | Propionic acid, 2-isopropoxy-, methyl ester | 59, 43, 71, 41, 45, 89, 42, 60, 69, 58 | 5.68 | 816.7 | 159103 | 195153 | 224015 | | | | |
| 18 | 2-Propyn-1-ol, propionate | 57, 63, 55, 103, 56 | 5.70 | 817.5 | | | | | | | 16098 |
| 19 | Pentanal, 2,4-dimethyl- | 43, 58, 57, 45, 41, 55, 71, 74, 73, 69 | 5.75 | 820.1 | | | 19234 | | | | |
| 20 | Oxirane, (methoxymethyl)- | 45, 58, 41, 43, 57, 49, 59, 42, 70, 85 | 5.82 | 821.8 | 26458 | 43160 | 34751 | 18176 | 30100 | 12657 | |
| 21 | Butane, 2,3-dichloro-2-methyl- | 41, 77, 43, 69, 76, 55, 79, 57, 71, 70 | 5.82 | 822.1 | 20655 | 46402 | 43052 | | | | |
| 22 | 2,6-Octadiene-4,5-diol | 71, 95, 82, 96, 53 | 5.99 | 828.4 | | | 6648 | | 3575 | | |
| 23 | 1,3-Propanediol | 58, 43, 57, 45, 41, 74, 59, 70 | 6.03 | 829.7 | 34793 | 53462 | 40477 | | | | |
| 24 | 2H-Pyran, 2-(bromomethyl)tetrahydro- | 85, 43, 55, 57, 54, 53, 45, 56, 82 | 6.07 | 831.2 | 14542 | 31800 | 36424 | | | | |
| 25 | Boronic acid, ethyl-, dimethyl ester | 73, 72, 71 | 6.15 | 834.2 | 7011 | 9710 | 13754 | | | | |
| 26 | 2-Pentanone, 4-hydroxy-4-methyl- | 43, 59, 101, 83, 58, 55, 82, 98 | 6.20 | 836.1 | | 15110 | | | | | |
| 27 | α-Chloroethyltrimethylisilane | 73, 93, 55, 43, 57, 95, 107, 41, 45, 49 | 6.26 | 838.0 | 90742 | 169047 | 143313 | | | | |
| 28 | Thiophene | 84, 58, 43, 45, 207, 69, 57, 56 | 6.29 | 839.1 | | | | | | | |
| 29 | Cyclopropane, 1-methyl-2-(3-methylpentyl)- | 70, 43, 55, 56, 69, 57, 41, 83, 42, 111 | 6.31 | 839.9 | | | | 4628 | 9446 | 3795 | |
| 30 | Benzene, chloro- | 112, 77, 114, 50, 51, 74, 73, 75, 73, 113 | 6.37 | 842.1 | 21006 | 45451 | 44071 | | | | |
| 31 | Cyclohexane, azido- | 83, 55, 69, 41, 42, 43 | 6.45 | 845.0 | | | | | 8902 | | |
| 32 | 1-Chloro-2-methyl-2-propanol | 59, 57, 41, 93, 95, 58, 43, 45, 60, 55 | 6.49 | 846.4 | 12999 | 13913 | 25093 | 6319 | 7356 | 4905 | 214407 |
| 33 | 3-Hexen-1-ol | 41, 67, 82, 55, 69, 42, 57, 70, 53, 54 | 6.57 | 849.5 | | | | | 36146 | | |
| 34 | 2-Hexenal | 41, 55, 69, 83, 42, 57, 70, 43, 98, 56 | 6.59 | 850.3 | | | 29403 | | 1162155 | | |
| 35 | 5,9-Dodecadien-2-one, 6,10-dimethyl-, (E,E))- | 43, 72, 57, 71, 42, 58, 67, 82 | 6.62 | 851.4 | | | | | | | |
| 36 | Phosphoric acid, bis(1-methylethyl) ester | 83, 109, 42, 69, 55, 43, 57 | 6.62 | 851.5 | | | | 6007 | | | |
| 37 | Isopropyl Alcohol | 45, 43, 41, 71, 55, 44, 42, 69, 46, 57 | 6.65 | 852.3 | | | | | | | 197108 |
| 38 | 3-Hexen-1-ol, (Z)- | 67, 41, 82, 55, 69, 42, 57, 54, 53, 70 | 6.66 | 852.8 | 103427 | 378790 | 670233 | 2863608 | 7149176 | 66580 | 12252 |
| 39 | 3-Pentanol | 59, 41, 57, 93, 43, 45, 58, 60, 95, 63 | 6.75 | 856.2 | | | | | | | 262931 |
| 40 | 2(3H)-Furanone, dihydro-5-propyl- | 85, 43, 56, 57 | 6.76 | 856.4 | | | | 15703 | | | |
| 41 | Ethylbenzene | 91, 106, 51, 65, 77, 92, 78, 105, 79, 63 | 6.81 | 858.3 | 157042 | 506502 | 362736 | 189868 | 237994 | 128529 | 5760 |
| 42 | 2-Butanone, 4-hydroxy-3-methyl- | 43, 61, 42, 41, 57, 85, 71 | 6.87 | 860.7 | | | | | | 7254 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 | 1-Methyloxy-2-propyl acetate | 43, 45, 72, 55, 90, 91, 41, 58, 57, 87 | 6.94 | 862.9 | 28561 | 49858 | 66070 | 13857 | | | | |
| 44 | 2-Hexen-1-ol, (E)- | 57, 41, 82, 67, 44, 43, 55, 56, 71, 42 | 6.97 | 864.3 | | | | | 764609 | | | |
| 45 | 2-Furanmethanol, tetrahydro-5-methyl-, trans- | 85, 57, 41, 43, 67, 56, 55, 42, 69 | 6.99 | 864.7 | | | | 21053 | | | | |
| 46 | 1-Hexanol | 56, 55, 43, 41, 42, 69, 57, 84, 44, 54 | 7.03 | 866.2 | 20178 | 15253 | 33821 | 15517 | 1129848 | | | |
| 47 | o-Xylene | 91, 106, 105, 77, 51, 92, 79, 103, 65, 78 | 7.06 | 867.4 | 178386 | 641200 | 405235 | 266997 | 285137 | 163145 | | |
| 48 | 4-Penten-2-ol | 45, 43, 41, 71, 44, 46, 69, 42, 49, 55 | 7.07 | 867.9 | 13259 | 15666 | 19510 | 17281 | 18252 | 12110 | 317181 | |
| 49 | p-Xylene | 91, 106, 105, 77, 51, 92, 79, 103, 65, 78 | 7.10 | 868.9 | 87118 | 247966 | 167875 | 118349 | 123591 | 72870 | | |
| 50 | 2-Butene-1,4-diol | 57, 41, 70, 69, 42 | 7.12 | 869.9 | | | | 3110 | | | | |
| 51 | 1-Butanol, 3-methyl-, acetate | 43, 70, 55, 41, 42, 61, 87, 73, 69, 71 | 7.28 | 875.7 | 73832 | | | | 12626 | | | |
| 52 | 2,3-Dimethyloxirane-2-carboxylic acid, methyl ester | 43, 59, 83, 45, 55, 74, 57, 82, 48 | 7.30 | 876.4 | | | | | | | | |
| 53 | 1-Pentene, 4,4-dimethyl- | 57, 83, 69, 41, 55, 56, 48 | 7.42 | 880.6 | 6672 | 19621 | | | | | | |
| 54 | 2,2-Bis(chloromethyl)-1-propanol | 90, 55, 53, 41, 92, 84, 67, 54, 89, 51 | 7.58 | 886.6 | 170654 | 308363 | 285527 | | | | | |
| 55 | Diazene, dimethyl- | 43, 58 | 7.65 | 889.0 | 4158 | | | | 6580 | | | |
| 56 | Styrene | 104, 103, 78, 51, 77, 50, 102, 52, 63, 74 | 7.69 | 890.7 | 15831 | 23098 | 60748 | 49924 | 55180 | 28288 | | |
| 57 | 2H-Pyran, 2-[(5-chloropentyl)oxy)]tetrahydro- | 43, 85, 56, 105, 41, 77, 79, 65, 70, 63 | 7.71 | 891.2 | 9614 | | 17891 | | | | | |
| 58 | Benzene, 1,3-dimethyl- | 91, 106, 105, 77, 92, 51, 65, 79, 63, 78 | 7.72 | 891.8 | 66285 | 196609 | 153891 | 87443 | 98075 | 40945 | | |
| 59 | Cyclopentanone, 2-methyl- | 55, 98, 42, 49, 70, 41, 69, 54, 83, 53 | 7.74 | 892.5 | | | 27672 | | | | | |
| 60 | Oxime-, methoxy-phenyl- | 59, 133, 43, 151, 71, 41, 135, 123, 45, 89 | 7.81 | 894.9 | 20589 | 48513 | 37092 | | | | | |
| 61 | 3-Acetyl-2,5-dimethyl furan | 123, 81, 138 | 7.88 | 897.5 | | | | | | | 18113 | |
| 62 | Acetic acid, 1-methylcyclopentyl ester | 43, 72, 84, 82, 83, 85, 100, 59, 67, 127 | 7.99 | 901.2 | 18608 | 57256 | 88121 | 88884 | 56181 | | | |
| 63 | Heptanal | 70, 44, 41, 55, 42, 71, 81, 68, 86, 96 | 8.02 | 902.1 | 38815 | | 87170 | | | | | |
| 64 | Ethylene glycol monoisobutyl ether | 57, 45, 87, 41, 75, 56, 58, 73 | 8.09 | 904.1 | | | | | | | | |
| 65 | Ketene | 42, 41, 45, 85 | 8.19 | 906.9 | | | | | | | | |
| 66 | 2-Penten-1-ol, acetate, (Z)- | 43, 67, 68, 41, 86, 57, 53, 69, 85 | 8.37 | 912.4 | | | | 7922 | 55729 | | | |
| 67 | Acetic acid, pentyl ester | 43, 70, 42, 61, 55, 73, 41 | 8.40 | 913.1 | | | | | 31165 | | | |
| 68 | 2-Pentenal, (E)- | 84, 57, 69, 55, 83, 41, 67, 56, 43, 53 | 8.50 | 916.0 | 31001 | 62194 | 71654 | | | | | |
| 69 | 1,3,2-Dioxathiolane, 2-oxide | 108, 78, 65 | 8.51 | 916.2 | | | | 5791 | | | | |
| 70 | Ethane, 1,1,2,2-tetrachloro- | 83, 85, 95, 61, 87, 60, 131, 133, 96, 168 | 8.57 | 918.1 | | | | | | | | |
| 71 | Cyclopentene, 1,2,3,4,5-pentamethyl- | 123, 81, 41, 67, 138, 91, 77, 109, 79, 95 | 8.55 | 917.5 | 5692 | 11772 | 13030 | | | | 30684 | |
| 72 | Acetophenone | 105, 77, 120 | 8.70 | 921.8 | | 4574 | | | | | | |
| 73 | Propane, 1,2,3-trichloro- | 75, 43, 99, 77, 110 | 8.74 | 923.0 | | | | | | | | |
| 74 | Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl- | 93, 91, 92, 79, 77, 121, 136, 41, 105, 94 | 8.76 | 923.6 | | | 53185 | 342559 | | | | |
| 75 | Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- | 93, 91, 77, 92, 79, 41, 43 | 8.85 | 926.2 | | | | 30706 | | | 8344 | |
| 76 | 4-Hexyn-3-ol | 69, 41, 83, 55, 73 | 8.94 | 928.9 | | | | | | | | |
| 77 | 1R-α-Pinene | 93, 91, 92, 77, 79, 41, 121, 105, 80, 94 | 9.07 | 932.6 | 29512 | 66376 | 861332 | 344615 | 156033 | 23804 | 362764 | |
| 78 | Butane, 1,1'oxybis[3-methyl- | 43, 70, 71, 69, 41, 53, 54, 140, 83, 67 | 9.47 | 944.2 | 27753 | | 50865 | | | | | |
| 79 | Sulfurous acid, di(cyclohexylmethyl) ester | 97, 55, 57, 96 | 9.51 | 945.5 | 5741 | | | | | | | |
| 80 | Camphene | 93, 70, 121, 43, 79, 41, 91, 67, 193, 107 | 9.63 | 948.8 | | | 275727 | 158421 | | | | |
| 81 | N-Benzyl-2-phenylethylamine | 91, 120, 71 | 9.69 | 950.6 | | | | | | | | |
| 82 | 2-Methyl-2,3-pentanediol | 43, 59, 45, 71, 57, 88, 41, 58, 72, 89 | 9.72 | 951.5 | | | | | | | | |
| 83 | 1-Propanamine, N,N-dimethyl-3[[1-(phenylmethyl)-1H-indazol-3-yl]oxy]- | 57, 85, 86, 91, 43, 55, 120, 72, 58, 42 | 9.76 | 952.7 | | | | | 86228 | | | |
| 84 | Benzene, (1-methylethyl)- | 105, 106, 77, 120, 51, 91, 78, 50, 103, 79 | 9.98 | 959.1 | 9971 | 51438 | 86787 | 48946 | | 12900 | | |
| 85 | 2(5H)-Furanone, 5-ethyl- | 83, 55, 57, 84, 112, 56, 53 | 9.99 | 959.4 | | | | | 43340 | | | |
| 86 | Benzene, (1-methylethyl)- | 105, 77, 106, 51, 120, 78, 50, 91, 79, 52 | 10.05 | 960.9 | | | 17650 | 12420 | 15725 | 25105 | | |
| 87 | Dichlorodifluoromethane | 105, 120 | 10.17 | 964.5 | | | | | 14523 | | | |
| 88 | Furan, tetrahydro-2,2,5,5-tetramethyl- | 43, 113, 59, 95, 55, 70, 58, 68, 114 | 10.23 | 966.4 | | | | | | | | |
| 89 | Ethaneperoxoic acid, 1-cyano-1-phenylpentyl ester | 105, 49, 120, 86 | 10.30 | 968.3 | | | | | 7596 | | | |
| 90 | Hexano-dibutyrin | 71, 43, 59, 99, 41, 85, 53, 55, 69, 67 | 10.32 | 968.8 | 38873 | | 626933 | | | | | |
| 91 | Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- | 93, 91, 77, 79, 41, 136, 94, 80, 92, 69 | 10.42 | 971.8 | | | 413236 | 133673 | 100268 | | 1047287 | |
| 92 | Benzamide, N-[6-(2-furyl)-2-oxo-2H-pryan-3-yl]- | 105, 120, 281, 91, 77, 79, 94, 69, 55, 70 | 10.58 | 976.4 | | | | | | | | |
| 93 | β-Pinene | 93, 41, 69, 91, 79, 77, 94, 121, 80, 67 | 10.58 | 976.5 | | | 767421 | 30260 | 36152 | | 4694051 | |
| 94 | 3-Pentenoic acid, 4-methyl- | 99, 43, 55, 56, 59, 42, 71, 70 | 10.61 | 977.2 | | | 8470 | | | | | |
| 95 | 5-Hepten-2-one, 6-methyl- | 43, 41, 55, 108, 69, 67, 58, 111, 71, 68 | 10.85 | 984.2 | 706021 | | 1313261 | 27337 | 190933 | 23690 | 399606 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 96 | β-Myrcene | 93, 69, 41, 91, 79, 77, 67, 53, 92, 94 | 11.02 | 989.3 | 607330 | | 4403132 | 287136 | 137942 | | 207445 |
| 97 | Heptane, 2,2,4,6,6-pentamethyl- | 57, 56, 41, 93, 69, 43, 71, 55, 85, 99 | 11.06 | 990.3 | | | | 890102 | 904142 | 444492 | |
| 98 | dl-6-Methyl-5-hepten-2-ol | 95, 41, 105, 69, 67, 45, 55, 43, 71, 120 | 11.12 | 992.1 | 72847 | | | | | | |
| 99 | Benzene, 1,2,3-trimethyl- | 105, 120, 77, 91, 79, 119, 43, 106, 103, 51 | 11.15 | 993.0 | | | 51616 | 30034 | 37577 | | |
| 100 | Decane | 57, 71, 85, 70, 56, 142, 58, 98, 99 | 11.41 | 1000.5 | | 104148 | | | | | |
| 101 | Octanal | 43, 41, 56, 57, 44, 55, 84, 42, 69, 68 | 11.51 | 1003.2 | | | 153911 | | | 7047 | |
| 102 | 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- | 91, 119, 134, 77, 92, 105, 67, 79, 117, 65 | 11.55 | 1004.2 | | | 288134 | | | | |
| 103 | Urea, phenyl- | 93, 136, 94 | 11.57 | 1004.9 | | | 29374 | | | | |
| 104 | 3-Hexen-1-ol, acetate, (E)- | 67, 43, 82, 41, 54, 55, 81, 68, 53, 83 | 11.59 | 1005.5 | 1346252 | 7931555 | | 16667464 | 62839121 | 916976 | 1465931 |
| 105 | Benzene, p-dichloro- | 146, 148, 111, 75, 74, 50, 73, 113, 150, 147 | 11.85 | 1012.6 | | | 15083 | | | | |
| 106 | Acetic acid, hexyl ester | 43, 56, 61, 55, 84, 69, 41, 42, 73, 58 | 11.88 | 1013.2 | | | | 9508 | 3925320 | | |
| 107 | 2-Hexen-1-ol, acetate, (Z)- | 43, 67, 82, 41, 100, 55, 57, 54, 71, 53 | 11.96 | 1015.4 | | | | | 639199 | | |
| 108 | 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 121, 93, 136, 91, 79, 119, 77, 107, 105, 92 | 11.97 | 1015.8 | | | 71680 | | | | |
| 109 | Propanedinitrile, (2-methylpropylidene)- | 105, 120, 119 | 12.07 | 1018.5 | | | | | | | |
| 110 | Benzene, 1-methyl-4-(1-methylethyl)- | 119, 134, 91, 117, 120, 77, 115, 65, 103, 41 | 12.25 | 1023.2 | | | 640499 | | | | |
| 111 | Butane, 2,2,3,3-tetramethyl- | 57, 99, 41, 56, 43, 113, 55 | 12.42 | 1028.0 | | | | 43543 | 50584 | 17697 | |
| 112 | Limonene | 68, 93, 67, 79, 94, 91, 92, 77, 53, 121 | 12.44 | 1028.5 | 11177 | | 13737 | 684081 | 85319 | | 1035189 |
| 113 | Eucalyptol | 43, 81, 108, 71, 111, 84, 69, 55, 93, 154 | 12.52 | 1030.8 | | | 131321 | | | | |
| 114 | Tricyclo[3.2.1.0(2,,4)]oct-6-ene, 8-methylene-(1α,2α,4α,5α)- | 117, 79, 118, 41, 108, 77, 69, 71, 51, 53 | 12.57 | 1032.1 | | | | | | | |
| 115 | 1,3,6-Octatriene, 3,7-dimethyl-, (E)- | 93, 91, 92, 79, 77, 41, 80, 105, 53, 121 | 12.70 | 1035.6 | 212666 | 78231 | 8787862 | | 5045997 | | 60003 |
| 116 | 1,3,6-Octatriene, 3,7-dimethyl- | 93, 91, 79, 80, 77, 92, 41, 105, 121, 53 | 13.09 | 1046.4 | 25229564 | 3175233 | 1.01E+08 | 68303 | 1897405 | 195506 | 4531157 |
| 117 | Acetic acid, dichloro- | 84, 86, 105, 91, 85 | 13.43 | 1055.6 | | | | | | | |
| 118 | 2,2'-Bifuran, octahydro- | 71, 43, 70, 91 | 13.44 | 1055.7 | | | | | 7291 | | |
| 119 | 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 93, 91, 77, 136, 121, 92, 79, 43, 41, 105 | 13.49 | 1057.0 | | | 802434 | | | | |
| 120 | 2,2-Dimethylpropanoic anhydride | 57, 85, 55, 41, 43, 67, 53, 81, 86, 58 | 13.56 | 1059.1 | | | | | 412208 | | |
| 121 | Acetophenone | 105, 77, 120, 51, 50, 69, 78 | 13.70 | 1062.9 | 6053 | | 26080 | | 8278 | | |
| 122 | Cyclohexanol, 2-(1-methylethyl)- | 57, 41, 99, 56, 55, 81, 67, 71, 109, 82 | 13.96 | 1069.9 | | | | | | | |
| 123 | 1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)- | 107, 41, 91, 69, 119, 79, 77, 134 | 14.26 | 1078.1 | 10547 | | | 13377 | | | |
| 124 | 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- | 91, 119, 134, 77, 117, 41, 79, 92, 65, 105 | 14.26 | 1078.1 | | | 238499 | | | | |
| 125 | 1,4-Hexadiene, 5-methyl-3-(1-methylethylidene)- | 121, 105, 136, 77, 79, 91, 93, 80 | 14.50 | 1084.7 | | | | | *26496* | | |
| 126 | cis-Linaloloxide | 59, 94, 43, 55, 68, 111, 93, 67, 41, 83 | 14.58 | 1086.7 | | | | | 108861 | | |
| 127 | Benzoic acid, methyl ester | 105, 77, 136, 51, 41, 50, 106, 78, 135, 91 | 14.76 | 1091.6 | 73093 | | | | | | |
| 128 | 1,5-Heptadiene, 3,3-dimethyl-, (E)- | 69, 41, 79, 107, 81, 53, 77, 43, 67, 94 | 14.82 | 1093.3 | 27672 | | 20969 | 41612 | | | 22086 |
| 129 | 4,7-Methano-1H-indene, octahydro- | 95, 94, 51, 79, 136, 67, 68, 121 | 14.88 | 1095.1 | | | | 8905 | | | |
| 130 | 3-Hexen-1-ol, propanoate, (Z)- | 67, 57, 82, 54, 81 | 15.01 | 1098.4 | | | | 23853 | 42980 | | |
| 131 | 1,6-Octadien-3-ol, 3,7-dimethyl- | 71, 93, 41, 43, 55, 69, 80, 67, 121, 83 | 15.02 | 1098.9 | 316123 | | | 24296 | 127747 | | 590209 |
| 132 | (±)-Lavandulol, acetate | 121, 69, 41, 93, 53, 42, 91, 68, 83, 81 | 15.07 | 1100.0 | | | | 4760 | | | |
| 133 | Undecane | 57, 43, 71, 41, 85, 56, 55, 70, 42, 84 | 15.08 | 1100.3 | | 16616408 | | | | | |
| 134 | n-Undecane | 57, 43, 71, 41, 85, 56, 55, 70, 42, 84 | 15.13 | 1101.7 | | | | | | | |
| 135 | Nonanal | 57, 41, 43, 56, 55, 44, 70, 69, 98, 68 | 15.22 | 1104.1 | 221903 | | 446440 | 56393 | 9093 | | |
| 136 | Furan, 2-methyl- | 82, 43, 81, 109, 54, 53, 97, 79, 83, 59 | 15.30 | 1106.4 | | | 116697 | | | | |
| 137 | Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene- | 69, 41, 81, 79, 53, 67, 82, 107, 135, 150 | 15.56 | 1113.5 | 10160247 | | | 5346863 | 120968 | 2055062 | 7392043 |
| 138 | 1-Propanol, 2-benzyloxy- | 91, 107, 43, 92, 79, 135, 77, 65, 119, 53 | 15.70 | 1117.3 | | | 114054 | | | | |
| 139 | 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- | 119, 91, 134, 77, 79, 105, 92, 117, 41, 93 | 15.76 | 1119.1 | 38844 | | 324832 | | | | |
| 140 | 1H-1,2,4-Triazole, 3-thiol-5-methyl- | 115, 56, 42 | 15.83 | 1121.1 | | | | | | | |
| 141 | 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)- | 121, 105, 136, 79, 91, 77, 93, 41, 106, 122 | 16.07 | 1127.7 | 11229 | | 578390 | | 1131363 | | |
| 142 | 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- | 119, 91, 134, 77, 79, 92, 41, 117, 65, 55 | 16.10 | 1128.5 | 606394 | | 4301040 | | | | |
| 143 | 1,3-Cyclohexadiene, 1,5,5,6-tetramethyl | 121, 105, 136, 91, 77, 93, 119, 106, 122, 65 | 16.48 | 1138.9 | | | 205767 | | | | |
| 144 | Butanoic acid, 3-hexenyl ester, (Z)- | 67, 82, 43, 71, 41, 55, 83, 81, 54, 68 | 16.62 | 1142.8 | | | | 107939 | | | |
| 145 | Benzene, (2-methylcyclopropyl)- | 132, 115, 95, 117, 108, 67, 91 | 16.70 | 1145.0 | | | | | | | |
| 146 | Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-, (1S)- | 95, 81, 41, 108, 69, 55, 109, 83, 152, 67 | 16.76 | 1146.7 | | | | 5719 | 677511 | | |
| 147 | Cyclobutane-1,1-dicarboxyamide, N,N'-di-benzoyloxy- | 105, 77, 122, 51, 50 | 17.04 | 1154.4 | | | | | | | |
| 148 | Acetic acid, phenylmethyl ester | 108, 91, 90, 43, 79, 77, 150, 107, 89, 51 | 17.30 | 1161.4 | | | | | | 57264 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 149 | p-Metha-1,5-dien-8-ol | 59, 94, 79, 91, 43, 93, 77, 41, 92, 55 | 17.62 | 1170.5 | | | 233571 | | 80243 | | | |
| 150 | Decanal | 57, 43, 41, 55, 56, 69, 82, 71, 81, 95 | 17.65 | 1171.1 | | | | | | 23924 | | |
| 151 | Butanoic acid, 3-hexenyl ester, (Z)- | 67, 82, 71, 43, 41, 55, 83, 54, 81, 42 | 18.17 | 1185.3 | | | | 448671 | 1703047 | | | |
| 152 | Methyl salicylate | 120, 92, 152, 121, 65, 93, 64, 63, 53, 153 | 18.29 | 1188.8 | 617346 | 759514 | | | | | | |
| 153 | Butanoic acid, hexyl ester | 71, 43, 89, 56, 41, 84, 55, 69, 42, 60 | 18.40 | 1191.8 | | | | | 212397 | | | |
| 154 | Ethene, tetramethoxy- | 133, 86, 148, 91, 115 | 18.44 | 1192.9 | | | | | | | | |
| 155 | Butanoic acid, 2-hexenyl ester, (E)- | 71, 43, 67, 41, 55, 82, 54, 83, 100, 53 | 18.50 | 1194.4 | | | | | 205143 | | | |
| 156 | 2,6-Dimethyl-1,3,5,7-octatetrae-2-ol, E,E- | 91. 43, 119, 109, 79, 77, 93, 152, 134, 53 | 18.69 | 1199.7 | | | 76233 | | 3183 | | | |
| 157 | Decanal | 57, 43, 41, 71, 70, 55, 56, 82, 69, 83 | 18.75 | 1201.4 | | | | 3018 | | | | |
| 158 | 5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol | 43, 95, 59, 55, 81, 41, 69, 67, 71, 97 | 18.81 | 1203.2 | 277036 | | 96231 | | | | | |
| 159 | 2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E- | 43, 91, 81, 95, 109, 55, 77, 41, 79, 119 | 18.86 | 1204.8 | 317299 | | 6570737 | | 3220 | | | |
| 160 | Decanal | 57, 41, 43, 55, 70, 44, 71, 82, 56, 68 | 18.90 | 1205.7 | | | 1357769 | | 3710 | | | |
| 161 | 2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E- | 43, 91, 109, 119, 81, 77, 79, 93, 152, 67 | 18.92 | 1206.3 | 1545459 | | 8150987 | | | | | |
| 162 | 5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol | 43, 95, 93, 55, 69, 41, 97, 71, 98, 59 | 19.04 | 1209.7 | | | 85191 | | | | | |
| 163 | 3-Thujen-2-ol, stereoisomer | 83, 119, 91, 134, 77, 109, 41, 105, 79, 117 | 19.10 | 1211.5 | | | 112126 | | | | | |
| 164 | n-Valeric acid cis-3-hexenyl ester | 82, 67, 57, 41, 85, 55, 83 | 19.77 | 1230.9 | | | | 66541 | 65215 | | | |
| 165 | 2-Butenoic acid, 3-hexenyl ester, (E,Z)- | 67, 82, 69, 41, 55, 85 | 19.90 | 1234.5 | | | | | 23352 | | | |
| 166 | Hexanedioic acid, dimethyl ester | 59, 114, 111, 101, 43, 143, 74, 83, 56, 69 | 20.13 | 1241.2 | | | | | | | | |
| 167 | Bicyclo[2.2.1]heptane, 2-(2-propenyl)- | 95, 67, 41, 79, 65, 80, 43, 77, 96, 108 | 20.28 | 1245.5 | | | 125312 | | | | | |
| 168 | Carbonic acid, bis(1-methylethyl) ester | 131, 146 | 20.30 | 1246.0 | | | | | | | | |
| 169 | Sulfurous acid, octyl 2-pentyl ester | 43, 71, 59, 113, 55, 41, 85, 73, 70, 83 | 20.72 | 1258.2 | | | 36744 | | | | | |
| 170 | 3-Hexen-1-ol, 2-ethyl- | 41, 43, 97, 72, 95, 67, 68, 79, 57, 81 | 21.01 | 1266.4 | | | 926942 | | | | | |
| 171 | Furan, tetrahydro-2,2,4,4-tetramethyl- | 43, 71, 59, 113, 55, 42, 67, 83, 53, 95 | 21.23 | 1272.8 | | | 15181 | | | | | |
| 172 | Benzene, pentamethyl- | 133, 148, 91, 43, 85, 71, 41 | 21.31 | 1275.0 | | | | | | | | |
| 173 | Phenacylidene diacetate | 105, 77, 51, 50, 170, 106, 135, 78, 74 | 21.31 | 1275.2 | | | | | | | | |
| 174 | trans-2-Undecen-1-ol | 57, 55, 41, 81, 70, 43, 71, 67, 82, 95 | 21.43 | 1278.5 | | | | | | | | |
| 175 | Bromonitromethane | 95, 43, 93, 86 | 21.64 | 1284.4 | | | | | | | | |
| 176 | Spiro[2.4]heptane-5-methanol, 5-hydroxy- | 43, 111, 55, 91, 41, 77, 93, 67, 95, 109 | 22.03 | 1295.8 | | | 135145 | | | | | |
| 177 | Octane, 2,4,6-trimethyl- | 57, 43, 71, 85, 41, 55, 56, 69, 70, 42 | 22.21 | 1301.0 | | 66745 | | 5616 | 77577 | 7658 | | |
| 178 | Undecanal | 43, 41, 57, 55, 82, 56, 68, 67, 69, 71 | 22.43 | 1307.5 | | | | | | | | |
| 179 | Cyclohexane, (1,3-dimethylbutyl)- | 82, 83, 55, 67, 85, 84, 43 | 22.90 | 1321.8 | | | | | 6982 | | | |
| 180 | Benzenemethanol, α,4-dimethyl- | 93, 121, 136, 91, 77 | 23.22 | 1331.6 | | | | | | | | 6458 |
| 181 | 1,2,3-Propanetriol, diacetate | 43, 103, 145, 116, 115, 42, 86, 57 | 23.51 | 1340.5 | | | | | | | | |
| 182 | Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester | 71, 43, 56, 83, 89, 98, 41, 55, 57, 73 | 23.68 | 1345.5 | | | | | | | | |
| 183 | 7-Hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohepta[d,e]naphthalene | 193, 207, 415, 44, 208, 74, 327, 191, 49, 41 | 23.88 | 1351.5 | | | | | | | | |
| 184 | Tetrahydropyran Z-10-dodecenoate | 85, 55 | 24.01 | 1355.6 | | | | | | | | |
| 185 | Bicyclo[3.3.1]nonan-3-one, 7-methylene- | 43, 81, 92, 82, 41, 107, 55, 79, 53, 67 | 24.34 | 1365.5 | | | | | | | | |
| 186 | Nonaneperoxic acid, 1,1-dimethylethyl ester | 57, 141, 71, 41, 58, 56, 59 | 24.38 | 1366.9 | | | | | | | | |
| 187 | Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester | 71, 56, 43, 89, 41, 73, 55, 72, 85, 173 | 24.51 | 1370.9 | | | | | | | | |
| 188 | α-Cubebene | 119, 105, 161, 93, 91, 92, 81, 41, 120, 77 | 24.69 | 1376.1 | | | | | 232560 | | | |
| 189 | Hexanoic acid, 3-hexenyl ester, (Z)- | 82, 67, 99, 43, 41, 55, 71, 83, 81 | 24.81 | 1379.9 | | | | | 52726 | | | |
| 190 | 1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene | 67, 93, 79, 189, 107, 121, 68, 82, 69, 53 | 25.09 | 1388.6 | | | 4847 | | | | | |
| 191 | Octane, 2,4,6-trimethyl- | 57, 43, 71, 85, 41, 55, 56, 70, 69, 84 | 25.51 | 1401.4 | | | | | 87784 | | | |
| 192 | Z-4-Dodecenol | 57, 43, 82, 55, 41, 69, 95, 56, 44, 96 | 25.75 | 1409.1 | | | | | | | | |
| 193 | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)- | 91, 150, 107, 135, 79, 77, 108, 105, 41, 109 | 25.92 | 1414.4 | | | 62316 | | | | | |
| 194 | 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R,S*)]- | 119, 93, 105, 91, 41, 69, 161, 77, 55, 92 | 25.95 | 1415.2 | | | | | | | | |
| 195 | Caryophyllene | 93, 91, 69, 41, 133, 79, 105, 77, 107, 67 | 26.04 | 1418.2 | | | 7349 | 4488 | 2760013 | 401180 | 302960 | |
| 196 | Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)- | 79, 108, 77, 43, 80, 41, 95, 82, 107, 91 | 26.17 | 1422.2 | 35037 | | 769244 | | | | | |
| 197 | 1-Butanol, 3-methyl-, benzoate | 105, 70, 77, 123, 55, 51, 41, 105, 42, 122 | 26.59 | 1435.8 | 173118 | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 198 | 5,9-Undecadien-2-one, 6-10-dimethyl-, (E)- | 43, 69, 41, 107, 151, 136, 67, 93, 125, 53 | 26.91 | 1445.9 | 266480 | 23886 | 444376 | | | | 11166 |
| 199 | α-Caryophyllene | 93, 80, 121, 91, 79, 41, 92, 147, 77, 107 | 27.21 | 1455.6 | | | | | 536757 | 7304 | |
| 200 | 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 177, 41, 220, 135, 67, 149, 57, 163, 91, 205 | 27.31 | 1458.7 | 9333 | 143416 | | 345452 | 127434 | 13108 | 9885 |
| 201 | N-Morpholinomethyl-isopropyl-sulfide | 100, 41, 56, 101, 42, 43, 70, 55, 98, 57 | 27.56 | 1466.8 | | | 51094 | 91445 | 15876 | | |
| 202 | Succinic acid, butyl isobutyl ester | 101, 57, 56, 41, 119, 55, 83, 43, 157, 73 | 27.81 | 1474.7 | | | | | | | |
| 203 | 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- | 161, 105, 91, 81, 119, 79, 41, 77, 93, 120 | 27.92 | 1478.4 | | | 130147 | | 218893 | | 387878 |
| 204 | Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methyethenyl)-,[1S-(1α,4α,7α)]- | 107, 93, 67, 79, 81, 105, 133, 91, 147, 95 | 27.96 | 1479.5 | | | | | | | 65021 |
| 205 | 1,3,6-10-Dodecatetraene 3,7,11-trimethyl-, (Z,E)- | 93, 119, 41, 69, 79, 91, 55, 107, 77, 105 | 28.23 | 1488.1 | 326591 | | 79991 | | | | 111032 |
| 206 | Hexadecane | 57, 43, 71, 85, 41, 55, 56, 70, 69, 42 | 28.63 | 1501.0 | | | 33616 | | 39181 | | |
| 207 | α-Farnesene | 93, 41, 69, 107, 55, 79, 119, 91, 123, 77 | 28.67 | 1502.5 | 10638877 | 7307662 | 152670 | | 26488 | 45476 | 10778780 |
| 208 | Benzoic acid, 1,2,3,4,5-pentamethylcyclopenta-2,4-dienyl ester | 134, 204, 105, 91, 41, 119, 43, 115, 81 | 29.13 | 1518.1 | | | | | 8431 | | |
| 209 | 1,6-Dioxacyclododecane-7,12-dione | 55, 54, 84, 100, 41, 129, 56, 71, 42, 111 | 29.80 | 1540.6 | | | | | | | |
| 210 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- | 69, 93, 41, 43, 107, 71, 55, 81, 67, 79 | 30.35 | 1559.1 | 420688 | | | | | | |
| 211 | Diethyltoluamide | 119, 91, 190, 105, 191, 82, 67, 77, 65 | 30.61 | 1567.8 | | | | | | | |
| 212 | 3-Hexen-1-ol, benzoate, (Z)- | 105, 82, 67, 77, 51, 106, 83, 78, 54, 123 | 30.64 | 1568.9 | 338827 | | | | 25895 | | |
| 213 | Furan, 3-(4,8-dimethyl-3,7-nondienyl)-, (E)- | 69, 81, 41, 79, 53, 67, 95, 94, 93, 55 | 30.72 | 1571.6 | 549479 | | | 9276 | | | 1182889 |
| 214 | Butanedioic acid, methyl-, bis(1-methylpropyl) ester | 115, 57, 41, 87, 42, 171, 114, 56, 43, 86 | 30.82 | 1575.0 | | | | | | | |
| 215 | 2(5H)-Furanone, 5-(bromomethyl)-5-pentyl- | 159, 105, 69, 77 | 30.92 | 1578.3 | | | | | | | 3024 |
| 216 | Diethyl Phthalate | 149, 177, 76, 150, 65, 105, 176, 93, 104, 50 | 31.08 | 1583.9 | | | | | 11784 | | |
| 217 | Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester | 71, 43, 41, 56, 111, 69, 72, 55, 159, 83 | 31.15 | 1586.2 | 21657 | 34879 | 19418 | | 70006 | | 83303 |
| 218 | Hexadecane | 57, 43, 71, 85, 41, 55, 56, 70, 69, 99 | 31.59 | 1601.2 | | | | | 13491 | | |
| 219 | 1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane | 95, 150, 151, 93, 81, 119, 69, 91, 79, 43 | 31.76 | 1607.1 | | | | | | | |
| 220 | 2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol | 233, 57, 247, 262, 234, 181, 217 | 32.22 | 1623.4 | | | | | | | |
| 221 | Decanoic acid, decyl ester | 173, 55, 99, 84, 56, 111, 41, 42, 112, 83 | 32.35 | 1628.2 | 5991607 | 10332540 | 7844398 | 17200643 | 8551907 | 2079108 | 6026936 |
| 222 | 6-Undecylamine | 100, 43, 41, 101, 56, 55, 42, 57, 70, 98 | 33.32 | 1662.5 | | | | | | | 310885 |
| 223 | 3-Cyclohexene-1-ethanol, α-ethenyl-α,3-dimethyl-6-(1-methylethylidene)- | 43, 81, 133, 91, 80, 41, 77, 69, 93, 55 | 33.47 | 1668.0 | 54246 | | | | | | |
| 224 | 1-(4-Hydroxy-3,5-di-tert.-butylphenyl)-2-methyl-3-morpholinopropan-1-one | 100, 57, 41, 233, 222, 207, 101, 43, 55, 56 | 33.66 | 1674.5 | 248611 | 1155927 | 690323 | 1579023 | 688982 | 105354 | 57549 |
| 225 | 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl) | 207, 222, 57, 41, 221, 165, 111, 223, 208, 137 | 33.70 | 1676.0 | | | | | | | |
| 226 | Adipic acid, isohexyl 2-methoxyethyl ester | 113, 187, 55, 111, 84, 114, 59, 129, 112, 143 | 33.74 | 1677.4 | | | | 17940 | | | |
| 227 | Caprolactam | 113, 55, 187, 111, 41, 84, 56, 42, 112, 43 | 34.02 | 1687.4 | | | | 20481 | | | |
| 228 | 1,3,2-Dioxaborinane, 2-ethyl-4-methyl- | 113, 55, 84, 112, 111, 56, 187, 41, 42, 100 | 34.22 | 1694.8 | | | | | | | |
| 229 | Octane, 2,4,6-trimethyl- | 57, 71, 43, 85, 41, 55, 56, 69, 99, 70 | 34.37 | 1700.0 | | | | | | | |
| 230 | 2-Ethylhexyl salicylate | 120, 138, 121, 57, 70, 41, 43, 71, 65, 55 | 36.86 | 1793.1 | | | | | | | 131573 |
| 231 | Butane, 2,2-dimethyl- | 43, 71, 55, 57, 41, 56, 85 | 37.05 | 0.0 | | | | | | | |
| 232 | Phthalic acid, bis(2-pentyl) ester | 149, 167, 71, 57, 41, 70, 150, 113, 105 | 37.30 | 1810.0 | | | | | | | |
| 233 | Isopropyl Myristate | 43, 60, 102, 41, 57, 55, 228, 73, 71, 229 | 37.65 | 1823.8 | | 7622 | | 31354 | | | |
| 234 | p-Toluic acid, 2-ethylhexyl ester | 119, 70, 112, 91, 137, 83 | 37.73 | 1826.8 | | | | | | | |
| 235 | 4,8,12-Tetradecatrienal, 5,9,13-trimethyl- | 69, 41, 81, 55, 93, 67, 136, 95, 91, 53 | 37.81 | 1830.2 | | | | | | | |
| 236 | 2-Heptanone, 5-methyl- | 43, 58, 95, 71, 83, 69, 70, 55, 109, 85 | 38.05 | 1839.3 | | | 18012 | | | | |
| 237 | Homosalate | 69, 109, 138, 124, 120, 83, 82, 55, 67, 65 | 38.26 | 1847.9 | | | | | | | 49655 |
| 238 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 149, 57, 150, 41, 104, 76, 223, 56, 65, 167 | 38.44 | 1854.7 | 172239 | 116594 | 56778 | 7483 | 175530 | 15022 | |
| 239 | Propanamide, N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)- | 259, 57, 274, 175, 218, 203, 245, 247, 217, 41 | 38.53 | 1858.4 | | | | | | | |
| 240 | N-Morpholinomethyl-isopropyl-sulfide | 100, 101, 41, 43, 56, 55, 42, 57, 70, 87 | 38.74 | 1866.4 | | | | | | | 1883281 |
| 241 | Homosalate | 138, 69, 109, 120, 121, 83, 124, 41, 55, 82 | 38.88 | 1872.0 | | | | | | | 854761 |
| 242 | Morpholine, 4-octadecyl- | 100, 101, 41, 43, 56, 55, 42, 57, 70, 44 | 39.12 | 1881.6 | 1413688 | 5268118 | 3059737 | 7923463 | 2961258 | 379691 | |
| 243 | 2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol | 233, 234, 247, 262, 119 | 39.22 | 1885.5 | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 244 | Benzenehexanitrile, β,β-dimethyl-ε-oxo- | 105, 58, 57, 55, 91, 106 | 39.74 | 1906.0 | | | | | | | |
| 245 | Pentadecanoic acid, 14-methyl-, methyl ester | 74, 87, 43, 55, 41, 75, 69, 143, 57, 83 | 40.16 | 1923.4 | | | | | | | |
| 246 | Dibutyl phthalate | 149, 150, 41, 76, 104, 223, 56, 57, 65, 205 | 40.78 | 1949.1 | 108891 | 20276 | | | 43967 | 4964 | |
| 247 | n-Hexadecanoic acid | 73, 60, 43, 56, 61, 41, 57, 55, 69, 71 | 40.94 | 1955.6 | | 11602 | 26698 | | | | |
| 248 | Morpholine, 4-octadecyl- | 100, 101, 43, 41, 55, 56, 42, 57, 70, 87 | 44.10 | 2085.5 | 253298 | 1439392 | 733058 | 1462838 | 462987 | | 185630 |
| 249 | 6-Undecylamine | 100, 101, 43, 41, 56, 55, 57, 42, 87, 69 | 50.95 | 2367.2 | | 12970 | | | | | |

**CURRICULUM VITAE**


# Laura Hansen

SUNY-ESF
1 Forestry Drive
Syracuse, New York

Entomology, Chemical Ecology, Biochemistry, Molecular Techniques and Chemical Analysis of VOCs

1-607-768-1944
Lehans01@syr.edu


## I. EDUCATION

- Entomology PhD expected in Spring 2020. SUNY-ESF.
  Dissertation Title: "*Host Olfactory Percepts in Two Polyphagous Sibling Species of Longhorned Beetles.*"

- Biochemistry BS. 2012. SUNY Geneseo.
  Research Project: "*Survey of Collembola in an Early Successional Forest in Western New York: Comparing Sampling Methods*."

## II. APPOINTMENTS AND WORK EXPERIENCE

- Graduate Teaching Assistant (SUNY-ESF) **Fall & Spring 2012-2016, Fall 2017**
  Genetics Laboratory, Biology Laboratory, Aquatic Entomology, Environmental & Forest Biology Orientation Seminar, Comparative Vertebrate Anatomy, and Physics of Life. Designed and presented course materials, supervised laboratory instruction, and supervised additional teaching assistants.

- Graduate Research Assistant (SUNY-ESF) **Summer 2013-2016, 2018-2019**
  Collaborative international field work in China on identification of semiochemicals for invasive and potentially invasive Longhorned beetles. Insect field trapping, handling, identification, and bioassays. VOC collection, GC-MS, GC-EAD, and GC-FID analysis.

- Office Assistant 2 (Keyboarding) (SUNY-ESF) **Spring 2017, 2018**
  Compiled information on prospective graduate students and facilitated the admission process according to department protocols and regulations. Performed additional clerical and secretarial duties.

## III. GRANTS

- East Asia and Pacific Summer Institute (NSF) **Summer 2017**
  Grant Title: "Host Olfactory Percepts in Two Polyphagous Sibling Species of Longhorned Beetles."
  Award Amount: $5400.
  National Science Foundation Grant. Participated in a jointly funded USA-Chinese governmental research institute. International field work and collaboration.

## IV. PUBLICATIONS

- **Hansen L**, Xu T, Wickham J, Chen Y, Hao D, Hanks LM, Millar JG, Teale SA. Identification of a male-produced pheromone component of the citrus longhorned beetle, *Anoplophora chinensis*. PloS one. 2015 Aug 4;10(8):e0134358.

- Zou Y, **Hansen L**, Xu T, Teale SA, Hao D, Millar JG. Optimizing pheromone-based lures for the invasive red-necked longhorn beetle, *Aromia bungii*. Journal of Pest Science. 2019 Jun 1;92(3):1217-25.

- Xu T, **Hansen L**, Teale SA. Female calling behaviour in the Asian longhorned beetle (Coleoptera: Cerambycidae). The Canadian Entomologist. 2019 Aug:1-8.

- Xu T, Yasui H, Teale SA, Fujiwara-Tsujii N, Wickham JD, Fukaya M, **Hansen L**, Kiriyama S, Hao D, Nakano A,

Zhang L. Identification of a male-produced sex-aggregation pheromone for a highly invasive cerambycid beetle, *Aromia bungii*. Scientific reports. 2017 Aug 4;7(1):7330.

- Sun L, Zhang YN, Qian JL, Kang K, Zhang XQ, Deng JD, Tang YP, Chen C, **Hansen L**, Xu T, Zhang QH. Identification and expression patterns of *Anoplophora chinensis* (Forster) chemosensory receptor genes from the antennal transcriptome. Frontiers in physiology. 2018 Feb 13;9:90.

## V. AFFILIATIONS

- International Society of Chemical Ecology (ISCE) Member
- Entomological Society of America (ESA) Member

## VI. AWARDS

- Gerald Lanier Memorial Award in Forest Entomology and Chemical Ecology (2015)

## VII. RELEVANT COURSEWORK

| Entomology | Advanced Chinese | Microscopes | Molecular Techniques |
|---|---|---|---|
| Arthropod Diversity | | Electron Microscopy | Cell Biology |
| Aquatic Entomology | Chemistry | Microscope Techniques | Genetics |
| Behavioral Assays | Biochemistry | | Genome Analysis |
| Forest Entomology | Chemistry | Statistics | Molecular Biology |
| Insect Physiology | Chromatography | Analysis of Variance | Molecular Techniques |
| Insect Chemical Ecology Methods | Natural Products | Multivariate Techniques | |
| | Physical Chemistry | Regression Techniques | |
| Plant-Herbivore Interactions | Spectroscopy | | |

## VIII. PRESENTATIONS

- 2012. 6th Annual GREAT (Geneseo Recognizing Excellence, Achievement & Talent) day. Laura Hansen and Jennifer Apple. Survey of Collembola in an Early Successional Forest in Western New York: Comparing Sampling Methods. (Poster)
- 2012. 12th Northeast Natural History Conference. Laura Hansen and Jennifer Apple. Survey of Collembola in an Early Successional Forest in Western New York: Comparing Sampling Methods. (Poster)
- 2014. Entomological Society of America's Entomology 2014. Laura Hansen, Tian Xu, Jacob Wickham, Sarah Pocock, and Stephen Teale. Discrimination of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) host and non-host tree species by antennally active volatiles. (Poster)
- 2015. 26th USDA Interagency Research Forum on Invasive Species. Laura Hansen, Tian Xu, Jacob Wickham, Sarah Pocok, and Stephen Teale. Discrimination of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) host and non-host tree species by antennally active volatiles. (Poster)
- 2019. International Society of Chemical Ecology 2019 Meeting. Laura Hansen, Dejun Hao, Tian Xu, and Stephen Teale. Host Volatile Percepts of *Anoplophora chinensis* and *Anoplophora glabripennis* (Citrus and Asian Longhorned Beetle) (Poster)

## IX. SELECTED SKILLS

Conversational Chinese
GC-MS, GC-FID, and GC- EAD Analysis of VOCs
Spectrometric Analysis
Insect Identification
SAS, Minitab, Statistica, and R Statistical Programs
ANOVA, Multivariate, and Regression Analysis
Insect Identification
PCR & Gel Electrophoresis
Cell Culture and Transformation
Insect Field Trapping
International Field Work and Collaboration