

**Visual, Acoustic, and Volatile Cues to Improve Performance of Trapping  
Ambrosia Beetles (Coleoptera: Curculionidae)**

by

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## Abstract

Three widely distributed species of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae), *Xylosandrus germanus* Blandford, *Xylosandrus crassiusculus* Motschulsky, and *Cnestus mutilatus* Blandford are introduced pests that cause damage to various tree cultivars by engraving tunnel networks and inoculating trees with their symbiotic fungi and secondary pathogens. Populations of these species have established in several regions of the United States. After a series of field experiments investigating the influence of visual and volatile cues and their capability to attract Scolytinae, it was demonstrated that trap captures can be influenced by the availability of specific nanometer wavelengths of light as well as chemical cue pairing. Different attractant cues were evaluated such as ratios of ethanol to methanol (0:100, 25:75, 50:50, 75:25, 100:0), conophthorin, and various LED color wavelengths: 395 nm (UV), 470 nm (blue), 525 nm (green), 625 nm (red). Visual cues and their role in orientation were also assessed in field experiments, showing the importance of peak diurnal flight activity occurring between 16:00 and 18:00 hours. Using *Cnestus mutilatus* as a model species, the acoustic sound produced by walking was characterized in laboratory experiments. These demonstrated that it is possible to isolate the unique signal derived from walking by an ambrosia beetle, which could be used for species detection in traps deployed in ornamental nurseries. This research suggests that *X. germanus*, *X. crassiusculus*, *C. mutilatus*, and other Scolytinae have species-specific attractant cue specifications and acoustic production, both of which can be used to further understand and manage these organisms. By integrating some of these concepts, it could lead to better trapping

efficiency to improve the timing of insecticide applications. An extensive analysis looking at the physiological mechanisms underlying attractant cue specifications and acoustic production could further our understanding of ambrosia beetles.

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## CHAPTER 1

### GENERAL INTRODUCTION

#### Biology of Ambrosia Beetles

There are 211 species of bark and ambrosia beetles (Coleoptera: Curculionidae) in the southeastern United States, 207 in the subfamily Scolytinae and 4 in the subfamily Platypodinae (Atkinson 2013). Of these beetles, 43 have been introduced from Europe, Asia, Africa, and the tropical Americas. The research primarily focuses on three main pest species, but occasionally encompasses the broader population present. The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), is an invasive species found throughout the United States that frequently causes damage in tree nurseries (Ree & Hunter 1995). *Xylosandrus germanus* (Blandford) is native to eastern Asia and is one of the most economically important exotic ambrosia beetles in U.S. nurseries (Oliver & Mannion, 2001). The camphor shot borer, *Cnestus mutilatus* (Blandford) is a recently introduced species of Asian origin which has unknown pest potential (Gandhi et al. 2009, Oliver et al. 2012). All three species were inadvertently introduced from Eastern Asia, with *C. mutilatus* being the most recent and was first detected in the United States within Oktibbeha County, Mississippi in 1999 (Schiefer & Bright 2004). The spread of these species was likely due to their broad host range, ability to efficiently find hosts (Ranger et al. 2013), and absence of natural enemies. Although the current range in the United States for

these species is vast, they continue to spread to new locations with *C. mutilatus* recently detected in Washington and Oregon in 2002 (LaBonte et al. 2005).

### *Morphological Characters*

Morphological characters are typically used in the separation of ambrosia beetle species. There are numerous characters utilized for this process and were essential to the identification of the prolific beetle diversity found within the two different trapping regions. The keys chosen included: the Screening Aid for Eastern United States Scolytinae (LaBonte et al. 2007), Bark Beetles of the Southeastern United States (Baker et al. 2009), and a guide from the USDA Horticultural Insects Research Laboratory in Wooster, OH (personal communication). The body length of the female *C. mutilatus* ranges from 2.6 – 3.9 mm and is typically black and shiny (Figure 1.1), while the female *X. crassiusculus* ranges from 2.1 – 2.9 mm with variable reddish black coloration (Figure 1.2), and the *X. germanus* female has an approximate body length of 2.3 mm and either all black or slightly reddish black appearance (Figure 1.3).



**Figure 1.1.** The body length of the female *C. mutilatus* ranges from 2.6 – 3.9 mm and is typically black and shiny (LaBonte et al. 2007).

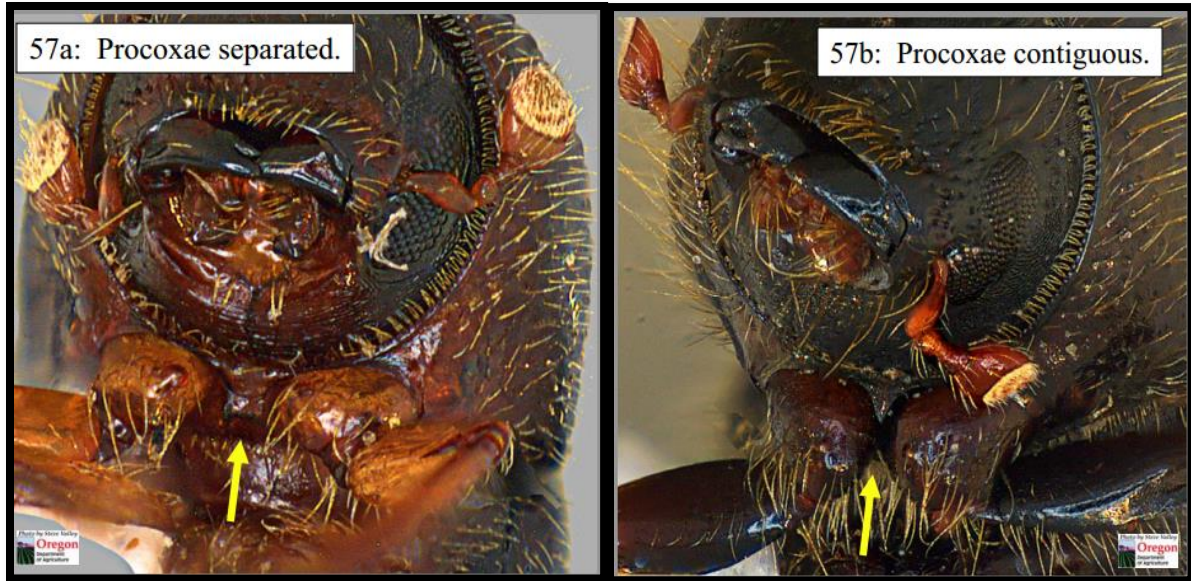
While the *Xylosandrus* spp. are considered stout, robust beetles, they are dwarfed by *Cnestus mutilatus* and its larger than average size. The pronotum length is another distinct characteristic for separating these two genera. The *Xylosandrus* spp. pronotum is always shorter than their elytra, having slightly less head protection, whereas the *C. mutilatus* specimen will always have a longer pronotum than elytra. Furthermore, the procoxae of all *Xylosandrus* spp. are distinctly separated with a small gap (Figure 1.4), while in *Cnestus mutilatus* the procoxae are contiguous (Figure 1.5).



**Figure 1.2.** The body length of the female *X. crassiusculus* ranges from 2.1 – 2.9 mm with variable reddish black coloration (LaBonte et al. 2007).



**Figure 1.3.** The body length of the female *X. germanus* has an approximate body length of 2.3 mm and either all black or slightly reddish black appearance (LaBonte et al. 2007).



**Figure 1.4.** The procoxae of all *Xylosandrus* spp. are distinctly separated with a small gap, whereas the procoxae of *Cnestus mutilatus* are contiguous, with no separation present (LaBonte et al. 2007).

Using these characteristics, identification of *Cnestus mutilatus* as being distinct from *Xylosandrus* spp. was achieved. The level of detail for separation of *X. germanus* from *X. crassiusculus*, as well as other potential beetle genera increases in difficulty while creating potential for error. Populations of *X. germanus* and *X. crassiusculus* varied greatly in both size and color. Samples from AL contained 60% *X. crassiusculus* and no *X. germanus*, however samples from OH contained 35% *X. germanus* and only 1% *X. crassiusculus*. When faced with the rare *X. crassiusculus* specimen, size and coloration were exemplary enough to make an accurate identification.

#### *Seasonal Activity*



In the United States, both *X. crassiusculus* and *X. germanus* have two to three generations per year and one to two in Europe or Japan (Weber & McPherson 1983, 1984, Oliver and Mannion. 2001). They seem to have a similar life cycle of 55-60 days depending on host availability and temperature, with the generalist adult female infesting a damaged or stressed host and overwintering inside of it as an adult (Atkinson 2007). *Cnestus mutilatus* is univoltine with emerging females making host selection flights once a summer (Schiefer & Bright 2004). Dispersal flights for each species of ambrosia beetle occur over an extended period of time, from as early as March to as late as September. Temperature plays an important role in the emergence of *X. germanus* and other ambrosia beetles from their overwintering galleries (Reding et al. 2013). Reding et al. (2012) found a strong relationship exists between maximum daily temperatures of 20°C and 21°C and *X. germanus* attack and flight activity, with no attacks occurring without at least 1 day of 20°C. Temperature plays an important role in the emergence of *X. germanus* and other ambrosia beetles from their overwintering galleries (Reding et al. 2013). Knowledge of *Xylosandrus* spp. activity in spring is important for nursery growers to effectively time their protective sprays (Reding et al. 2013). Regional variability occurs for the flight period, with the southern United States generally beginning earlier and ending later (Weber & McPherson 1983, Schiefer & Bright 2004.).

### *Host selection*

The phases most bark and ambrosia beetles experience during host selection includes: dispersal, selection, concentration, and establishment. Dispersal begins with emergence from the tree and ends with the response to host cues, selection acts upon the stimuli and ends with

feeding on the host phloem, concentration is the release of sequestered host pheromones and volatiles by the invading beetle, and establishment is onset of their fungal inoculum and oviposition (Wood 1982).

As the winter or hibernation period comes to an end, newly emerging bark and ambrosia beetles are tasked with locating a suitable host from the few stressed trees scattered throughout the forest. The host tree must be an ideal species and in most cases the insects seek stressed, less resistant trees, or trees that are in the beginning stages of decline. It is expected that species have evolved behavioral responses to specific volatile host-plant chemicals that indicate the presence of a specific host as being inhabitable. The decision by the insect whether to accept the plant is dependent on external (olfaction, vision, mechanoreception, and gustation) stimulatory and inhibitory inputs balanced against internal excitatory and inhibitory inputs (Miller & Strickler 1984). As ambrosia beetles continue flight using up energy reserves in the form of lipids (Thompson & Bennet 1971), they become increasingly desperate to find a host, and will ultimately accept the host based on an inverse correlation of host suitability and fatigue level of the beetle (Miller & Strickler 1984).

The importance of host volatiles during host location by insects was initially questioned, but numerous studies have since confirmed their importance (Bruce et al. 2005). Visser (1986) proposed that the recognition of a host plant by olfactory signals could occur by using either (1) species-specific compounds or (2) specific ratios of general odor compounds. Bruce et al. (2005) states most studies favor the second scenario, with strong evidence that plant discrimination is due to central processing of olfactory signals by the insect, rather than their initial detection.

*Life cycle*

Inside the host ambrosia beetles will excavate a series of tunnels, introducing its symbiotic fungi harbored within a specialized organ known as the mycangium (Hulcr et al. 2012). Eggs, larvae, and pupae are all found together in the tunnels, and all feed exclusively on their ectosymbiotic fungi. Ambrosia beetle galleries harbor all life stages of the insect. The resulting female to male ratio is typically female dominated, being 10:1 within *X. germanus* colonies (Weber & McPherson 1983). Male ambrosia beetles usually have short life duration, are flightless, and fulfill the role within the brood chamber of mating with its siblings (Weber & McPherson 1983). Male *C. mutilatus* illustrates the dramatic sexual dimorphism present between the two sexes, being 2.4 mm long, less robust, and a brown coloration (Figure 1.6).



**Figure 1.5.** Illustrates the dramatic sexual dimorphism in *Cnestus mutilatus*.

Males (left photo) is 2.4 mm in length, less robust, and has a brown coloration.

## Damage by Ambrosia beetles to Trees in Ornamental Trees

Ornamental tree nurseries face numerous pest issues due to their wide variety of cultivars grown on site, in rows, and in variable states of development while frequently being stressed by abiotic factors. Damage estimates vary each year based on species and regional differences. For example *X. crassiusculus* can cause losses in excess of US \$5,000 per nursery for nurseries across the southeastern United States and in Texas (Ree & Hunter 1995). The damage potential of this pest is further amplified by *X. crassiusculus* having in excess of 120 potential tree host species, ranging from hardwoods to pine in both the nursery setting and forest (Hudson & Mizell 1999, Oliver & Mannion 2001). *Xylosandrus germanus* has a world-wide host range >200 species (Weber and McPherson 1983b) with a preference for deciduous trees and is a serious pest in ornamental nurseries (Reding et al. 2010). *Cnestus mutilatus* has been reported as a pest on avocado trees (*Persea americana*), but has unknown pest potential within the United States (Schiefer & Bright 2004). Symptoms of infestation include frass toothpicks sticking out from the trunk from the tunnel entrances, sap oozing, wilting foliage, branch dieback, and profuse basal sprouts. The estimated total economic impact of fruit and tree nuts alone in Alabama was \$11.7 million in 2012 (USDA Quickstats, <http://quickstats.nass.usda.gov/>), making up only a small percentage of the nursery stock grown.

### *Trapping Methods*

Current methods for monitoring ambrosia beetle seasonal dispersal flight patterns involves traps baited with ethanol (Oliver and Mannion 2001, Reding et al. 2010, 2011). The Baker bottle trap and the Lindgren funnel trap are the most commonly used traps for field sampling of ambrosia beetles (Lindgren 1983, Oliver & Mannion 2001). Traps are generally

most effective when placed low to the ground. Reding et al. (2010) tested the effect of varying trap heights on ambrosia beetle capture and found that 0.5 m caught more *X. crassiusculus* and *X. germanus* than traps positioned at 1.7 m and 3.0 m traps. This suggests that *X. germanus* and *X. crassiusculus* fly low to the ground while attempting to locate a host. Ethanol-baited bottle and Lindgren funnel traps mainly attract ambrosia beetles, but also non-target arthropods. Thus, trap collections regular maintenance and ambrosia beetles must be separated from other arthropods and debris for identification purposes.

### *Volatile Attractant Cues*

Abiotic and biotic stressors can induce the emission of stress-related volatiles from trees, including acetaldehyde, acetone, ethane, ethanol, ethylene, and methanol (Kimmerer and Kozlowski, 1982; Kimmerer and MacDonald, 1987; MacDonald and Kimmerer, 1991; Holzinger et al., 2000). Several of these volatile compounds act as bio-indicators of vulnerable trees to secondary ambrosia beetles and other wood-boring insects (Kelsey and Joseph, 2001; Ranger et al., 2013). In particular, ethanol plays a role in attracting ambrosia beetles to attack specific trees (Ranger et al., 2010, 2012, 2013; Kelsey et al., 2013).

Ethanol is the most attractive individual compound currently known for *X. crassiusculus* and *X. germanus* (Ranger et al. 2010, 2011). Some Scolytinae have demonstrated increased attraction through synergistic semiochemical baiting, as demonstrated with the coffee berry borer *Hypothenemus hampei* (Ferrari) (Da Silva et al. 2006). The synergy of ethanol and methanol resulted in a minimum increase of 2.7 times the capture rate compared to an ethanol-baited trap (Da Silva et al. 2006). Montgomery & Wargo (1983) did not find mixtures of ethanol with methanol, acetaldehyde, and/or turpentine were more attractive than ethanol alone to various

Scolytinae. Yet, recent studies demonstrated conophthorin, a volatile associated with hardwoods, synergistically enhanced the attractiveness of *X. crassiusculus* (Van DerLaan and Ginzel 2013) and *X. germanus* (Ranger, pers. comm.) to ethanol-baited traps.

Release rate of volatile attractants, particularly ethanol, is a critical factor to consider when trapping ambrosia beetles. Klimetzek et al. (1986) first demonstrated a positive concentration response to ethanol for *X. germanus* and other ambrosia beetles. Additional studies have demonstrated ethanol release rate plays an important role in attracting ambrosia beetles (Oliver et al. 2004, Ranger et al. 2011, 2012, Reding et al. 2011). Oliver et al. (2004) and Ranger et al. (2011) found that traps with the highest ethanol release rates were more effective overall for attracting ambrosia beetles than traps baited with low release rate lures. A positive correlation has also been demonstrated between concentration of ethanol injected into a tree and the corresponding ambrosia beetle attacks (Ranger et al. 2012). However, too high of a release rate can have a repellent effect on ambrosia beetle attraction. Montgomery & Wargo (1983) found ethanol released at 2 g/day attracted more Scolytinae than higher release rates. This indicates that emission rate of an attractant is an important consideration when trapping for specific species.

### *Visual Attractant Cues*

Visual cues play an important role during orientation by species within the Scolytinae. For instance, Da Silva et al. (2006) found green plastic bottle traps increased captures of *H. hampei* and other Scolytinae species compared to clear traps or transparent red traps. Similarly, Strom et al. (1999) demonstrated that both the southern pine beetle *Dendroctonus frontalis* (Zimmerman) and *Thanasimus dubius* (Fabricius) are affected by altering visual silhouettes on traps, finding that white paint and white trap paneling caught fewer southern pine beetles than

black or transparent coloration. This work on visual cues in the Scolytinae has been expanded upon as part of the current thesis research, further detailing the underlying mechanisms and quantifying their importance.

## **Bioacoustics**

The wing beat frequency of an insect species is a typical acoustic behavioral factor investigated for the use of sensor production. As seen in previous research (Cator et al. 2011), this principle is demonstrated with the mosquito species *Aedes aegypti* (Linnaeus) described using a specially designed cone that registers the appropriated wing beat frequency via acoustic sensor while in flight. However, not all insects are suited to being classified through wing beat frequency, but rather a distinctive behavior intrinsic to their lifestyle. The behavior termed “stridulation” is common within the Orthoptera, and is significantly accessible for recording purposes as opposed to wing beat. Using the method of stridulation, crickets (Orthoptera: Gryllidae) generate different song patterns for acoustic communication (Hedwig 2000). This unique acoustical behavior is an ideal candidate for identifying a unique frequency and decibel recording, and can later be used as a potential species signature.

## *Detection Systems*

Many systems have demonstrated the capability to detect specific insects based on their acoustic sound production. Mankin et al. (1997) utilized the accurate sensitivity of 16 piezoelectric sensors to detect the differing rates of intensities and sound production of adult insects in stored wheat. These small and delicate sensors suit the needs of research performed on small subterranean insects, stored grain pests, and many other economically important

arthropods. Prior to recent material developments allowing for a more flexible sensor design, detection of insect infestation in agricultural commodities was provided by piezoelectric means which generates electricity when stimulated by a frequency above 500 hertz (Litzkow et al. 1990). This acoustic approach allows for another cue to be incorporated into a trap for early detection and perhaps even species specificity.

### **Current strategy for managing ambrosia beetles in ornamental nurseries**

Ethanol-baited traps are typically deployed in ornamental nurseries for monitoring ambrosia beetle flight activity. Upon capture of a suspected ambrosia beetle pest, preventative applications of conventional insecticides are made to prevent attacks. Increasing the attractiveness of trees to ambrosia beetles using ethanol injections (Ranger et al. 2010, 2011) has allowed for screening the efficacy of conventional (Frank and Sadof 2011, Reding et al. 2013) and botanically based insecticides (Ranger et al. 2011).

Permethrin-based insecticides have been found to be most effective in minimizing attacks (Reding et al. 2013). However, in order to apply insecticide treatments to nursery trees, most growers use an inefficient airblast sprayer that forcefully releases insecticide mist that billows through nursery beds (Frank & Sadof 2011). While manual applications require 12 times more labor than airblast applications, the benefit of not spraying tree canopies resulted in 50% more natural enemies and 50% fewer spider mites present in nursery beds (Frank & Sadof 2011). This tradeoff exemplifies the benefit/cost of implementing a more precise treatment application system, which could lead to an economically feasible strategy for integrated pest management of ambrosia beetles.



Botanically based products, an alternative to conventional insecticides, could potentially supplement a grower's treatment options for managing ambrosia beetles. Rather than using highly toxic conventional insecticides to kill ambrosia beetles, disrupting their host selection behavior with repellent or deterrent botanically based compounds, or both, has the potential to be a useful management tool (Ranger et al. 2011). Furthermore, identification and implementation of repellents could be used as another tool and possible alternative to conventional treatments. Verbenone, a bark beetle anti-aggregation pheromone, reduces ambrosia beetle attacks on individual trees or on a small spatial scale, but deployment of verbenone might be most effective when integrated as part of a “push-pull” strategy (Ranger et al. 2013).

Implementation of monitoring tactics and preventative applications of conventional insecticides are useful for minimizing ambrosia beetle attacks. However, most pest ambrosia beetles, including *X. germanus* and *X. crassiusculus*, preferentially land on and attack physiologically-stressed hosts (Ranger et al. 2013). Therefore, abiotic and biotic stressors that induce the initial attack should be taken into consideration. In essence, minimizing the impact of stressors known to induce the production of ethanol and predispose trees to attack should be the primary foundation of a management plan (Ranger 2013).

## **Objectives**

The ambrosia beetle presence in the southeastern United States is a growing issue with the newly established species *Cnestus mutilatus*. Although ambrosia beetles have been a problem in this region for some time, there are limited methods developed for effective trapping and control. The long-term goal of this project is to evaluate an effective trapping system that will provide insight into ambrosia beetle attractant cues and flight patterns, which will lead to a more

effective first response control effort. This will allow growers the ability to pinpoint their pest management strategies to a first response leading to better timing of preventative spraying. In the project, there will be three supported objectives to accomplish the long-term goal.

1. Identify the visual and volatile cues involved with attracting key ambrosia beetles, specifically methanol, conophthorin, and LEDs.
2. Find an effective method of detecting ambrosia beetles in the trap, thereby allowing for effective first response pest management plans.
3. Develop a working trap model that will be effective in sampling nurseries for pest ambrosia beetles that are simple and inexpensive for widespread use.

For the three objectives, there are three separate hypotheses. 1. Integrated volatile and visual cues should increase trap capture and potentially specificity of ambrosia beetles. 2. We hypothesize that through the use of acoustic detection, a more specific cue could be developed for use in traps. 3. Early detection trapping will lower the damage cost created by populations of ambrosia beetles on target host tree sites. The results will be used to further understand the complex interactions of ambrosia beetles with their hosts. By gaining insight into these fundamental mechanisms, an improved monitoring and management strategy can be implemented, thereby reducing environmental damages and financial costs.

## CHAPTER 2

### CAPTURE OF *XYLOSANDRUS CRASSIUSCULUS* AND OTHER SCOLYTINAE (COLEOPTERA: CURCULIONIDAE) IN RESPONSE TO VISUAL AND VOLATILE CUES

#### Abstract

In Jun and Jul 2011, traps were deployed in the Tuskegee National Forest, Macon County, Alabama to test the influence of chemical and visual cues on the capture of bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). The first experiment investigated the attractiveness of traps baited with different ratios of ethanol to methanol (0:100, 25:75, 50:50, 75:25, 100:0). No differences occurred in total Scolytinae trap captures for any of the various ratios of ethanol to methanol. The second experiment tested the attractiveness of ethanol-baited traps supplemented with light emitting diodes (LED) of various wavelengths to Scolytinae. Ethanol-baited traps supplemented with UV (395 nm) and green (525 nm) LEDs were more attractive than traps baited only with ethanol, but they were not more attractive than ethanol-baited traps supplemented with blue (470 nm) and red (625 nm) LEDs. These results suggest that ethanol-baited traps supplemented with UV or green LEDs would be more effective than traps baited with just ethanol for detecting various Scolytinae.

#### Introduction

The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae), is an ambrosia beetle of Asian origin that was first detected in the

continental United States near Charleston, South Carolina (Anderson 1974). While *X. crassiusculus* is considered only a nuisance pest in some areas, this insect has caused significant losses (in excess of US\$ 5,000 per nursery) in several nurseries across the southeastern United States and in Texas (Ree & Hunter 1995). The damage potential of this pest is further amplified by *X. crassiusculus* having in excess of 120 potential tree host species, ranging from hardwoods to pine in both the nursery setting and forest (Weber & McPherson 1983, Solomon 1995, Hudson & Mizell 1999, Oliver & Mannion 2001). Scolytinae pests also include bark beetles, which are the most significant pest of coniferous forests, but can also attack deciduous trees.

Adult ambrosia beetles cause mechanical damage to trees as they tunnel into the heartwood of their hosts. Spores of a symbiotic fungus, which serves as a source of nutrition for the larvae and adult ambrosia beetles, are found within a pouch-like structure called a mycangium. As ambrosia beetles tunnel, the tunnel walls are coated with spores of the symbiotic fungus giving rise to colonial proliferation (Stone et al. 2007). The symbiotic fungus can block parenchyma cells within the vascular tissue of the trees, but Dute et al. (2002) found ambrosia beetle fungi (*Ambrosiella* spp.) were not particularly pathogenic. However, secondary pathogens such as *Fusarium* spp. can also be introduced to the host during the process of tunneling, or due to the open wounds created in trees. Secondary pathogens are suspected of playing a role in the eventual dieback and the death of infested hosts (Reding et al. 2010).

The physiological condition of a potential host tree plays an important role in the selection behavior of *X. crassiusculus* and other ambrosia beetles (Ranger et al. 2012a). When a tree is stressed, it can produce and emit acetaldehyde, acetic acid, acetone, ethane, ethanol, ethylene, and/or methanol (Kimmerer & Kozlowski 1982, Kimmerer & MacDonald 1987, Holzinger et al. 2000, Ranger et al. 2012a). Water damage, improper planting, drought stress,

pollutants, low temperature, impaired root function, and diseases are some of the factors that can lead to the emission of ethanol and other stress-related volatile cues (Kelsey 2001, Ranger et al. 2012). This gaseous ethanol is important for attracting ambrosia beetles to invade host trees (Ranger et al. 2010, Ranger et al. 2012a,b). Ethanol is an attractant for a number of ambrosia beetles (Ranger et al. 2010, 2011, 2012b). Mixtures of ethanol and methanol have also increased trap captures of the bark beetle *Hypothenemus hampei* (Ferrari) compared to ethanol alone (Da Silva et al. 2006), indicating there is potential for interactions among volatile compounds.

In addition to olfactory cues, visual cues can also play a role during host selection by the Scolytinae. For instance, Da Silva et al. (2006) found green plastic bottle traps increased captures of *H. hampei* and other Scolytinae species compared to clear traps or transparent red traps. Similarly, Strom et al. (1999) demonstrated that both the southern pine beetle *Dendroctonus frontalis* (Zimmerman) and *Thanasimus dubius* (Fabricius) are affected by altering visual silhouettes on traps, finding that white paint and white trap paneling caught significantly fewer southern pine beetles than black or transparent coloration. In order to improve the detection and monitoring of various bark and ambrosia beetles, the objective of this research was to examine olfactory and visual cues for their ability to enhance the attractiveness of ethanol-baited traps. We hypothesize integrated volatile and visual cues should increase trap capture and potentially specificity of ambrosia beetles. Current management of ambrosia beetles in nurseries relies primarily on preventive treatments of insecticides applied to the trunks of trees, which can be improved with a greater knowledge of their seasonal activity and ecological interactions (Reding et al. 2010, Hudson & Mizell 1999).

## **Materials and Methods**

### *Experiment 1: Influence of Olfactory Cues*

The ability of methanol to enhance the attractiveness of ethanol to various Scolytinae was investigated using Baker traps (Bambara et al. 2002, Oliver et al. 2004) (Figure 2.1). Traps consisted of 2 L transparent bottles cut with three windows above the bottom and hung from a shepherd's hook, resulting in the traps being  $\approx 1.2$  m above the ground. A mixture of water and liquid dish detergent (100:1; v:v) was added to the bottom of the container to subdue and kill the entering beetles. An 8 dram (29.5 ml) clear glass vial with a cotton wick ( $8 \times 2$  cm, Cotton<sup>®</sup> American Fiber and Finishing Inc., St. Albemarie, NC) containing the volatile attractants (28 mL) held in place with parafilm was hung inside each individual bottle trap. Release rates for the volatiles were held constant at 3.8 g/day across all treatments by filling the uniform bottle (diameter 25 mm) completely with a cotton wick.



**Figure 2.1.** A Baker bottle trap design was used for testing the attractiveness of varying ratios of ethanol and methanol to ambrosia beetles.

Trap treatments included the following ratios of 95% ethanol to 99.8% methanol (EMD chemicals Inc. Darmstadt, Germany) (v:v): 0:100, 25:75, 50:50, 75:25, 100:0. Attractant test solutions were replenished every 3 days over the course of the experiment.

Traps were deployed in a randomized complete block design in the Tuskegee National Forest, Macon County, Alabama (N 32° 29' 19" W 85° 35' 39") from 12 Jun to 6 Jul 2011. There were seven replicates per treatment, and collections were made every 3 days. Traps within each block were 6 m apart and were located in separate parts of Tuskegee National Forest. The trap sites were densely populated with a mixture of pine and hardwood trees and were partially shaded. Traps were returned to the laboratory for specimen identification. *Xylosandrus crassiusculus* was identified to species, but the remaining specimens were grouped as total Scolytinae.

### *Experiment 2: Influence of Visual Cues*

A second trapping experiment was conducted to examine the attractiveness of different wavelengths to *X. crassiusculus* and other Scolytinae. Embedded into the top of the aforementioned traps and spaced equally along the top rim of the bottle were four 1.5 watt light-emitting diode (LED) bulbs (Boesch Built LLC, Waterford Township, MI) powered by a 6 volt battery (McMaster-Carr<sup>®</sup> Elmhurst, IL) that was fastened directly underneath the traps (Figure 2.2). The LED color wavelengths included 395 nm (UV), 470 nm (blue), 525 nm (green), 625 nm (red), and a blank control (Figure 2.3). The LED lights were powered continuously throughout the entire duration of the experiment. Each trap was also baited with the



aforementioned 95% ethanol lure. Traps were arranged in randomized complete blocks in Tuskegee National Forest. The field test was conducted from 12 Jun to 6 Jul 2011. There were 7 replicates per treatment, and collections were made every 3 days.



**Figure 2.2.** An improved bottle trap for testing the attractiveness of volatile cues and various light emitting diode (LED) wavelengths, including 395 nm (UV), 470 nm (blue), 525 nm (green),





**Figure 2.3.** Light emitting diodes (LED) (1.5 watts) were attached to traps to test the attractiveness of different wavelengths, including 395 nm (UV), 470 nm (blue), 525 nm (green), and 625 nm (red) (Boesch Built LLC, Waterford Township, MI).

### *Statistics*

A one-way analysis of variance (ANOVA) was performed on  $\log_{10}(x+1)$  transformed data to compare the attractiveness of varying ratios of ethanol to methanol on Scolytinae trap captures ( $\alpha = 0.05$ ; PROC GLM; SAS Institute 2003). Differences between transformed means were separated using Tukey's honestly significant difference (HSD) test ( $P < 0.05$ ). A one-way ANOVA ( $\alpha = 0.05$ ; PROC GLM; SAS Institute 2003) on  $\log_{10}(x+1)$  transformed data and Tukey's HSD test ( $P < 0.05$ ) was also used to compare Scolytinae trap captures associated with the various LED colors.

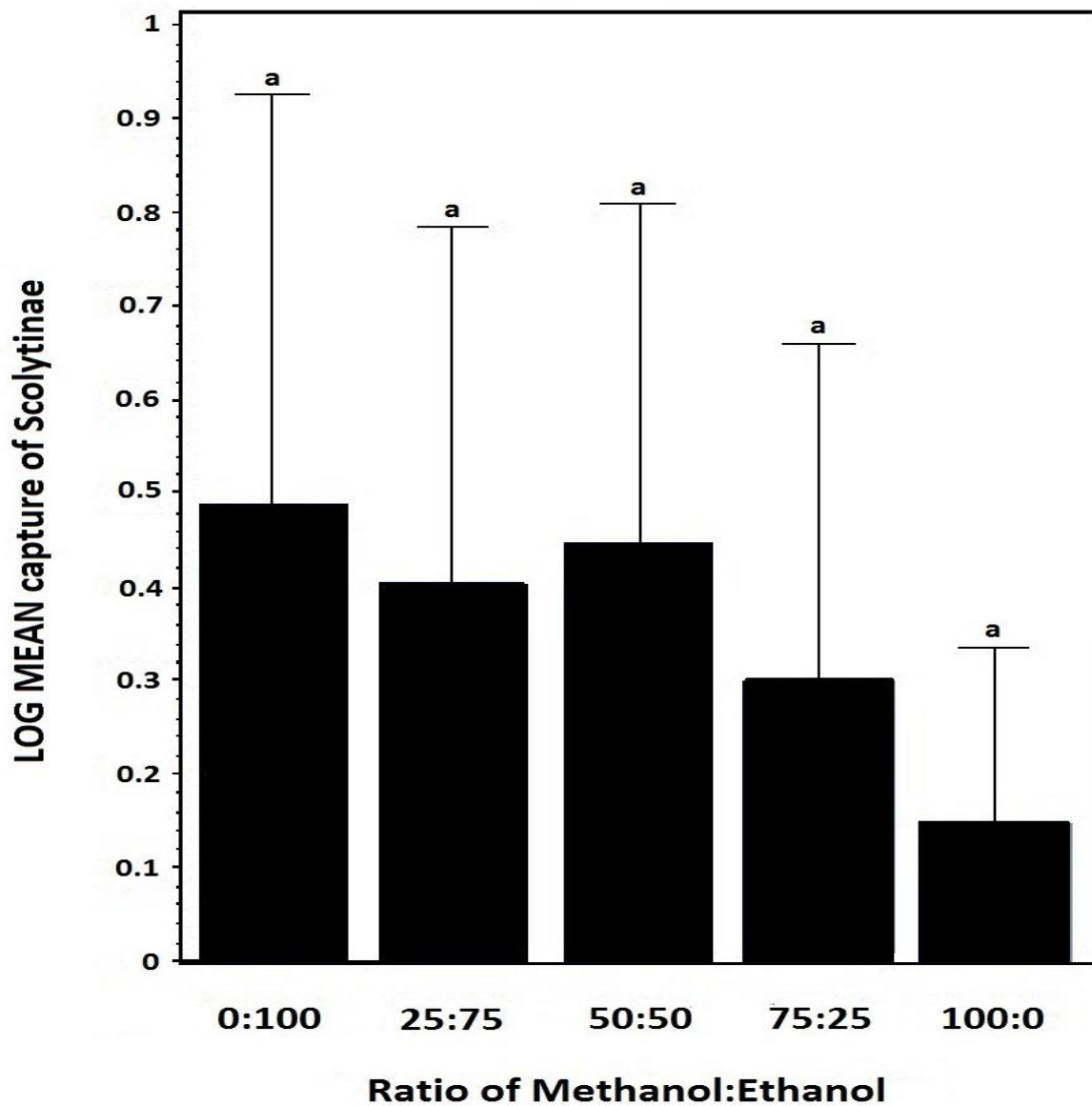
## **Results**

### *Experiment 1: Influence of Olfactory Cues*

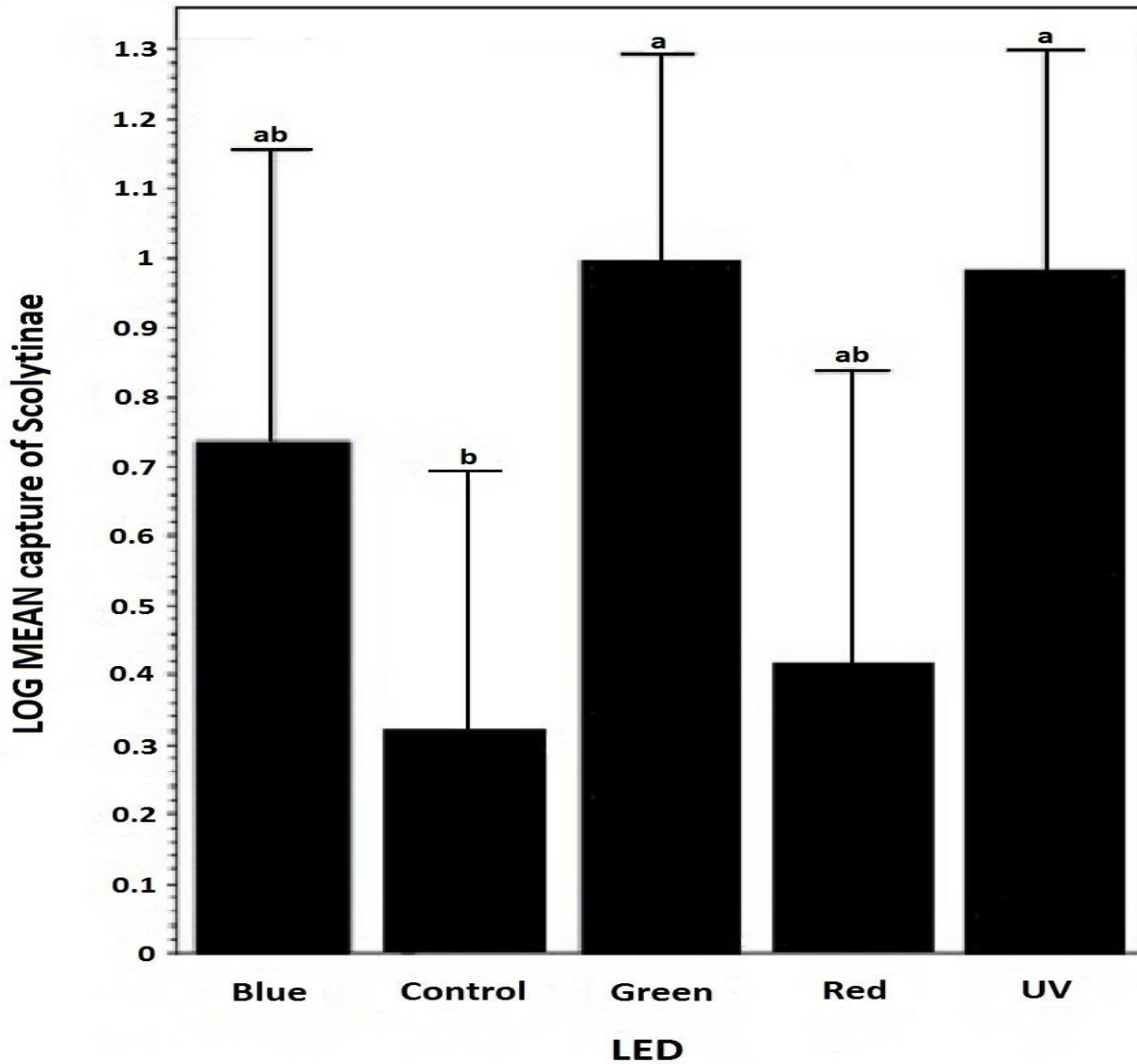
No significant difference was detected in captures of all Scolytinae among traps baited with varying ratios of ethanol to methanol (100:0; 75:25; 50:50; 25:75; 0:100) ( $F_{4,30} = 0.99$ ,  $P = 0.43$ ) (Figure. 2.4). No significant differences in mean ( $\pm$  SE) captures of *X. crassiusculus* were detected among traps baited with ethanol alone ( $0.57 \pm 0.30$ ), methanol alone ( $1.0 \pm 0.38$ ), or varying ratios of ethanol to methanol at 75:25 ( $1.0 \pm 0.53$ ), 50:50 ( $1.57 \pm 0.53$ ), 25:75 ( $0.86 \pm 0.59$ ) ( $F_{4,30} = 0.54$ ,  $P = 0.71$ ).

### *Experiment 2: Influence of Visual Cues*

Ethanol-baited traps supplemented with green (525 nm) and UV (395 nm) LED lights captured significantly more Scolytinae than traps baited with ethanol alone ( $F_{4,30} = 4.34$ ,  $P = 0.007$ ) (Figure. 2.5). However, no significant difference was detected in total *X. crassiusculus* trap captures among ethanol-baited traps supplemented with UV (395 nm) ( $0.71 \pm 0.29$ ), blue (470 nm) ( $0.57 \pm 0.43$ ), green (525 nm) ( $1.0 \pm 0.44$ ), and red (625 nm) ( $0.14 \pm 0.14$ ) LEDs ( $F_{4,30} = 0.99$ ,  $P = 0.43$ ).



**Figure. 2.4.** LOG transformed number (mean  $\pm$  SE) of Scolytinae adults collected with bottle traps baited with various ratios of ethanol to methanol (0:100, 25:75, 50:50, 75:25, 100:0) deployed from Jun to Jul 2011 in Tuskegee National Forest, AL, USA. Means with different letters indicate significant differences (one-way ANOVA;  $P < 0.05$ ).



**Figure. 2.5.** LOG transformed number (mean  $\pm$  SE) of Scolytinae adults collected with ethanol-baited traps supplemented with various LED lights during Jun to Jul 2011 in Tuskegee National

Forest, Alabama, USA. Means with different letters indicate significant differences (one-way ANOVA;  $P < 0.05$ ).

## **Discussion**

Methanol alone was just as attractive as ethanol alone (Figure 1), in contrast to findings from other studies involving Scolytinae. Montgomery & Wargo (1983) did not find a mixture of 50% ethanol, 5% methanol, and 5% acetaldehyde to be more attractive than ethanol alone to various Scolytinae. Similarly, Ranger et al. (2010) found methanol-baited traps were more attractive to *Xylosandrus germanus* (Blandford) than acetaldehyde- and acetone-baited traps, but less attractive than ethanol-baited traps. Injecting sweetbay magnolia, *Magnolia virginiana* L. (Magnoliales: Magnoliaceae), with methanol did not induce more pest ambrosia beetle attacks than a non-injected control, but over 120 attacks per plant were induced following ethanol-injection (Ranger et al. 2010). The lack of significant differences among any of the treatments involving various ratios of ethanol to methanol could be due to low trap counts from deploying traps after peak flight, which typically takes place in Mar to Apr in Macon County, Alabama (Gorzlancyk, pers. obs.) The relatively high release rate of ethanol and methanol from our lure apparatus may also have resulted in low trap counts. A positive concentration response to ethanol has been demonstrated for a few ambrosia beetles (Klimetzek et al. 1986; Ranger et al. 2011, 2012b), but too high of a release rate could have a repellent effect on ambrosia beetle orientation. Montgomery & Wargo (1983) found ethanol released at 2 g/day attracted more Scolytinae than higher release rates. Thus, a mixture of ethanol and methanol cannot be recommended as an alternative or replacement of ethanol alone as an attractant for *X. crassiusculus* and other Scolytinae.

Ultraviolet and household floodlights are currently used in general insect traps (Syms & Goodman 1987, Ferreira et al. 2012). Results from our current study demonstrated Scolytinae showed a higher attraction to ethanol-baited traps supplemented with green (525 nm) and UV (395 nm) LED lights compared to ethanol-baited traps alone. Attraction to green wavelengths has also been noted to occur in other members of the Curculionidae. For instance, Nakamoto & Kuba (2004) found evidence that the weevil *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) preferred green LEDs to the UV light traps.

Our results suggest ethanol-baited traps supplemented with green or UV LEDs would be useful for detecting various Scolytinae in a nursery or forest setting. The integration of specific LED wavelengths into other trap configurations could potentially yield species specific responses. A more detailed examination is therefore warranted to elucidate the physiological mechanisms underlying the role of visual cues in behavioral responses by *X. crassiusculus* and other Scolytinae.

## CHAPTER 3

### CAPTURE OF *CNESTUS MUTILATUS* (BLANDFORD), *XYLOSANDRUS CRASSIUSCULUS* (MOTSCHULSKY), AND OTHER SCOLYTINAE (COLEOPTERA, CURCULIONIDAE) IN RESPONSE TO GREEN LIGHT EMITTING DIODES, ETHANOL, AND CONOPHTHORIN

#### Abstract

Traps were deployed to determine the attractiveness of three different treatments to bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae), namely, traps baited with ethanol alone, ethanol plus conophthorin, and ethanol, conophthorin, and green LEDs. Ethanol-baited traps integrated with conophthorin and green LEDs (525 nm) were more attractive to *Xylosandrus crassiusculus* and other Scolytinae than traps baited with ethanol alone. In contrast, *Cnestus mutilatus* capture was not affected by the addition of conophthorin, or conophthorin and green LEDs to ethanol-baited traps. This study indicates that ethanol-baited traps supplemented with conophthorin and green LEDs can be useful for improving the attractiveness of ethanol-baited traps to *X. crassiusculus* and other Scolytinae.

#### Introduction

The camphor shot borer, *Cnestus mutilatus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), is an ambrosia beetle of Asian origin (Schiefer & Bright 2004). It was first detected in the United States within Oktibbeha County, Mississippi in 1999 (Schiefer & Bright 2004). In Asia, *C. mutilatus* is known to attack many hardwood species, including *Acer*, *Albizia*,

*Carpinus*, *Castanea*, *Cornus*, *Fagus*, *Lindera*, *Osmanthus*, and *Swietenia* spp. (Wood & Bright 1992, Schiefer & Bright 2004). In the United States, *C. mutilatus* has undetermined pest potential, but has been found in Alabama, Arkansas, Florida, Georgia, Louisiana, North Carolina, Ohio, Tennessee, Texas, and West Virginia (Gandhi et al. 2009, Oliver et al. 2012). The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae), was first detected in the United States near Charleston, South Carolina (Anderson 1974). Currently *X. crassiusculus* is found within the northeastern, mid-Atlantic, southeastern, southern, midwestern, and northwestern United States (Ree & Hunter 1995). It can attack more than 120 hosts, ranging from hardwood to pine (Hudson & Mizell 1999, Oliver & Mannion 2001).

Olfaction plays an important role during host-location by ambrosia beetles. Due to its emission from living, but weakened trees, ethanol acts as an important attractant for *X. crassiusculus* and a number of other ambrosia beetles (Oliver & Mannion 2001, Ranger et al. 2010, 2012, 2013, Kelsey et al. 2013). Ethanol-baited traps are commonly used for detecting and monitoring ambrosia beetles, and efforts are underway to identify compounds that enhance trap attractiveness. One promising compound, conophthorin, is associated with the bark of a variety of angiosperm trees and is also produced by several Scolytinae (Huber et al. 2001). Conophthorin disrupts the response of several bark beetles (Huber et al. 2001), but recent studies by Dodds & Miller (2010) found it increased the attractiveness of trap trees to *Xylosandrus germanus* (Blandford).

In addition to olfactory cues, visual cues also play a role in host location by various Scolytinae (Campbell and Borden 2009) and may be useful for improving trap attractiveness to ambrosia beetles. A study by Mathieu et al. (1997) demonstrated the preference of

*Hypothenemus hampei* (Ferrari) for red or white traps depending on the release rate of trap volatiles. Chapter 2 demonstrated ethanol-baited traps supplemented with green (525 nm) light-emitting diode (LED) bulbs were more attractive to Scolytinae than traps baited only with ethanol. In order to improve the detection and monitoring of various bark and ambrosia beetles, the objective of this research was to examine conophthorin and green LEDs for their ability to enhance the attractiveness of ethanol-baited traps. We hypothesize integrated pheromonal and visual cues should increase trap capture and potentially specificity of ambrosia beetles.

## Materials and Methods

We conducted a field-based trapping study to test the ability of conophthorin and green LEDs to enhance the attractiveness of ethanol-baited traps to *X. crassiusculus* and *C. mutilatus* (Figure 3.1). Three trap treatments were tested, namely: (1) ethanol alone, (2) ethanol and conophthorin, and (3) ethanol, conophthorin, and green LEDs. Lindgren-type traps consisted of eight black opaque funnels with a detachable white plastic collection cup hung from a metal shepherd's hook, resulting in the traps being  $\approx 1.2$  m above the ground. A mixture of water and





**Figure 3.1.** Modified Lindgren funnel trap used for testing the attractiveness of ethanol, conophthorin, and LEDs to Scolytinae. Arrow notes the placement of around the top rim of the trap

liquid dish detergent (100:1; v:v) (Joy, Procter & Gamble, Cincinnati, OH) was added to the bottom of the collection cup to subdue and kill the entering beetles.

An 8 dram (29.5 ml) clear glass vial containing 28 ml of ethanol (EMD Chemicals Inc. Darmstadt, Germany) and a cotton wick (8 cm × 2 cm, Cotton<sup>®</sup> American Fiber and Finishing Inc. St. Albemarle, NC) held in place with parafilm was fastened to the top funnel of each Lindgren trap. Release rate for ethanol was 3.8 g/day at 25°C. Ethanol test solutions were replenished every 3 days over the course of the experiment.

Conophthorin was emitted from eppendorf tubes sealed with a permeable cap containing 250 µl of test substance (Contech Inc. Victoria, British Columbia, Canada), which were fastened adjacent to the ethanol vial (Figure 3.2). The release rate of conophthorin was 0.5 mg/day at 25°C and replenishment was not necessary.



**Figure. 3.2.** An 8 dram ethanol vial and 250  $\mu$ L conophthorin eppendorf tube attached to the upper portion of a Lindgren trap used for testing their attractiveness to Scolytinae.

Four, 1.5 watt green LEDs (Boesch Built LLC, Waterford Township, MI) were spaced equally along the top rim of the Lindgren funnel traps. LEDs were powered continuously throughout the entire duration of the experiment by a 6 volt battery (McMaster-Carr<sup>®</sup> Elmhurst, IL) that was fastened directly underneath the trap.

Traps were deployed in a randomized complete block design in Tuskegee National Forest, Macon County in Alabama (32-29'19" N, 085-35'39" W) from April 14 – 22, 2012. Traps within the block were 6 m apart, and the trap site was characterized by a mixture of partially-shaded pine and hardwood trees. Samples were returned to the laboratory after collection for specimen identification. *Xylosandrus crassiusculus* and *C. mutilatus* were identified to species, but the remaining scolytine specimens were identified and grouped as 'other Scolytinae'. There were 5 replicates per treatment, each collection counting as a replicate.

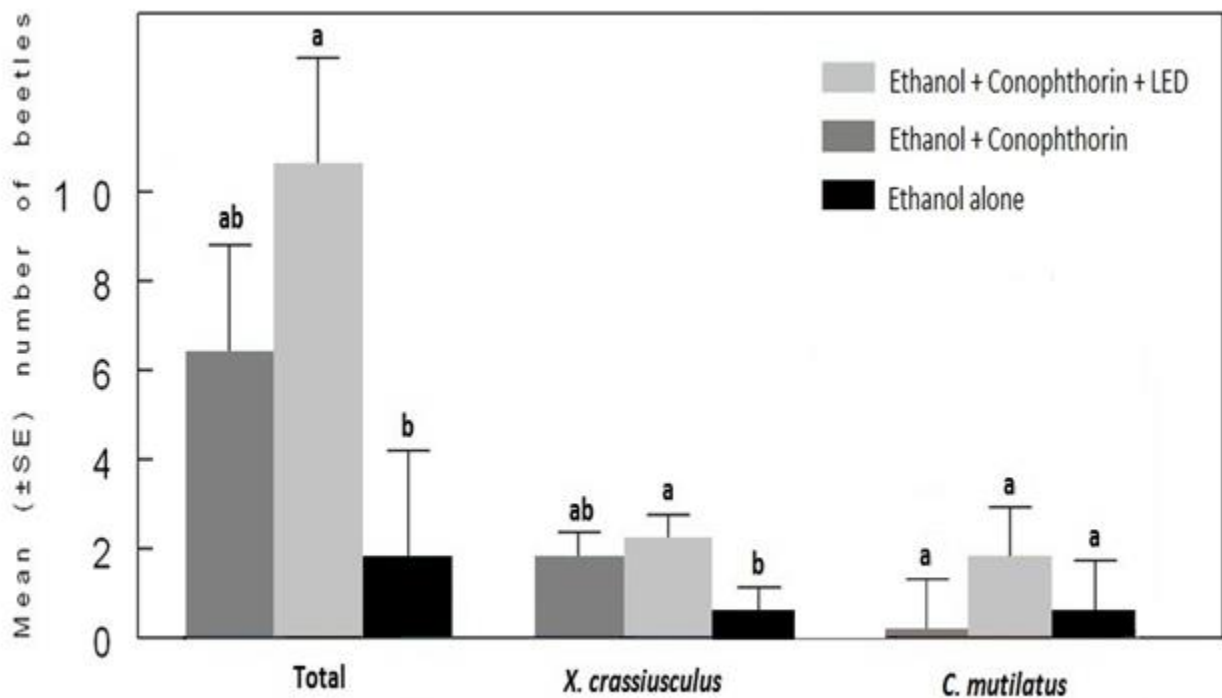
## Statistics

Samples from the entire period were pooled before analysis. A one-way analysis of variance (ANOVA;  $\alpha = 0.05$ ; PROC GLM; SAS Institute 2003) on normal data and Wilcoxon test ( $P < 0.05$ ) was used to compare trap captures between treatments.

## Results

Ethanol-baited traps supplemented with conophthorin and green LEDs attracted significantly more *X. crassiusculus* ( $Z = 2.3$ ,  $df = 2$ ,  $P = 0.02$ ) than traps baited with ethanol alone, but no difference was detected between traps baited with ethanol plus conophthorin and

ethanol alone. Similarly, traps integrating ethanol, conophthorin, and green LEDs attracted significantly more other Scolytinae ( $Z = 2.1$ ,  $df = 2$ ,  $P = 0.036$ ) than traps baited with ethanol alone (Figure. 3.3), but no difference was detected between traps baited with ethanol plus conophthorin and ethanol alone. No difference in *C. mutilatus* counts were detected among any trap treatments ( $df = 2$ ,  $P = 0.74$ ) (Figure. 1). Captures of *X. crassiusculus* ( $P = 0.044$ ) and *C. mutilatus* ( $P = 0.016$ ) were significantly correlated with total Scolytinae captured per treatment.



**Figure 3.3.** Mean  $\pm$  SE number of Scolytinae collected with traps baited with ethanol alone, ethanol plus conophthorin, and ethanol, conophthorin, plus green LED lights (525 nm). Traps were deployed from April 14th – 22nd, 2012 in Tuskegee National Forest, Alabama, USA. Means with different letters indicate significant differences within a species (Wilcoxon;  $P < 0.05$ )

## Discussion

This indicates that integrating green LEDs and conophthorin improved the overall trap captures, which in turn increased captures of these target species. Thus, results from our current study found ethanol-baited traps supplemented with green LEDs and conophthorin were more attractive to *X. crassiusculus* and ‘other Scolytinae’ than traps baited with ethanol alone. The integration of specific LED wavelengths into other trap configurations could increase captures and possibly yield more details regarding visual cue responses.

## CHAPTER 4

### ACOUSTIC SIGNAL DETECTION DETAILING WALKING IN *CNESTUS MUTILATUS* (BLANDFORD)

#### Abstract

Laboratory tests were performed to detect the acoustic signal production of *Cnestus mutilatus* (Coleoptera: Curculionidae: Scolytinae). Using *C. mutilatus* as a model species, the acoustic sound produced by walking was determined and recorded in laboratory experiments. This study investigated the frequency and decibel level of acoustic emission through the use of two microphones in a constructed anechoic environment. A distinct acoustic signal associated with an adult female *C. mutilatus* while walking was detected within a frequency range from 120-200 Hz and 10-25 dB. This study suggests acoustic signals produced by ambrosia beetles could be incorporated into traps in a nursery setting for early pest detection and perhaps species identification.

#### Introduction

Insect species in many orders communicate using complex acoustic and vibrational signals. Acoustic signals are functionally produced in a variety of behavioral contexts including mate attraction, predator-prey interactions, and aggression/territoriality (Drosopoulos & Claridge 2006). Acoustic signals that function for communication must be efficiently and effectively transmitted, received, processed, and conducive of a behavioral response within the receiver.

This interaction has been repeatedly demonstrated and studied in several orthopteran, hemipteran, lepidopteran and hymenopteran species (Drosopoulos and Claridge 2006). In contrast, studies of acoustic communication in Coleoptera have received less attention and breadth of details (Alexander et al. 1963, Arrow 1942, Wessel 2006).

Sound production in beetles has been described in approximately 30 families and has evolved several times (Wessel 2006). Sounds are typically produced through a mechanism of stridulation, which is unanimous across Coleoptera where "file-and-scraper" stridulatory structures are scraped together (Alexander et al. 1963, Arrow 1942, Wessel 2006). Stridulatory organs consists of two highly sclerotized structures, namely a "pars stridens" (file), and a "plectrum" (scraper), which is either a single sclerotized peg, a series of pegs, a single ridge, multiple ridges, or tubercles (Wessel 2006). Given the highly sclerotized exoskeletons, where simple movements can cause body parts to strike and produce vibrations, stridulatory organs have evolved on almost every part of the beetle body (Wessel 2006).

Michelsen (1966) described the synchronization of various coordinated rhythmic movements during courtship behavior in the long-horned beetles (Cerambycidae), and suggested that the 'ritualization' of these movements can lead to different stridulatory organs independently evolving. Currently, 14 different types of stridulatory mechanisms have been described in beetles (Wessel 2006), and many are thought to have evolved from movements produced during walking, wing-folding, biting, and struggling behaviors (Claridge 1968, Hyder and Oseto 1989, Lyal and King 1996, Masters 1980, Michelsen 1966, Miller 1971).

One large family of beetles that has received relatively little attention in terms of acoustic communication are the ambrosia beetles (Scolytinae), a subfamily of the true weevils (Curculionidae). Adult ambrosia beetles produce acoustic signals during flight, walking,

chewing, grooming, and causing mechanical damage to trees as they tunnel into the heartwood of their hosts. Acoustic communication is believed to be widespread across Scolytinae, as stridulatory structures have been documented and described in numerous species across several genera (Barr 1969). To date, studies on acoustics in bark beetles have primarily focused on the mechanisms of sound production, as well as some of the characteristics of signals and how they are associated with different behavioral contexts.

The camphor shot borer, *Cnestus mutilatus* (Blandford) is a recently introduced invasive ambrosia beetle of Asian origin that was first detected in the United States in Oktibbeha County, Mississippi in 1999 (Schiefer & Bright 2004). *Cnestus mutilatus* has since been found in Alabama, Arkansas, Florida, Georgia, Louisiana, North Carolina, Ohio, Tennessee, Texas, and West Virginia (Gandhi et al. 2009, Oliver et al. 2012). This exotic species has unknown pest potential (Gandhi et al. 2009, Oliver et al. 2012), but in Asia, *C. mutilatus* is known to attack many hardwood species, including *Acer*, *Albizzia*, *Carpinus*, *Castanea*, *Cornus*, *Fagus*, *Lindera*, *Osmanthus*, and *Swietenia* spp. (Wood & Bright 1992, Schiefer & Bright 2004). In the United States, *C. mutilatus* has undetermined pest potential, but has been found in Alabama, Arkansas, Florida, Georgia, Louisiana, North Carolina, Ohio, Tennessee, Texas, and West Virginia (Gandhi et al. 2009, Oliver et al. 2012). To date, no stridulatory structures have been detected or acoustic investigation has been performed on this ambrosia beetle. This study suggests acoustic signals produced by ambrosia beetles could be incorporated into traps in a nursery setting for early pest detection and perhaps species identification. In order to improve the detection and monitoring of various bark and ambrosia beetles, the objective of this research was to find an effective method of detecting ambrosia beetles in the trap, thereby allowing for effective first response pest

management plans. We hypothesize that through the use of acoustic detection, a more specific cue could be developed for use in traps.

## Materials and Methods

### *Insect source and rearing*

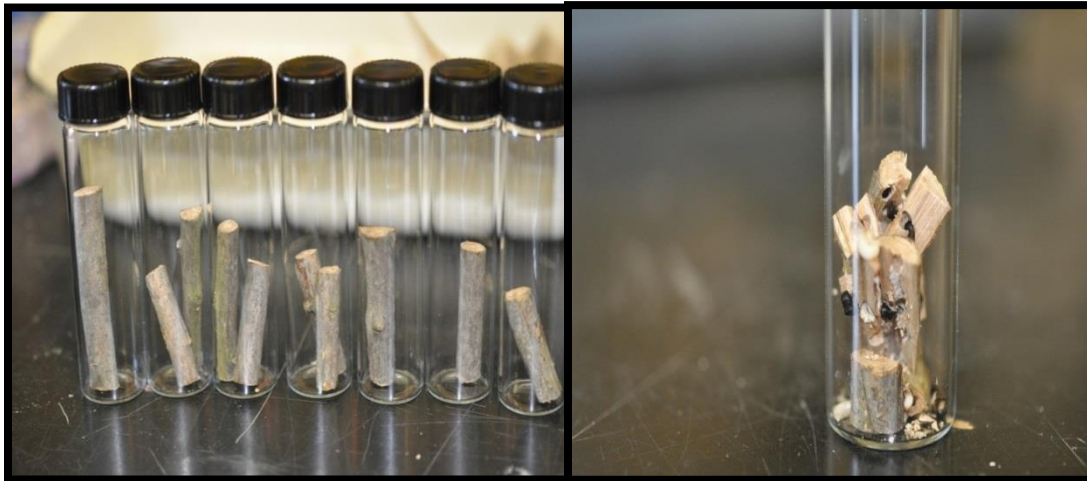
Adult *C. mutilatus* specimens were field-collected from April – July 2012 using a modified version of an ethanol injected trap trees (Ranger et al. 2010, 2012) placed in a wooded lot adjacent to the Auburn University campus. Adult *C. mutilatus* infested the crape myrtle tree (Lythraceae: *Lagerstroemia* spp.) (Figure 4.1) in large numbers over the time period of 3 months. Once infested, trees were returned to the lab and adult *C. mutilatus* were subsequently extracted and collected as live specimens.





**Figure. 4.1.** A-B (A) An ethanol-injected crape myrtle tree (Lythraceae: *Lagerstroemia*) infested by *C. mutilatus* (B) A frass toothpick produced by an ambrosia beetle.

All collected individuals were immediately transferred to 8 dram glass vials kept at 28° C with a section of their engraved tunnel structure still intact, and then were given 5 mL of de-ionized water every 2 days for continued fungal development (Figure 4.2). The 8 dram vials containing the fresh adult *C. mutilatus* were kept covered by black felt 24 hours a day to simulate environmental conditions. Their nutritional requirements were fulfilled by their symbiotic fungal development which proliferated in the simulated conditions. Three total generations were produced from this method, yielding on average 4-5 adults per individual brood chamber, along with the rare *C. mutilatus* male (Figure 4.3).



**Figure. 4.2.** Eight dram glass vials kept at 28° C containing wood sections used for reproducing a suitable environment for the captured ambrosia beetles.



**Figure. 4.3.** (A) A developing *C. mutilatus* larva and (B) rare *C. mutilatus* male.

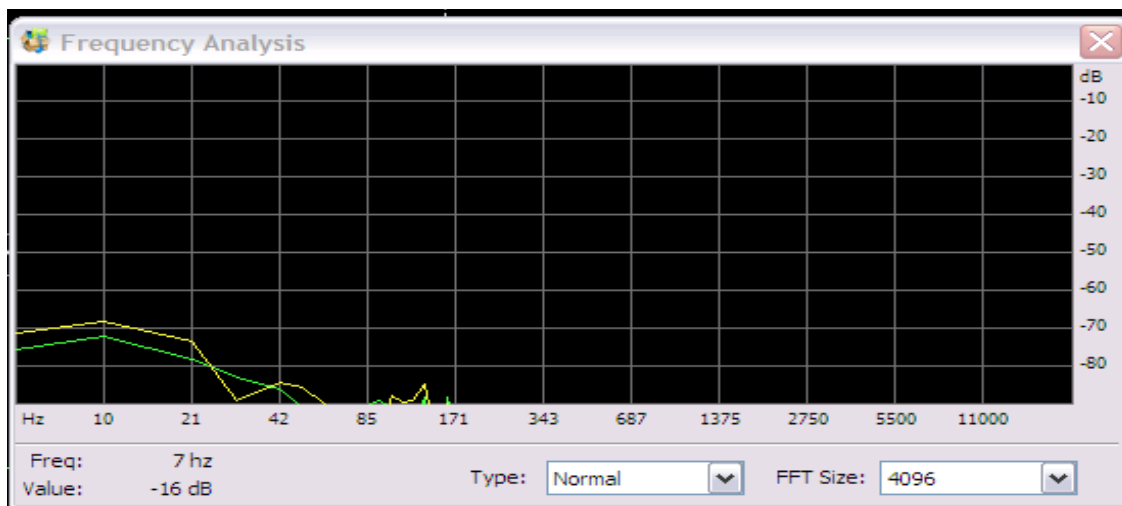
#### *Preliminary testing*

Preliminary acoustic testing began in February 2012 and continued through June 2012. Initial tests incorporated the use of commercially available equipment and were performed in the Forestry-Agronomy-Horticulture-Botany-Entomology (FAHBE) building at Auburn University. The FAHBE building is primarily dedicated to organismal biology and does not have an acoustically sound proofed recording room, therefore a micro environment was constructed. An insulated box with a plexiglass covering served as the micro-environment, and all electrical devices were turned off while recording. *Tribolium castaneum* (Herbst), the red flour beetle, was the test organism used because of its rigorous activity and availability (Figure 4.5). Approximately 50 *T. castaneum* were recorded during a session at 20-22°C.



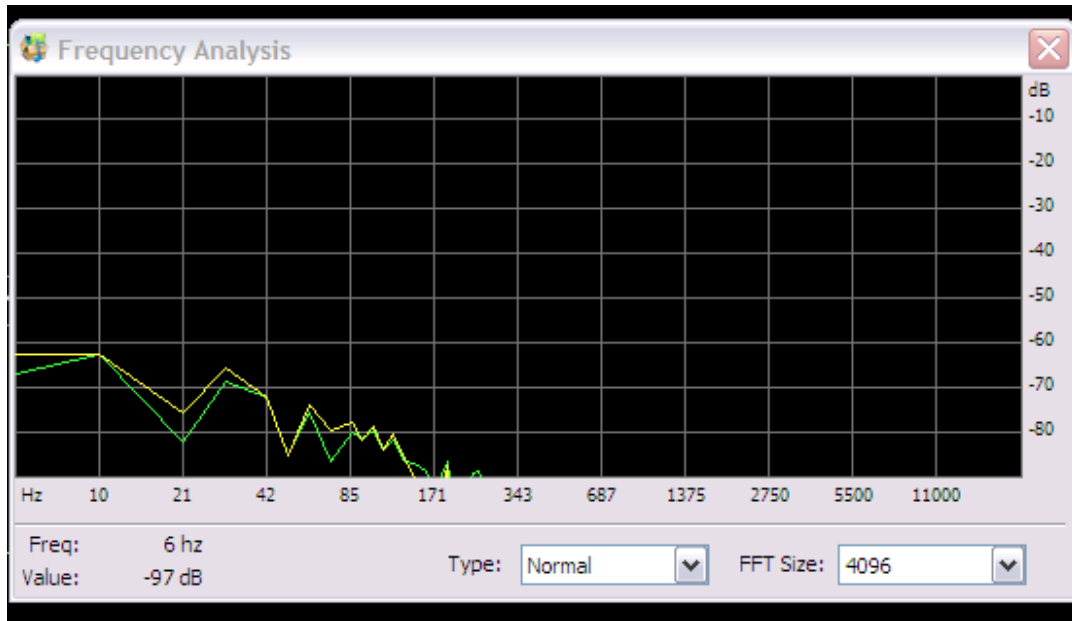
**Figure. 4.5.** An insulated box with a plexiglass served as the initial recording chamber, using a commercially available microphone and *T. castaneum* as a sample organism.

Numerous tests (7 sessions) and environmental reconfigurations produced two specific signals. Background interference fluctuations occurred and actively changed during the recording session and were variable throughout the day. However, a general range of Hz and dB was estimated (Figure 4.6).



**Figure. 4.6.** The real-time background signal (yel. max., gr. min.) recorded in FAHBE building.

The second recording included the *T. castaneum* swarm and produced a constantly fluctuating wave form that was distinctly audible above the background noise (Figure 4.7).



**Figure. 4.7.** The *T. castaneum* acoustic signal (yel. max., gr. min.) recorded from within the FAHBE building.

The initial recording sessions demonstrated the presence of a distinct signal being produced by the test organism, *T. castaneum*. However, the limitations of the software and the hardware used in addition to the constantly fluctuating background interference rendered the data unusable. In order to separate the insect's signal from the background noise in real time, a more sophisticated method capable of producing high resolution comparative data points would need to be implemented.

### *Experimental setup*

The ability of *C. mutilatus* to produce a distinct acoustic signal while walking was examined using a customized recording laboratory in the materials engineering department at

Auburn University. The room temperature was maintained between 20-22°C. Ten active adult female *C. mutilatus* were confined per chamber for recording purposes. Due to the nature of the acoustic chamber walls, the room in which recording was performed would be considered a reverberant space. However, the addition of a heavily insulated box produced more of an anechoic environment where the sound measurements were taken (Figure 4.4). The instrumentation used for data acquisition consisted of two microphones, one accelerometer,



**Figure. 4.4.** Acoustic chamber used for dampening background sound while simultaneously recording two different FFT's, the background and the ambrosia beetle activity.

one oscilloscope, and a Brüel & Kjaer PULSE system, model 3560. The PULSE system is capable of reading four channels of data simultaneously, and the system interfaces with a PC utilizing Brüel & Kjaer PULSE LabShop version 10.1. Two, ½ sized Brüel & Kjaer pre-polarized free-field microphones were used: microphone one was a model number 4188-L-001,

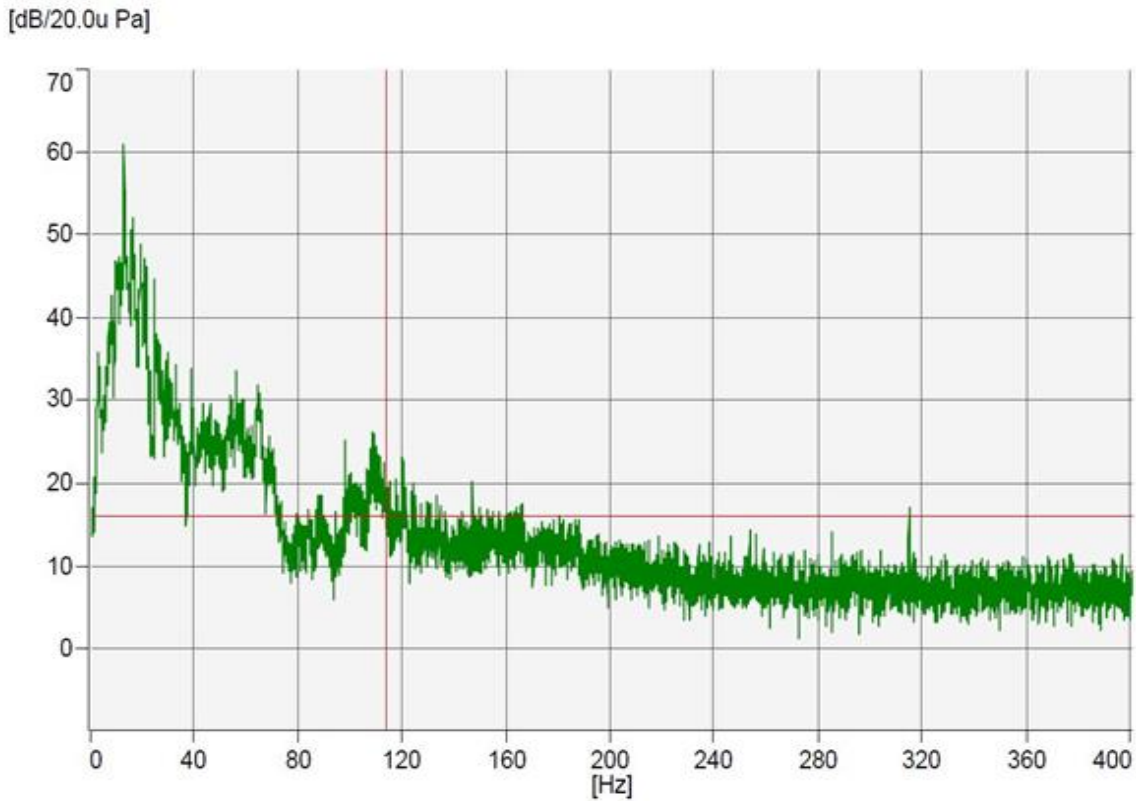
serial number 2381450, and microphone two was a model number 4188-L-001, serial number 2381451. Both microphones were calibrated using a Brüel & Kjaer 4230 sound level calibrator, serial number 1314630, prior to any testing. The calibrator produces a 93.8 dB, 1000 Hz signal.

The measurements recorded directly by the microphone test were performed using an FFT (Fast Fourier Transform) over the range of 0-400 Hz. Microphone 1 was responsible for recording the background acoustic signals given off by the environment, while simultaneously microphone 2 recorded the direct signal being produced by the 10 *C. mutilatus* performing a basic walking behavior. The fast fourier transform test gathered acoustic data points over the course of 12 seconds, and was performed numerous times over the course of 3 months in various sound reducing environments until a clear signal was detected.

## **Results**

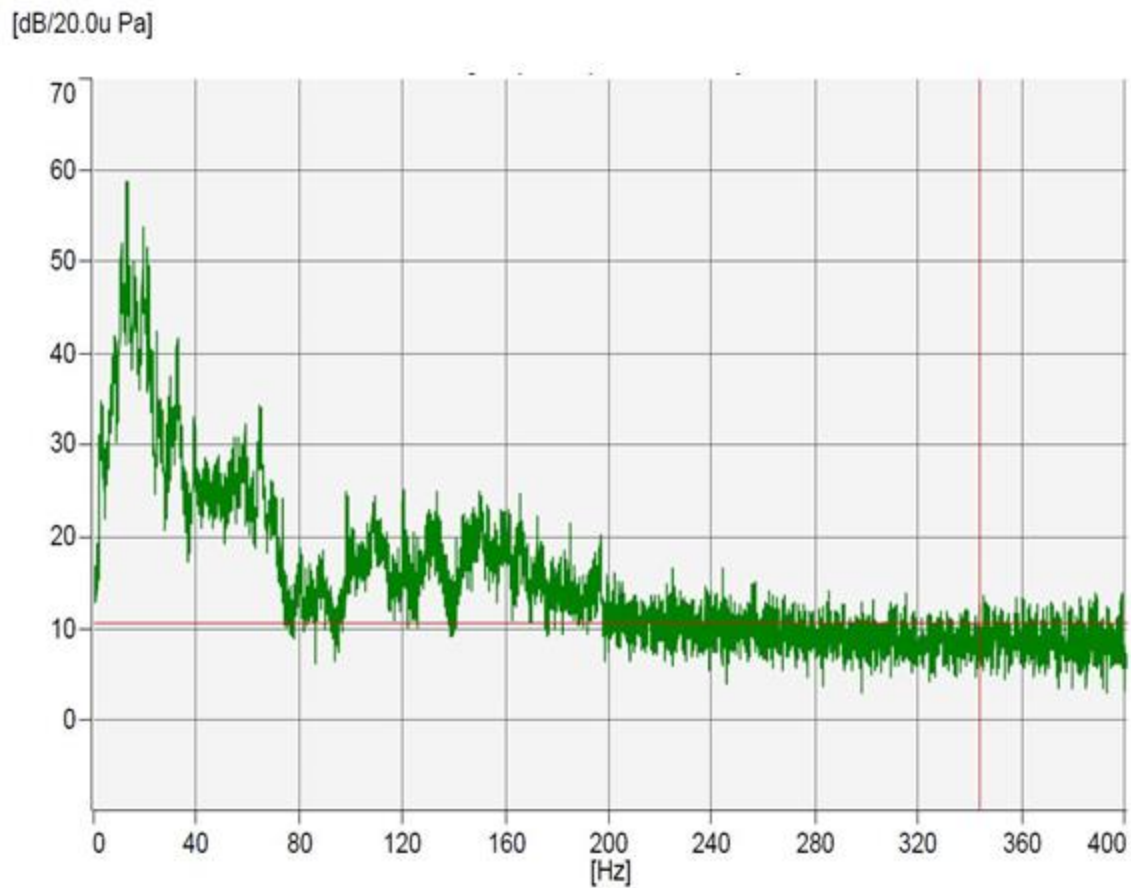
The direct acoustic recording of *C. mutilatus* within the closed, anechoic system produced a clear and recognizable signal from both microphone 1 and microphone 2. Microphone 1 recorded a background signal from 0 – 400 Hz, with any potential interference from background electronics or any other source clearly established (Figure 4.8). The maximum decibel rating was 61 dB and the minimum was 2 dB, encompassing a broad range of background noise. Most of the background interference was lower frequency levels < 120 Hz, which is attributable to several electrical appliances, lighting, and air conditioning present in the laboratory. Above 200 Hz, the decibel level became more uniform and stabilized around 10 Hz. Microphone 2 recorded the beetle walking activity within an enclosed cylinder, with several of the beetles either on the microphone or at a maximum distance from the microphone of 5 cm

(Figure 4.9). Decibel readings below 120 Hz and above 200 Hz were nearly identical for both tests, however the region ranging from 120 – 200 Hz was distinctly different.



**Figure. 4.8.** Microphone 1 characterized the background signal by recording from 0 – 400 Hz.

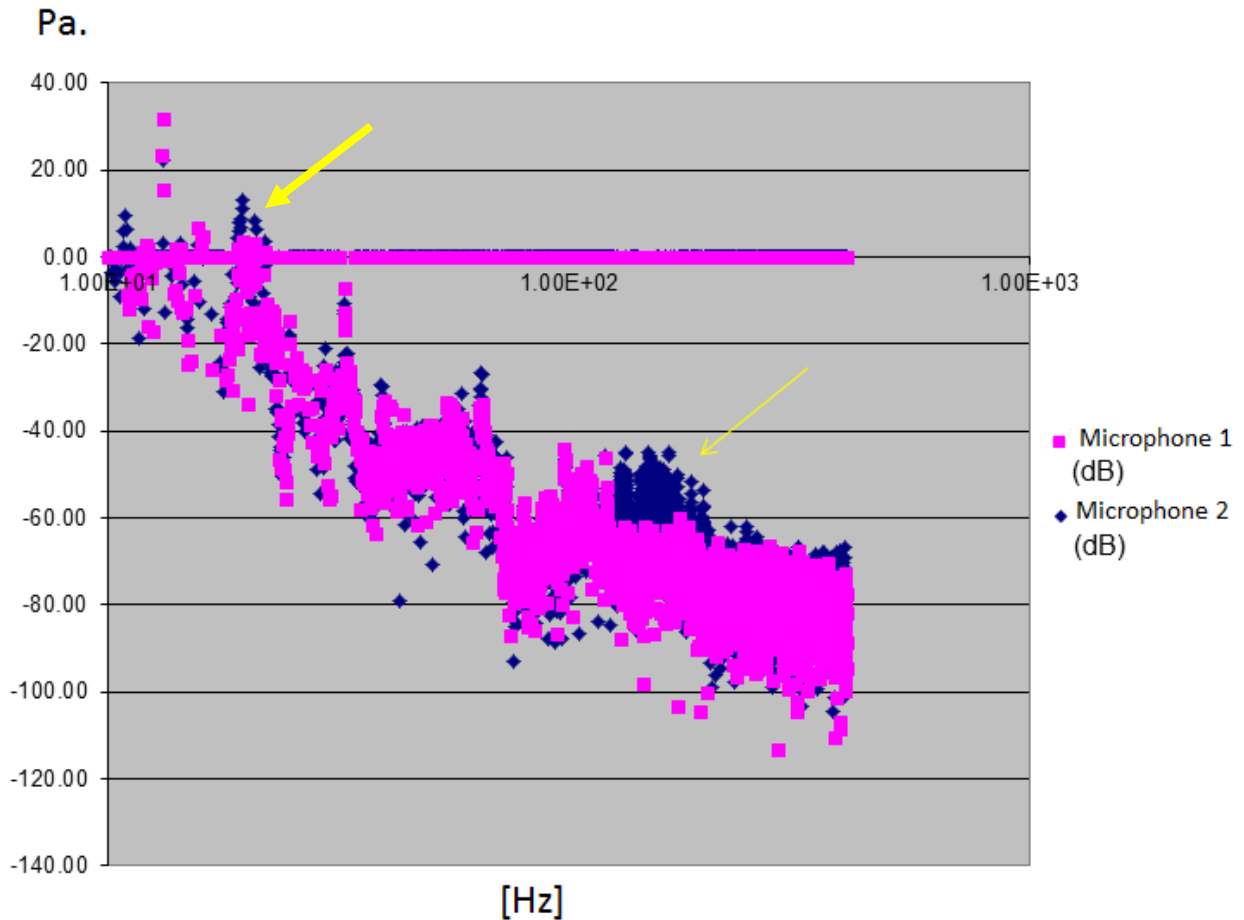




**Figure. 4.9.** Microphone 2 characterized the background signal by recording from 0 – 400 Hz

In order to accurately describe the signal produced by the *C. mutilatus* within the recording chamber, recorded data points from Microphone 1 and Microphone 2 were compared by overlaying the signal (Figure 4.10). The distinct range from 120-200 Hz and 10-25 dB stands out, highlighting the acoustic signal produced by an adult female *C. mutilatus* while walking.





**Figure. 4.10.** Data points recorded from the background interference (pink, Microphone 1) and the *Cnestus mutilatus* (blue, Microphone 2). A distinct range from 120-200 Hz and 10-25 Hz, highlights the acoustic signal produced by an adult female *C. mutilatus* while walking.

## Discussion

The economic damage to high value trees within ornamental nurseries due to invasive ambrosia beetles could potentially be mitigated using bioacoustic recognition to detect the initial phase of their flight period. By cataloguing behavioral acoustic signals produced by problematic pest species, a precise first response system could be developed around immediate species detection. *Cnestus mutilatus* is one of four invasive ambrosia beetles that attack woody plant

hosts in production nurseries and the landscape. We used *C. mutilatus* as a model species to determine the feasibility of defining the acoustic signals for this group of important pests. In general, female *C. mutilatus* acoustic signals were easily discernible compared to each other and all signals were comprised of broad frequency bandwidths. This opens up the potential for not only early detection, but also identifying active infestations within host trees during dormancy, as demonstrated within the red palm weevil (*Rhynchophorus* spp.) infestation of *Areaceae* spp. including the economically important coconut (*Cocos nucifera*), date (*Phoenix dactylifera*), and ornamental palms (Mankin et al. 2008, Hussein et al. 2010).

Some conditions under which bioacoustic detection would be difficult include cold weather in which ambrosia beetle activity is reduced. Noisy environments can add sufficient background interference, drowning out any ambrosia beetle activity from being detected. The low frequency nature of these signals also creates an issue with detection devices, specifically piezoelectric sensors, which rely upon a sensitive frequency range to properly detect activity.

Acoustic signal replication could produce additional cues for effective trapping of ambrosia beetles. This principle has been demonstrated in other insects such as the mole cricket (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.). Acoustic trapping broadcasts the real or imitation calling song of the male and attracts flying mole crickets of both sexes (Walker 1988), making it possible to acquire large numbers of living mole crickets. Before potential implementation, investigation into acoustic receptor organs of Curculionidae should be explored. Thus far, tympanal ears have only been described in two coleopteran families namely Scarabaeidae and Carabidae:Cicindelinae, and in both, they have been shown to function for detecting the ultrasonic cries of predatory bats (Spangler 1988, Forrest et al. 1995). Other insects in which acoustic trapping has been successful include: mosquitoes (Kahn & Offenhauser 1949),

chironomid midges (Ogawa 1992), cockroaches (Mistal et al. 2000), tephritid fruit flies (Webb et al. 1983), and others. Several studies have shown that when trees experience long periods of dehydration, the hydrogen molecules of the water columns in the xylem tissues cavitate, releasing ultrasonic sounds with frequencies ranging from 60 to 2000 kHz (Haack et al. 1988, Mattson and Haack 1987, Pena and Grace 1986). Mattson and Haack (1987) hypothesized that bark and wood-boring insects may exploit these acoustic cues during host-plant selection. Further understanding of sound production in ambrosia beetles and their host plants could potentially bring acoustic signals into the realm of viable attractants, as is currently semiochemical baits, pheromones, and visual cues (Chapters 2 and 3).

## CHAPTER 5

### INVESTIGATING AMBROSIA BEETLE (COLEOPTERA, CURCULIONIDAE) SPECIES DIVERSITY AND DIURNAL FLIGHT USING VARIOUS TRAPPING METHODS

#### Abstract

In field studies in AL and OH, we compared captures of ambrosia beetles in nurseries between an experimental trap baited with ethanol, conophthorin, and green LEDs and an ethanol-baited Baker trap to test the influence of chemical and visual cues on the capture of bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). No differences occurred in total Scolytinae trap captures between the two trap types at sites in AL or OH, however a large variety of species were represented within the samples in AL, and a slightly lessened variety was represented in the OH samples. The integration of a new detection system allowed for time sensitive capture data, making it possible to elaborate on the connections between ambrosia beetle flight, temperature. The traps in OH suggested the amount of ambrosia beetles making a host selection flight resulting in trap capture could be related to the time of day and temperature, with most ambrosia beetle captures occurring between 6 p.m. and 8 p.m. at 20°C. The integration of specific LED wavelengths into other trap configurations could increase captures and possibly yield more details regarding visual cue responses accompanying navigational mechanisms.

## Introduction

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are important pests of tree nursery production. Adult beetles bore into trees and proceed to excavate a gallery that consists of several tunnels including a brood chamber. During this process, they introduce their symbiotic fungi which serve as food for the adults and larvae (Weber & McPherson 1984, Roeper 1996). Ambrosia beetle galleries harbor all life stages of the insect. The resulting female to male ratio is typically female dominated, being 10:1 within *X. germanus* colonies (Weber & McPherson 1983). Male ambrosia beetles usually have short life duration, are flightless, and fulfill the role within the brood chamber of mating with its siblings (Oliver et al. 2012).

Most ambrosia beetles tend to have 1-3 generations per year, which is highly dependent upon seasonal conditions and host availability. In the United States, both *X. crassiusculus* and *X. germanus* have two to three generations per year and one to two in Europe or Japan (Weber & McPherson 1983). Dispersal flights for each species of ambrosia beetle occur over an extended period of time, from as early as March to as late as September. Temperature plays an important role in the emergence of *X. germanus* and other ambrosia beetles from their overwintering galleries (Reding et al. 2013). Regional variability is seen for determination of flight period, with generally the southern United States beginning earlier and ending later (Weber & McPherson 1983, Oliver and Mannion 2001, Schiefer & Bright 2004).

Effective trapping of ambrosia beetles requires monitoring their seasonal dispersal flight patterns and following a systematic approach to capture. Generally, attempts at field sampling of ambrosia beetle populations have included the implementation of various traps that utilize volatile attractants. The Baker bottle trap and the Lindgren funnel trap are the most widely used traps for field sampling of ambrosia beetles. Reding et al. (2010) tested the effect of varying trap

heights on ambrosia beetle capture and found that 0.5 m caught more *X. crassiusculus* and *X. germanus* than the competing 1.7 m and 3.0 m traps. A positive relationship exists between concentration of ethanol emitted from lures and attraction by ambrosia beetles (Klimetzek et al. 1986; Oliver et al. 2004; Ranger et al. 2011, Reding et al. 2011), along with emissions from ethanol-injected trees and corresponding attacks (Ranger et al. 2012).

In order to improve the detection and monitoring of various bark and ambrosia beetles, previously investigated olfactory and visual cues (Chapters 2 & 3) were implemented for their ability to enhance the attractiveness of ethanol-baited traps. Current management of ambrosia beetles in nurseries relies primarily on preventive treatments of insecticides applied to the trunks of trees with timing of chemical sprays dependent on trapping (Hudson & Mizell 1999). A more specific or efficient trap may therefore reduce losses and the number of sprays. In order to improve the monitoring of various bark and ambrosia beetles, the objective of this research was to develop a working trap model that will be effective in sampling nurseries for pest ambrosia beetles that are simple and inexpensive for widespread use. We hypothesize early detection trapping will lower the damage cost created by populations of ambrosia beetles on target host tree sites.

## **Materials and Methods**

We conducted a field-based trapping study to test the ability of conophthorin and green LEDs to enhance the attractiveness of ethanol-baited traps to local Scolytinae populations. This study also included an experimental sensor to detect when captures were occurring within the trap. Two trap treatments were tested, namely: (1) ethanol alone, and (2) ethanol, conophthorin, and green LEDs. This was examined using Baker traps (Bambara et al. 2002; Oliver et al. 2004) (Figure 5.1) baited with only ethanol, and our experimental trap baited with ethanol,

conophthorin, and green LEDs (Figure 5.2). Baker bottle traps consisted of a 2 L clear plastic bottle hung from a shepherd's hook, resulting in the traps being  $\approx 1.2$  m above the ground. Our custom designed traps ( $\approx 2$  m above the ground) consisted of: (1) Solar panel (Model SLCK-010-12, Solarland, Jiangsu, P.R.China) (2) 2 mm thick plexiglass vein (Plaskolite Inc. Columbus, OH) (3) 1.5 watt green LED lights (525 nm) (Boesch Built LLC, Waterford Township, MI) (4) 1/8 inch wire mesh (Midwest Air Technologies Inc. Lincolnshire, IL) (5) 100 mm and 160 mm plastic funnel (VWR International Radnor, PA) (6) IR sensor emitter P/N: 782-TSUS4300 and receiver P/N: 78-TEFD4300F (Mouser Electronics Inc. Mansfield, TX) (7) 250 mL collection bottle (VWR International Radnor, PA) (8) 12 volt rechargeable battery (Model Ps-121000 U, Power-Sonic Corp., San Diego, CA) (9) 250  $\mu$ l eppendorf tubes containing conophthorin with a release rate of 0.5 mg/day at 25°C (Contech Inc. Victoria, British Columbia, Canada) and permeable membrane ethanol bags with a release rate of 65 mg/day at 25°C (AgBio Inc. Westminster, CO) (10) Micro controller unit (Model Mega 2560, Arduino Inc., Ivrea, Italy) (11) Hobo Pro V2 U23-001 temperature logger (Onset Bourne, MA). A mixture of water and liquid dish detergent (100:1; v:v) (Joy, Procter & Gamble, Cincinnati, OH) was added to the bottom of the collection cup to subdue and kill the entering beetles. LEDs were powered continuously throughout the entire duration of the experiment by the 12 volt battery that was fastened directly underneath the trap.

Traps were deployed in a randomized complete block design in Alpine, Talladega County, AL (33-22'51" N, 086-20'00" W) from 25 March – 1 May 2013, as well as Avon, Loraine County, OH (41-25'28" N, 082-01'07" W) from 3 May – 17 June, and Madison, Lake County, OH (41-47'58" N, 081-04'41" W) from 3 May – 12 June. Traps within the block were 6 m apart, and the trap site was characterized by a standard nursery setting which included various

stages of trees in production. At the AL nursery site, the trees (*Acer* spp., *Cedrus* spp., *Cercis* spp., *Cryptomeria* spp., *Ilex* spp., *Lagerstroemia* spp., *Magnolia* spp., *Prunus* spp., *Thuja* spp.)



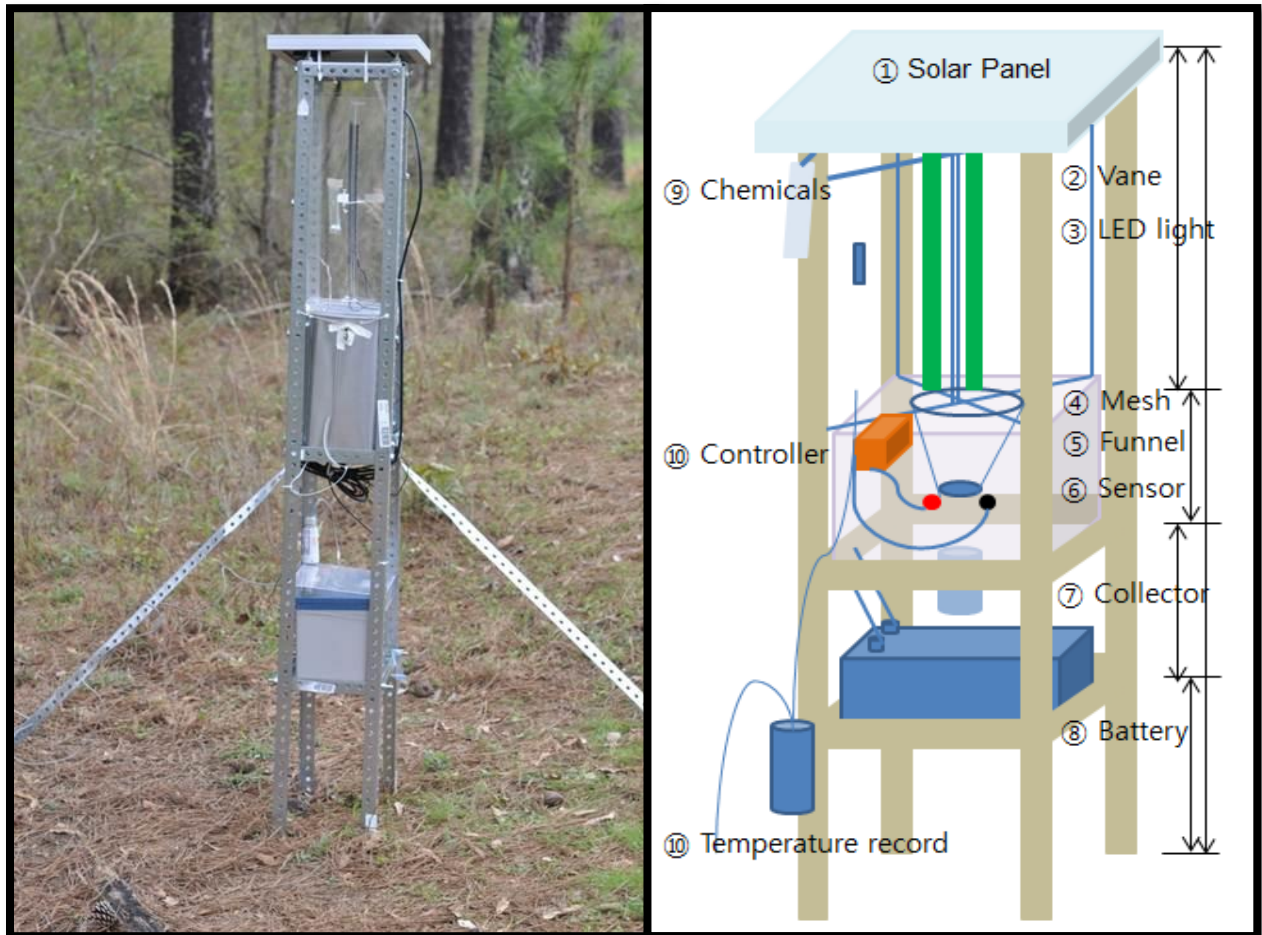
present in the block adjacent to the traps were scouted during every trap collection (3-4 d) to determine if and when attacks occurred. Blocks of trees in OH were scouted by research cooperators (nursery employees). Insecticide application records were also provided for these

**Figure. 5.1** A Baker bottle trap baited with an ethanol lure.

blocks. Beetle and sensor samples were returned to the laboratory at the end of the field experiment for specimen identification and sensor data unloading. Scolytine specimens were identified to species using the Screening Aid for Eastern United States Scolytinae (LaBonte et al. 2007), Bark Beetles of the Southeastern United States (Baker et al. 2009), and a guide from the



USDA Horticultural Insects Research Laboratory in Wooster, OH (personal comm.). There were 4 traps per treatment in Alpine and 3 traps per treatment at both OH locations.



**Figure. 5.2** A-B (A) Custom trap baited with ethanol, conophthorin, and green LEDs. (B) Schematic diagram of trap components

## Results

### *Experiment 1: Trapping in Alpine, Talladega County, AL*

Ethanol-baited traps supplemented with conophthorin and green LEDs did not attract more Scolytinae than traps baited with ethanol alone. *X. crassiusculus* and *X. saxeseni* were the most common species, yet 24 different species were captured (Table 5.1).

<b>Ambrosia beetle species</b>	<b># of individuals</b>
<i>Xylosandrus crassiusculus</i>	116
<i>Euwallacea fornicatus</i>	5
<i>Xyleborinus saxeseni</i>	19
<i>Scolytus rugulosus</i>	1
<i>Xyleborus volvulus</i>	1
<i>Hylocurus carinifrons</i>	1
<i>Xyleborus atratus</i>	6
<i>Pityophthorus scriptor</i>	2
<i>Xyleborus californicus</i>	4
<i>Dryocoetes betulae</i>	3
<i>Monarthrum mali</i>	3
<i>Anisandrus dispar</i>	2
<i>Cryptocarenum heveae</i>	6
<i>Cryptocarenum seriatus</i>	7
<i>Cryptocarenum diadematus</i>	2
<i>Cnestus mutilatus</i>	3
<i>Pityophthorus liquidambarus</i>	1
<i>Xyleborus ferrugineus</i>	4
<i>Hypothenemus crudiae</i>	1
<i>Hypothenemus interstitialis</i>	1
<i>Thysanoes fimbriicornis</i>	3
<i>Pseudothysanoes phoradendri</i>	1
<i>Pityophthorus annectans</i>	1
<i>Hypothenemus californicus</i>	1

**Table 5.1** Species and total amount of ambrosia beetles captured in traps in Alpine, Talladega County, AL.

*Xylosandrus crassiusculus* was the most common species totaling 60% of the overall capture, with *Xyleborinus saxeseni* being the second most common contributing 10%. Ethanol baited

bottle traps captured a wider diversity of ambrosia beetles with less specificity totaling 22 species (Table 5.2), whereas our experimental modified trap captured a less diverse sample with increased specificity totaling 9 species (Table 5.3). No attacks were recorded on any of the trees within this block.

<b>Ambrosia beetle species</b>	<b># of individuals</b>
Xylosandrus crassiusculus	90
Euwallacea fornicatus	5
Xyleborinus saxeseni	13
Scolytus rugulosus	1
Xyleborus volvulus	1
Hylocurus carinifron	1
Xyleborus atratus	5
Pityophthorus scriptor	1
Xyleborus californicus	3
Dryocoetes betulae	1
Cryptocarenum heveae	6
Cryptocarenum seriatus	7
Cryptocarenum diadematus	2
Cnestus mutilatus	3
Pityophthorus liquidambarus	1
Xyleborus ferrugineus	1
Hypothenemus crudiae	1
Hypothenemus interstitialis	1
Thysanoes fimbriicornis	3
Pseudothysanoes phoradendri	1
Pityophthorus annectans	1
Hypothenemus californicus	1

**Table 5.2** Species and total amount of ambrosia beetles captured in ethanol-baited bottle traps in Alpine, Talladega County, AL.

<b>Ambrosia beetle species</b>	<b># of individuals</b>
Xylosandrus crassiusculus	26
Xyleborinus saxeseni	6
Xyleborus atratus	1
Pityophthorus scriptor	1
Xyleborus californicus	1
Dryocoetes betulae	2
Monarthrum mali	3
Anisandrus dispar	2
Xyleborus ferrugineus	3

**Table 5.3** Species and total amount of ambrosia beetles captured in the modified trap supplemented with conophthorin and green LEDs in Alpine, Talladega County, AL.

*Experiment 2: Trapping in Avon, Loraine County, and Madison, Lake County, OH*

Ethanol-baited traps supplemented with conophthorin and green LEDs did not attract more Scolytinae than traps baited with ethanol alone. Several species of ambrosia beetles were identified from the baited and control traps in various amounts (Table 5.4).

<b>Ambrosia beetle species</b>	<b># of individuals</b>
Xylosandrus germanus	67
Xyleborus californicus	24
Xyleborinus saxeseni	65
Xyleborus ferrugineus	2
Hypothenemus dissimilis	4
Hypothenemus eruditus	1
Anisandrus maiche	1
Xyleborus atratus	18
Euwallacea validus	5
Xyleborus pubescens	1
Monarthrum mali	2
Xylosandrus crassiusculus	1

**Table 5.4** Species and total amount of ambrosia beetles captured in traps in Avon, OH (Loraine County) and Madison, OH (Lake County).

*Xylosandrus germanus* was the most common ambrosia beetle captured (35%), followed by *Xyleborinus saxeseni* (34%), *Xyleborus californicus* (13%), and finally *X. atratus*. These species dominated the trap sample although 12 species in total were captured, but all others accounted for 10% or less of the total capture collectively. Ethanol baited bottle traps captured a less diverse sample with increased specificity totaling 7 species (Table 5.5), whereas our experimental modified trap captured a wider diversity of ambrosia beetles with less specificity totaling 9 (Table 5.6). No attacks were recorded on any of the trees within this block.

Ambrosia beetle species	# of individuals
<i>Xylosandrus germanus</i>	45
<i>Xyleborus californicus</i>	3
<i>Xyleborinus saxeseni</i>	31
<i>Hypothenemus dissimilis</i>	4
<i>Hypothenemus eruditus</i>	1
<i>Xyleborus atratus</i>	9
<i>Xylosandrus crassiusculus</i>	1

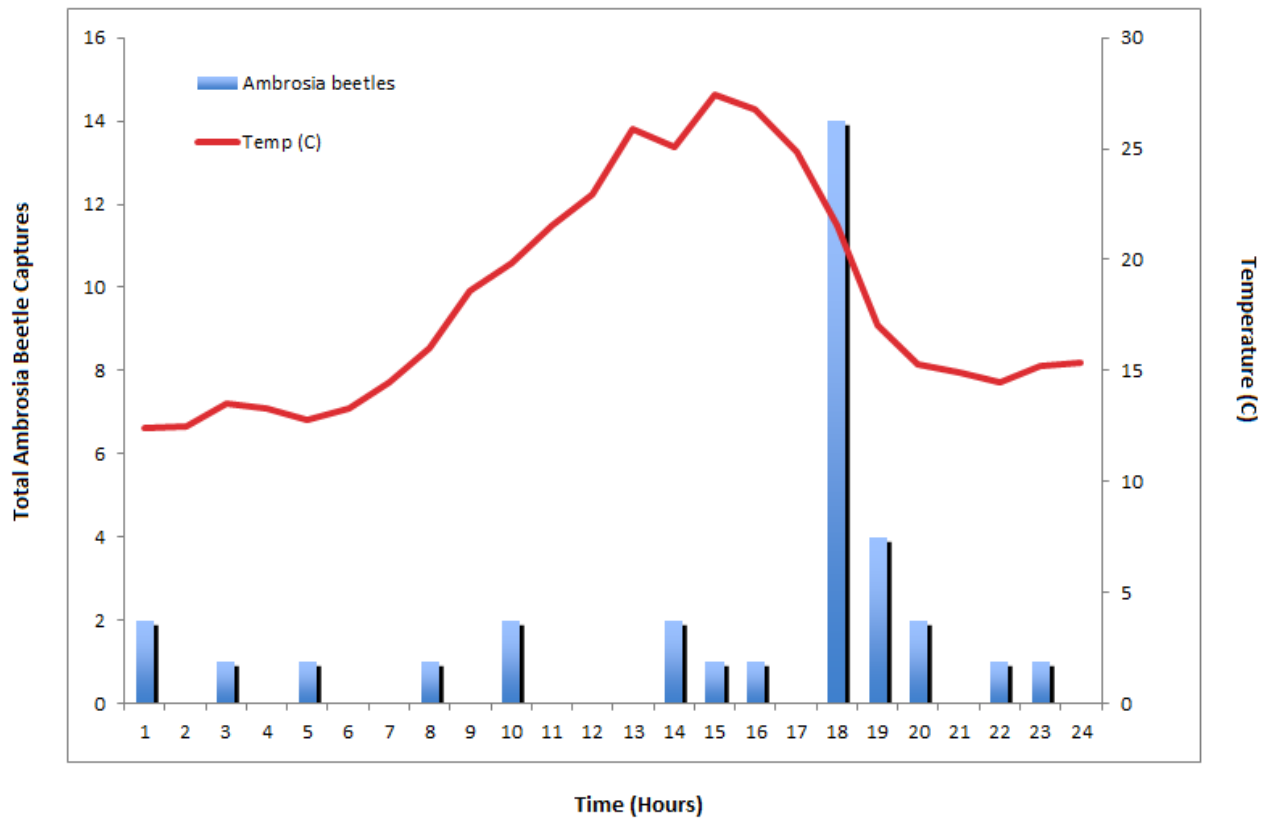
**Table 5.5** Species and total amount of ambrosia beetles captured in ethanol-baited bottle traps in Avon, OH (Loraine County) and Madison, OH (Lake County).

Ambrosia beetle species	# of individuals
<i>Xylosandrus germanus</i>	22
<i>Xyleborus californicus</i>	21
<i>Xyleborinus saxeseni</i>	34
<i>Xyleborus ferrugineus</i>	2
<i>Anisandrus maiche</i>	1
<i>Xyleborus atratus</i>	9
<i>Euwallacea validus</i>	5
<i>Xyleborus pubescens</i>	1
<i>Monarthrum mali</i>	2

**Table 5.6** Species and total amount of ambrosia beetles captured in the modified trap supplemented with conophthorin and green LEDs in Avon, OH (Loraine County) and Madison, OH (Lake County).

*Sensor data: Counter sensor performance at Willoway nurseries*

From May 3<sup>rd</sup> to May 7<sup>th</sup> the sensor counter acquired ambrosia beetle capture data. The amount of ambrosia beetles making a host selection flight resulting in trap capture is potentially related to the time of day and temperature as recorded by the data logger (Figure. 5.3). The majority of ambrosia beetle captures on this site occurred between 6 p.m. and 8 p.m.



**Figure. 5.3** Ambrosia beetles captured in Avon, OH from May 3<sup>rd</sup> to May 7<sup>th</sup>, with each day's capture placed over a 24 hour period.

**Discussion**

This study displays the wide diversity of ambrosia beetles present in the Midwestern (OH) and southern (AL) U.S. The modified trap aided in defining the peak diurnal flight period of ambrosia beetles.

It is likely the trap design was flawed in comparison to the standardized lindgren funnel trap and the baker bottle traps, however, additional experiments comparing the two traps with the same volatile and/or visual cues would be required to accurately compare their efficacy. The entrance to the detection zone on the custom trap was a single flat surface enveloped with mesh, whereas the multiple funnel gradations in a lindgren funnel trap and open direct access to killing water in the bottle trap more efficient.

Although many ambrosia beetles were captured in each trap throughout the 3 nurseries, there were few to no attacks upon the nursery stock. The captures confirm the presence of multiple species populations being present and readily seeking new hosts for infestation. Since there were few attacks in the nursery, this indicates that the stock was generally healthy and was not producing stress induced volatiles. Being that the traps were placed along the perimeter of the nursery, it is likely that the captured ambrosia beetles originated in the surrounding wooded areas which maintain a constant reservoir population.

The flight period for *X. germanus* at a nursery in OH in early May was from 6 to 8 p.m. The sharp decrease in temperature may allow for the ambrosia beetles to better manage its energy cost of flight and simultaneously avoid the potential for water loss during the hotter periods of the day. The peak capture point occurred at approximately 70°F. During twilight hours it is common for most light wavelengths present to become polarized and exhibit an elongated wavelength ranging between 550 to 700 nm (Ugolnikov et al. 2003). Shorter wavelengths > 550 nm become less common, which are representative of the blue green color which is typical of

reflected light from foliage. It has been demonstrated that copepods have peak photo behavioral responses at wavelengths corresponding to those available during twilight in coastal water (Cohen & Forward 2002). The sensitivity to this specific setting was demonstrated within ambrosia beetles to a 525 nm light source in a twilight setting. Thus, results from our current study reinforced that traps baited with green LEDs were capable of replicating a host tree during the host seeking flight behavior as demonstrated in Chapter 3. Therefore it is suggested that a chemical treatment plan would be best implemented by application to the trunks of trees in the morning.

The integration of specific LED wavelengths into other trap configurations could increase captures and possibly yield more details regarding visual cue responses. A physiological approach defining the underlying mechanisms of visual cues in ambrosia beetles could potentially further elucidate navigational mechanisms during host seeking behavior.



## CONCLUSIONS

Three widely distributed species of ambrosia beetles, *X. germanus*, *X. crassiusculus*, and *C. mutilatus*, are introduced pests that attack trees in mass and create tunnels into the heartwood and sapwood, introducing a symbiotic fungus and occasionally secondary pathogens. Current traps for these species use generic volatile cues that result in non-target beetle captures (by-catch). By-catch creates false positives resulting in unnecessary spray applications. In this project, there were three supported objectives to accomplish the long-term goal. 1. Identify visual and volatile cues, specifically volatile organic compounds and LEDs, that will either increase trap captures or reduce by-catch. 2. Identify an effective method(s) of detecting ambrosia beetles in the trap. 3. Field test a working trap model in nurseries to assess diversity and diurnal flight activity

Through field experiments, it was demonstrated that ambrosia beetle trap captures can be increased by the availability of specific nanometer wavelengths of light, specifically green (525 nm) LEDs as well as chemical cue pairing of ethanol and conophthorin. A species specific analysis was performed upon the trap captures, however we did not find cues that appealed more to individual species, but we did find cues that increased trap captures relative to ethanol only traps. In the final experiments utilizing alternative cues (Chapter 5), the customized trap did not outperform the standard bottle trap for total ambrosia beetle capture. It is likely the trap design was flawed in comparison to the baker bottle traps due to a limited area for the beetles to enter the detection zone efficiently.

Since beetles rarely fly in traps walking frequencies appear to be most useful for in-trap identification and to exclude false positives associated with by-catch. Piezoelectric sensors fabricated to resonate at the proper *C. mutilatus* walking frequency have not yet been tested under field conditions. Fabrication of the highly flexible piezoelectric sensor needed to obtain the low frequency and decibel rating may not be feasible within the limits of a portable trap design.

This work suggests that captures of *X. germanus*, *X. crassiusculus*, *C. mutilatus*, and other Scolytinae can be increased relative to the standard trap using green LEDs and conphthorin. Furthermore, sensors may also make it possible to enumerate or even identify beetles entering the trap using acoustic detection. These advances can lead to reduced by-catch, false positives in traps, and more judicious use of insecticides in managing ambrosia beetles in nurseries by timing insecticide applications more effectively. This also could potentially be advantageous in a nursery control program where only certain pest species pose a threat to the tree stock.

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