ABSTRACT

The economically damaging southern pine beetle (*Dendroctonus frontalis* Zimmermann) is one of the most destructive insect pests in southeastern United States. SPB populations are monitored using a racemic kairomone, α-pinene, and pheromone, frontalin to capture SPB and predator, *Thanasimus dubius* (Fabricius). I assessed whether SPB and *T. dubius* differentiate between enantiomers of α-pinene. Results indicated the response of female and male SPB to α-pinene enantiomers did not significantly differ, although males were somewhat more responsive to (+)-α-pinene. Captures of *T. dubius* increased with volumes of α-pinene, and *T. dubius* did not differentiate between enantiomers.

Typically SPB infest pines other southern pine bark beetle guild (SPBBG) members (which include *Dendroctonus terebrans* (Olivier) and *Ips* beetle species). Colonizing *Ips* species release either ipsdienol and/or ipsenol. I assessed the inter- and intraspecies attraction among SPBBG and their predators. Results indicate SPB and *T. dubius* are not attracted to *Ips* attractants and vice versa. BTB and *Ips calligraphus* (Germar) were attracted to *Ips* attractants. SPBBG predators (other than *Pycnomerus sulcicollis* LeConte) did not differentiate between SPB and *Ips* attractants.

Using linear regression, I assessed the relationship between lightning strike and SPB infestations. Results indicated a relationship between SPB infestations developing within 100-250 m of a negatively-charged lightning strike with a magnitude of ≥ 150 kilo amps. There was no relationship between the basal area pine stands and the likelihood of
lightning strike. There was no relationship between the distance of a strike and the number of trees infested with SPB.

The ecological impacts of forest management techniques used to control SPB populations in loblolly pine (*Pinus taeda* L.) stands were also assessed. Ground-foraging ant assemblages were used to indicate the ecological impacts of loblolly stands that were unmanaged, thinned, prescribed burned, or clear-cut and replanted with longleaf pine. Ant species diversity was highest in unmanaged stands. Species assemblages were more similar in prescribed burned or thinned stands and differed greatly between unmanaged stands and clear-cut, replanted stands. However, species assemblages and richness did not differ among stands overall.

INDEX WORDS:  
SOUTHERN PINE BEETLE (*Dendroctonus frontalis* Zimmermann): SEMIOCHEMICAL ECOTOLOGY, RELATIONSHIP OF OUTBREAK POPULATIONS AND LIGHTNING STRIKE, AND ECOLOGICAL IMPACTS OF SUPPRESSION AND CONTROL TECHNIQUES

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SOUTHERN PINE BEETLE (*Dendroctonus frontalis* Zimmermann):

SEMIOCHEMICAL ECOLOGY, RELATIONSHIP OF OUTBREAK POPULATIONS AND LIGHTNING STRIKE, AND ECOLOGICAL IMPACTS OF SUPPRESSION AND CONTROL TECHNIQUES

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DEDICATION

This dissertation is dedicated to the two people who have made every happiness and success in my life possible. I could not have accomplished grand academic and personal ventures without their encouragement, support, and tremendous love.

Bill and Sherry, thank you for picking up the phone.
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CHAPTER 1
DISSERTATION INTRODUCTION

1.1 Study Organism: Southern Pine Beetle

The southern pine beetle, *Dendroctonus frontalis* Zimmermann (SPB), is considered the most economically important native forest pest in eastern North America. In the United States, SPB occurs from New Hampshire in the North to Florida in the South, and Texas, Arizona, and New Mexico in the Southwest. SPB typically has cyclical population cycles in which populations become epidemic then crash approximately every 12 years in mountainous regions and 8-10 years within in the Piedmont region of Georgia with consistent, low population levels for 1-3 year intervals. Outbreak populations of SPB infest stressed or weakened pines (*Pinus* spp.) and create “spots” of dead or dying pines across the landscape. Within thirty years, cyclical population outbreaks of SPB have caused approximately $1.2 million in timber losses in the southeastern United States (Pye et al. 2011). SPB kill tress, first, by girdling their nutrient (phloem) system while excavating nuptial chambers and larval SPB feed on phloem in egg chambers. Further, SPB adults introduce a blue-stain fungus (*Ophiostoma minus* (Hedgcock)) which interrupts water transportation within the xylem.

SPB has complete metamorphosis (egg, larval, papa, and adult stages) and is multivoltine, although the number of generations depends upon local climatic conditions (Thatcher 1960). Adults are minute (2-4 mm) bark beetles with a rounded posterior (Osgood and Clark 1963). Adult females are identified by the mycangium, a transverse
ridge on the anterior pronotum. Males do not have a mycangium but are identifiable by a prominent frontal groove created by rough tubercles on their head (Osgood and Clark 1963, Payne 1980). Female colonizes the optimal conifer host first, and releases pheromones to attract males. Adults mate under the bark, and create serpentine egg galleries within the phloem, within which developing larvae completes four instars. Once larvae develop into the final instar, they move into the corky bark to pupate for approximately two weeks (Sullivan 2011). Teneral and newly emerged adults sclerotize to a brownish to black color within a week.

SPB typically infests all southern yellow pine tree species (18 species) in the southeastern United States (Hayes et al. 1992). SPB are known to colonize loblolly (*P. taeda* L.), pitch (*P. rigida* Mill.), pond (*P. serotina* Michx.), shortleaf (*P. echinata* Mill.), Virginia (*P. virginiana* Mill.), and white (*P. strobus* L.) pines (Feldman 1981, Georgia Forestry Commission 2009). Longleaf pines (*Pinus echinata* Mill.) appear to be least susceptible to SPB infestations. Pine trees release a blend of phenolics and monoterpenes, called oleoresin at the site of insect attack. Toxins within the oleoresin become more concentrated with varying ratios. Typically, necrotic lesions (pitch tubes) forms at the attack site, where the beetle has been expelled and phloem tissues may resin blisters or canal filled with concentrated allelochemicals that interferes with gallery formation (Ragenovich 1980). Pines can successfully stop an infestation by entombing or killing adults or at least interfering with oviposition or gallery construction. Pitch tubes are reddish to brown in color and may contain evacuated beetles. SPB “spots” are characterized by dead trees (brown needles), trees in declining health (greenish to brown needles), and weakened trees (green needles but pitch tubes are present) (Georgia
Forestry Commission 2009). However, the success of a SPB infestation depends on a number of host factors including tree health, forest community structure, weather, ambient temperature, beetle behavior, and reproductive success (Ragenovich 1980).

A significant chemical component of oleoresin released by pine tree is α-pinene (Erbilgin and Raffa 2001). Alpha-pinene is likely the most important monoterpene in the SPB behavioral complex (Blanche et al. 1984). SPB is attracted to and metabolizes α-pinene into two conspecific and intraspecific attractant pheromones, cis- and trans-verbenol. Once female beetles have arrested on a host, they bore into the phloem layer which stimulates emission of a conspecific attractant called frontalin by females and increases the release of host volatiles. The release of α-pinene and attractant pheromones of successfully attacking SPB, facilitate colonization by conspecifics. Upon mating, male SPB releases endo-brevicomin which deters other beetles from the point where endo-brevicomin is released (Pittman et al. 1969, Pureswaran et al. 2008, Sullivan et al. 2011) and redirects flying SPB to neighboring hosts where female SPB release frontalin (Sullivan et al. 2011). Hence, flying SPB are attracted to frontalin, trans-verbanol, and host volatiles, and their flight is directed to neighboring pines by the release of endo-brevicomin (Sullivan et al. 2011).

SPB infestations are identified by “pitch tubes” on the trunk of infested trees and spatially identified by “spots” across the forest landscape (Billings 2011). Pitch tubes are reddish to brown clumps of sap that are visible at the entry wound on the tree trunk; where the tree has released sap in an effort to remove borrowing beetles. As SPB populations increase, infestation spots enlarge destroying new pines until the population crashes or management practices stop spot expansion. In the East Texas,
encompassing ≤ 20 trees rarely enlarge unless there are neighboring trees (within 6-8 m) that have been freshly attacked. In the spring, spot growth is attributed to ambient temperatures that facilitate beetle dispersal and immigration and not stand conditions. Spot growth is associated with slow tree growth and high stand density. Spot growth slows in cooler months and is limited if either tree spacing is ample (which reduces inter-tree resource competition), or adult emergence and sex pheromone production is too low to continue spot growth (Schneider 1995).

A close relationship between SPB ecology and epidemiology to lightning strikes has been postulated (Coulson et al. 1983, Blanche et al. 1984). For example, Hodges and Pickard (1971) identified lightning as an important component in sustaining SPB populations in the Gulf Coastal Plain because it creates habitat in low risk stands for reservoir populations during enzootic population phases (Hodges and Pickard 1971). In Louisiana, Miller (1983) conducted ground-checks and determined that over a four-year period, 29% of active SPB spots were initiated by lightning strikes. It is postulated that endemic SPB populations typically reside in lightning-struck trees along with Ips beetles, and when the stand and weather conditions are optimal, they can use those trees as hot-spots to start an outbreak population in a stand.

1.2 Subcortical Beetles Associated with SPB

SPB are typically found with many other bark beetle species and predators within a single tree creating a subcortical insect community called the southern pine bark beetle guild (SPBBG). The SPBBG contains five Curculionidae species: SPB, D. terebrans (Olivier), I. avulsus (Eichhoff), I. grandicollis (Eichhoff), and I. calligraphus (Germar) (Smith et al. 1990, Payne et al. 1991). The main predators of SPBBG include: Anobium
*punctatum* (DeGeer), *Lasconotus pusillus* LeConte, *Monochamus* species (including *titillator* and *carolinensis* complex), *Namunaria guttulata* (LeConte), *Platysoma cylindrica* (Paykull), *Pycnomerus sulcicollis* (LeConte), *Temnochila virescens* (Fabricius), *Tenebroides marginatus* (Palisot de Beauvois), and *Thanasimus dubius* (Fabricius). Along with the bark beetles, there are a number of woodboring beetles in the families’ Buprestidae and Cerambycidae that may be present within the same trees (Miller 1986). The woodborers are typically secondary colonizers where they attack trees after infestations by bark beetles.

Male *Ips* beetles burrow into phloem and release conspecific pheromones, ipsdienol (*I. avulsus, I. calligraphus*) or ipsenol (*I. grandicollis*) (Vité et al. 1972). A cross-attraction between *Dendroctonus* and *Ips* attractant pheromones during SPB outbreaks is well documented (Byers 1989, Smith et al. 1990, Payne et al. 1991). This behavior enables location of susceptible hosts, and isolates SPBBG species within a host to avoid competition of phloem resources (Birch and Wood 1975, Byers and Wood 1980).

Host volatile, α-pinene, and frontalin are also exploited by the primary predator of SPB, *T. dubius* (Wood 1982, Mizell et al. 1984). *Thanasimus dubius* is a generalist predator and habitat specialist which utilizes the pheromone, frontalin, and volatiles released by damaged or dying pines to locate bark beetle infestations (Wood 1982, Mizell et al. 1984, Haberkern and Raffa 2003). Arriving within 24 hours of a bark beetle infestation, both adult and larval *T. dubius* feed on all life stages and species of bark beetles (Dixon and Payne 1979, Erbilgin and Raffa 2001), but they appear to respond more often to SPB’s semiochemical complexes than those of other species (Haberkern...
and Raffa 2003). Thanasimus dubius does not respond to lures only with α-pinene (Billings 1985, Erbilgin and Raffa 2000). As SPB colonization intensifies, T. dubius populations typically increase as well perhaps contributing to SPB decline with time (Dixon and Payne 1979, Moser and Dell 1979).

1.3 Management of SPB

In southeastern United States, Federal and State forest management agencies forecast SPB populations by monitoring local SPB and T. dubius each spring and fall. Both beetle species are captured using multiple-funnel traps baited with SPB and T. dubius attractants frontalin and the commercially available Sirex lure [70% (+)-α-pinene and 30% (-)-β-pinene] (Turchin and Odendaal 1996, Reeve 1997, Phillips et al. 1999, Billings 2011). The ratio of captured D. frontalis to the total catch of D. frontalis and clerids is used to predict D. frontalis population densities with ~75% accuracy (Billings 2011).

Foresters and land-managers may prevent SPB infestations through prescribed burning, non-commercial thinning, and restorative planting of longleaf pines. Prescribed burning and non-commercial thinning increase tree vigor and resistance by lowering density stand density. Prescribed burning promotes nutrient cycling, controls competing vegetation and removes woody debris (Hermann et al. 1998, Haywood et al. 2001, Sayer and Haywood 2006). Non-commercial thinning decreases tree competition and provides remaining trees adequate lateral area for growth (Guldin 2011). Planting SPB resistant longleaf pine reduces risk of SPB infestations, reintroduces longleaf pine to its historic range within the Southeast, and renews economic value to the property (Schowalter et al. 1981, Georgia Forestry Commission 2009).
The most popular SPB suppression method is salvage cutting or cut-and-leave. Salvage cutting is appropriate if infested trees are of merchantable size and pitch tubes are visible. In the cut-and-leave method, a 12-22 m buffer strip of green, living trees are felled surrounding the infested spot. If the trees are not merchantable, they are piled and chemical insecticide is applied. If the trees are not merchantable, they are piled and chemical insecticide is applied. This method is most effective from May to October in spots where 10-50 trees are infested. To cut-and-leave, foresters cut a buffer strip and fell infested trees towards the center of the spot. The buffer strip consists of green, uninfested trees and is as wide as the average height of the felled trees (~12-20 meters).

SPB infestations are also suppressed by attaching release devices filled with high volumes of the semiochemical, verbenone, to the bole of infested and uninfested pines. Verbenone is a multifunctional pheromone, predominantly released by male SPB. Low volumes of verbenone typically arrest male beetles walking along the bole at points where frontalin is released, such as the entrance of galleries where burrowing females release frontalin (McCarty et al. 1980). In high volumes, verbenone typically reduces the attraction of flying male SPB to sources of α-pinene and frontalin (i.e., a pine infested with female SPB), thus slowing or stopping the advancement of SPB infestations (Payne et al. 1978, Strom and Clarke 2011).

1.4 Thesis Objectives

The overarching goal of this thesis is to explore the population, community, and chemical ecology of SPB and associated subcortical beetles including predators and other bark and wood boring beetles. Results from this study could be used to guide surveying and management for SPB and their associates using semiochemicals in the southeastern
pine stands. As such, the thesis is centered on four major themes and their research objectives as follows:

1. Responses of SPB and its Predator *T. dubius* to α-Pinene Enantiomers

   Understanding the effect of α-pinene (a monoterpene) chirality on SPB behavior may improve prediction accuracy of SPB monitoring programs. Research objectives were to determine whether attraction of SPB and *T. dubius*: 1) differed between enantiomers (positive or negative); 2) were synergized by opposing enantiomers (combining positive and negative enantiomers); 3) differed by volume of enantiomers; and 4) differed by the gender of SPB; and 5) differed by season (spring versus fall).

2. Multi-trophic Interactions of an Endophytic Beetle Community Associated with Southern Yellow Pines

   The goal was to improve our understanding of the semiochemicals that mediate population dynamics of the southern pine bark beetle guild. The hypothesis was that when SPB and *Ips* populations are low, both species locate adequate hosts by participating in an interspecies exploitation of attractant pheromones. Hence, SPB would be more likely or equally attracted to *Ips* pheromones and *vice versa*. The hypothesis is that SPBBG predators may exploit SPB and *Ips* pheromones because bark beetle prey is more difficult to locate when bark beetle populations are low. Research objectives were to determine the responses of SPBBG members and predators to host kairomone, α-pinene, SPB pheromone, frontalin, and *Ips* species pheromones, ipsdienol and / or ipsenol.

3. Relationship between SPB Stand Infestations and Lightning Strikes in the Homochitto National Forest, Mississippi
A significant relationship between lightning strike and proximity of SPB spot formations may be used by land managers to prevent SPB outbreaks. Landowners may use lightning strike coordinates to locate points within a landscape susceptible where stands would be susceptible to SPB infestation. The hypothesis is that lightning is an important natural disturbance that facilitates the epidemiology and ecology of SPB, and there is a positive correlation between the two attributes. The research objective was to determine whether SPB infestations are more likely to occur within biologically significant spatial and temporal parameters of lightning strikes.


Forest management practices (such as burning, thinning, and planting longleaf pine) are effective and widely used to prevent SPB infestations (Billings 2011, Guldin 2011). Prescribed burning, thinning, and planting longleaf pine changes the ecological, floral, and structural function of a forested landscape. The hypothesis was that landscapes managed with SPB prevention techniques may ecologically impact local faunal taxa such as invertebrate indicator species (ants) that are active on the forest floor. The research objective was to understand the impact of three management techniques: prescribed burning, non-commercial thinning, and restorative planting of longleaf pine on ground-active ant assemblages in central Georgia.
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CHAPTER TWO

RESPONSES OF SOUTHERN PINE BEETLE (*Dendroctonus frontalis* Zimmernann) AND ITS PREDATOR *Thanasimus dubius* (Fabricius) TO ALPHA-PINENE ENANTIOMERS

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Abstract

The southern pine beetle *Dendroctonus frontalis* mass-attacks host trees in response to its aggregation pheromone combined with host resin odors that include the monoterpane α-pinene. We investigated discrimination of α-pinene enantiomers in olfactory responses of *D. frontalis* and in behavioral responses of both *D. frontalis* and its major clerid predator *Thanasimus dubius* (Fabricius). Trapping trials during both Spring and Fall assessed attraction of both species to the *D. frontalis* aggregation pheromone components frontalin and *endo*-brevicomin either alone or in combination with α-pinene lures of differing enantiomeric compositions. α-Pinene lures enriched with the (+)-enantiomer (97.5%) increased *D. frontalis* catches significantly more than (-)-enriched (93%) lures but did not differ from racemic lures. Sexes of *D. frontalis* did not significantly differ from one another in their responses to the enantiomers. Analysis of Variance tests (ANOVA) failed to detect a significant difference in lure discrimination by *D. frontalis* and *T. dubius*, and *T. dubius* did not distinguish lures with differing enantiomeric compositions of α-pinene. Dose-response electroantennogram (EAG) studies of *D. frontalis* indicated that their antenna had a slightly lower response threshold to (+) than (-)-α-pinene. Each enantiomer habituated the antenna more to itself than to its antipode, implying the existence of olfactory receptors with differing affinities for the enantiomers. The preference of *D. frontalis* for (+)-α-pinene may affect its host selection behavior and should be considered during formulation of population monitoring lures for this species.
Keywords: α-pinene, *Dendroctonus frontalis*, *endo*-brevicomin, enantiomer, frontalin, *Thanasimus dubius*
2.1 Introduction

*Dendroctonus frontalis* Zimmermann (the southern pine beetle) is one of the most economically important pests of *Pinus* in the southeastern U.S. (Billings 2011). This bark beetle colonizes 18 pine species with loblolly (*P. taeda* L.), pitch (*P. rigida* Mill.), pond (*P. serotina* Michx.), shortleaf (*P. echinata* Mill.), and Virginia (*P. virginiana* Mill.) pines being particularly susceptible hosts (Wood 1982). During outbreaks, *D. frontalis* successively colonize adjacent trees thereby creating characteristic “spots” of dying and dead pines scattered across forested landscapes (Franklin 1970, Schowalter et al. 1981). In the southeastern U.S., *D. frontalis* is endemic within pine landscapes but produces regional outbreaks at roughly 10-12 year intervals (Birt 2011). Activity by *D. frontalis* has caused an average of seven million dollars of loss in annual timber sales in Georgia alone (Georgia Forestry Commission 2009).

Mass colonization by *D. frontalis* of host pines is mediated by a complex of semiochemicals that facilitates host location, host selection, mass aggregation, attack density regulation, and interactions between the sexes (Smith et al. 1993, Sullivan 2011). The hindgut of newly emerged *D. frontalis* females (the gallery-initiating sex) contains pheromone components frontalin and *trans*-verbenol which are released after landing on a host and attract both sexes (Renwick and Vité 1969). Females that successfully penetrate the outer bark begin feeding on phloem; this stimulates increased production of frontalin by the female and triggers the damaged host tissues to release defensive oleoresin containing *α*-pinene (Sullivan 2011, Pureswaran and Sullivan 2012). *α*-Pinene is the predominant volatile constituent of the oleoresin of preferred hosts for *D. frontalis* (Mirov 1961, Phillips et al. 1999). *Trans*-Verbenol and *α*-pinene are apparently

*Thanasimus dubius* is a generalist predator of bark beetles that utilizes the pheromone component frontalin and volatiles released by attacked pines as kairomones for locating *D. frontalis* infestations (Vité and Williamson 1970, Billings and Cameron 1984, Mizell et al. 1984, Billings 1985, Costa and Reeve 2011). Adult *T. dubius* feed on arriving adult bark beetles whereas the predators’ larvae feed on bark beetle brood within the bark (Thatcher and Pickard 1966, Dixon and Payne 1979). Populations of *T. dubius* typically increase as *D. frontalis* colonization intensifies on the landscape and evidence suggests that they are a significant population regulating force for *D. frontalis* (Moser and Dell 1979, Turchin et al. 1991, Reeve 1997, Turchin et al. 1999).

Semiochemicals of *D. frontalis* are used to monitor their population levels each year in the southeastern U.S. (Turchin and Odendaal 1996, Reeve 1997, Billings 2011). During four weeks following the springtime blooming of dogwoods (*Cornus* spp.), *D. frontalis* and *T. dubius* are sampled by a network of multiple-funnel traps deployed
throughout the pine forested areas of the southern United States. These traps are uniformly baited with frontalin and a host-component lure that is composed predominantly of α-pinene. Numbers of captured *D. frontalis* and their ratios to *T. dubius* catches are entered into a predictive model that can forecast the summertime abundance of *D. frontalis* infestations with an estimated 75% accuracy (Billings 2011).

We conducted field trapping and electroantennogram studies to assess whether responses by *D. frontalis* and *T. dubius* to α-pinene are affected by its enantiomeric composition. Our research objectives were to determine whether enantiomeric preferences exist and whether these differ between species, sexes (for *D. frontalis* only), and seasons (Fall vs Spring). Pine species differ in the ratios of α-pinene enantiomers they produce (Marques et al. 2012), hence discrimination of α-pinene enantiomers could potentially influence *D. frontalis* host tree preferences and *T. dubius* prey finding efficiency. Furthermore if enantiomeric preferences are absent, it should be possible to change the enantiomeric composition of the α-pinene component of the population monitoring lure (as may be dictated by cost or availability) without reducing lure sensitivity or requiring recalibration of the predictive model.

### 2.2 Methods

#### 2.2.1 Study Sites

Field bioassays were conducted in the spring (19 April-4 May 2011) and fall (12-27 October 2011) within the Oconee National Forest in Greene County, Georgia (Piedmont region of Georgia; 33°39´ N, 83°16´ W). Traps were deployed in stands of mature loblolly and shortleaf pines that had a mean (± SD) diameter at breast height (DBH) of 53 ± 2 cm and were 31 ± 3 years old. The understory was composed of mixed
hardwood trees (*Liquidambar styraciflua* L. and *Quercus* species) in clayey-Udults soil (Griffith et al. 2001, National Resources Conservation Service 2013). The local annual mean temperature was 16.3 ± 0.9 °C and precipitation was 191.3 ± 0.4 cm (data supplied by Georgia Automated Environmental Monitoring Network 2013). At the time of the experiment, *D. frontalis* was not in outbreak status and spots had not been detected in this section of the Oconee National Forest, Georgia since 2000 (Georgia Forestry Commission 2009). Natural sources of semiochemicals that might interact with trap lures (e.g., pines damaged by wind, lightning, or insect attack) were not apparent within our sampled stands.

### 2.2.2 Lure Treatments for Field Trials

Twelve-unit multiple funnel traps (Lindgren 1983) (Contech International Inc., Delta, British Columbia) were hung from free-standing metal poles with the collection cup positioned 1-1.5 m aboveground. Trap collection cups were filled with 250-350 ml of propylene glycol (Prestone® Low Tox® Antifreeze/Coolant, Prestone Products Corporation, Danbury, Connecticut) and water (3:1 mixture) to arrest beetle movement and preserve specimens. Traps were baited uniformly with racemic *endo*-brevicomin and frontalin plus either: 1) α-pinene enriched in the (+)-enantiomer; 2) α-pinene enriched in the (-)-enantiomer; 3) racemic α-pinene; 4) racemic α-pinene at twice the release rate of treatment 3 (i.e., two racemic release devices), or 5) no α-pinene (control) (Table 2.1).

The α-pinene release devices were constructed from pieces of 3.2 cm diam. low-density polyethylene (LDPE) tubing (2 mil) sealed with an impulse sealer on both ends to create a 5.1 x 3.8 cm enclosure in the shape of an irregular tetrahedron (each seal was at right angles to the other). Each device was filled with 6 ml of α-pinene before the second seal
was made (Table 1.1). The elution rate of α-pinene devices was ~450 mg/d at 21°C for approximately one week. On each funnel trap, one α-pinene release device and two LDPE microcentrifuge tubes containing frontalin (Synergy Semiochemicals Corp., British Columbia) were secured to the lip of the fourth funnel above the collection cup. To enhance beetle attraction to baited traps, an endo-brevicomin bubble cap (Synergy Semiochemicals, Table 1.1) was attached to the tip of a bamboo gardening rod 1.5 m above the ground and 6 m southwest of each trap (Sullivan and Mori 2009).

**2.2.3 Experimental Design for Field Trials**

Four replicate transects of five traps each were established, and one of the five treatments was assigned at random to each trap within each transect. Interference among transects and traps was limited by installing transects $\geq 150$ m apart and traps $\geq 100$ m apart within transects (Turchin and Odendall 1996). Lure-induced attacks on adjacent trees were limited by installing traps $> 9$ m from all pine trees and $> 15$ m from any pines located in a northeastern (prevailing downwind) direction from each trap. Every three days catches were collected and treatments re-randomized without replacement to any previous position within transects so each treatment occurred at each trapping site once during five collection periods. Hence the experimental design was four complete Latin squares with each square containing five traps (columns) and five consecutive trapping intervals (rows). A random number table was used to re-assign treatments to columns and rows (Winer et al. 1991, SAS 2007).

Captures of adult *D. frontalis* and *T. dubius* were recorded and preserved in 70% ethanol. Female *D. frontalis* were identified by the presence of a bulging callus partially encircling the anterior portion of the pronotum, and males were identified by the deep
medial groove and paired tubercles of the frons (Osgood and Clark 1963, Payne 1980). *Thanasimus dubius* were not sexed. Voucher specimens were deposited at the Georgia Museum of Natural History, Athens, Georgia.

### 2.2.4 Electroantennogram Assays

Electroantennogram (EAG) assays were performed to identify differences in olfactory responsiveness by *D. frontalis* to the enantiomers of α-pinene when presented over a range of concentrations. To obtain test subjects, Lindgren multiple funnel traps were deployed on 5-9 August 2013 in the same location as field trials in Oconee National Forest in Georgia and baited either as treatments 1 or 2 of the field trials. Assayed beetles were derived in equal numbers from traps either with (+) or (−)-enriched α-pinene to detect whether individuals trapped with either enantiomer had inherently greater olfactory sensitivity to that enantiomer. Trap cups were lined with moistened paper towels, and the mouth of the bottom trap funnel of each trap was covered by a ~20 cm diameter disk of 3.2 mm mesh hardware cloth to prevent entry of beetle predators. Live *D. frontalis* were collected every 1-2 days, and held in refrigeration on pieces of moistened paper wipers until used in EAG tests (i.e., after 6-12 d).

Antennal preparations and signal recording apparatus for *D. frontalis* were as described previously in Sullivan (2005). Antennal preparations were exposed to a stream of humidified, purified air (400 ml/min) delivered from a 1 cm i.d. glass tube into which brief “puffs” (50 ml/min for 2 sec) of test odors were introduced from Pasteur pipettes. Each Pasteur pipette contained a 0.5 x 7 cm piece of Whatman #1 filter paper to which were added 35 µl of water (to equalize humidity with the delivery tube airstream) and 10 µl of test solution. This solution consisted of either α-pinene dissolved in mineral oil
(Smart Sense™, Hoffman Estates, Ill.) at $10^{-1}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$ by volume; frontalin dissolved in mineral oil ($10^{-2}$, as a positive control); or pure mineral oil (negative control). A separate dilution series was created for both (+) and (-) $\alpha$-pinene (both enantiomers: Aldrich, 99% chemical and 97% optical purity). In each 46 min trial, an antennal preparation was exposed sequentially to the positive and negative controls followed by all twelve $\alpha$-pinene dilutions in random order; this was followed again by the two controls. Each exposure was separated by 3 min, and the antennal voltage amplitudes coinciding with each exposure were recorded. Preparations of eight males and eight females [with half of each trapped with either the (+) or (-) enantiomer of $\alpha$-pinene] were tested. A single insect was used for only one trial.

Additionally, an EAG bioassay was performed to detect the presence of olfactory receptors with differing affinities for either enantiomer of $\alpha$-pinene. Antennal preparations were partially adapted by exposure for 20 sec to one of the enantiomers delivered from a pipette into the airstream passing over the preparation. A test stimulus consisting of a 2 sec puff of the same or opposite enantiomer was delivered into the airstream from a second pipette 15 sec after initiation of the adapting stimulus. Both pipettes contained a folded, 1 x 7 cm piece of filter paper treated with 35 µl purified water and 25 µl of a $10^{-2}$ dilution of either $\alpha$-pinene enantiomer in mineral oil, and the airflow through each was 50 ml/min during odor delivery. All four possible combinations of adapting and test stimuli were assayed once on each of 10 preparations (equal numbers of either sex), with a randomized order of presentation for each enantiomer of the adapting and test stimuli. The preparation was allowed to recover for 3.5 min between exposures to olfactory stimuli.
2.2.5 Statistical Analyses of Beetle Captures

Poisson regression was used to assess main effects and interactions for lure treatment, beetle species, sex (D. frontalis), and season. Transformation was not necessary because the natural log link function associated with Poisson regression addressed overdispersion and several zero catches within the data. Tukey-Kramer tests were used to analyze treatment main effects within species and within sex of D. frontalis and season (α = 0.05) (SAS 2007). Tukey-Kramer tests were used to analyze treatment main effects within species and within sex of D. frontalis and season (α = 0.05) (SAS 2007).

2.2.6 Statistical Analyses of Electroantennogram Assays

To compensate for typical decline in antennal responsiveness over time and its effects on amplitudes of responses to test stimuli during each trial, responses to α-pinene dilutions were normalized using the responses to the positive and negative controls. This was done by calculating the linear equations (with X as time and Y as response amplitude) which joined the positive and negative (respectively) control responses at the beginning and end of each trial. The Y-values of these equations which coincided with delivery of each test stimulus were used as the time-corrected amplitudes for positive and negative control responses. Estimated negative control amplitudes were subtracted from the α-pinene dilution response, and then this difference was divided by the estimated positive control response. Normalized responses were cube root transformed to remove heteroscedasticity and analyzed by a mixed model ANOVA (PROC MIXED, SAS 2007) with dilution concentration (dose), dilution enantiomer, sex, and live-trap lure enantiomer (and all possible interactions) as fixed factors and trial nested within live trap enantiomer
by sex as a random factor. A SLICE statement was used to generate pairwise contrasts ($\alpha = 0.05$) of responses to enantiomers within each dose, and the resulting $P$-values were adjusted with a Bonferroni correction. A $t$-test was performed on means of the cube root transformed, normalized responses to each dose and enantiomer to determine whether they exceeded the threshold of response (i.e., were significantly different from zero).

The arithmetic differences in amplitude of EAG peaks produced by the (-) and (+) test stimuli during exposure to either (+) or (-) adapting stimuli were contrasted with a paired $t$-test using antennal preparation as subject. Similarly the raw response amplitudes to (-) and (+) test stimuli were contrasted when either (-) or (+) was the adapting stimulus.

2.3 Results

2.3.1 Field Bioassays Results

The majority of $D.\ frontalis$ captures (Table 2.2) occurred in the Spring (93%) whereas $T.\ dubius$ captures were predominantly in the Fall (72%). There was not a significant interaction between treatment and species, implying that $D.\ frontalis$ and $T.\ dubius$ may not respond differently to the enantiomers of $\alpha$-pinene ($F=1.22; d.f.=4, 345.1; P=0.303$) (Fig. 2.1). There was a significant main effect for lure treatment within $D.\ frontalis$ ($F=19.43; d.f. =4, 167.1; P<0.000$). With sexes pooled, all $\alpha$-pinene lure treatments significantly increased catches of $D.\ frontalis$ (Fig. 2.1). The (+)-$\alpha$-pinene lure and the double racemic lure did not differ significantly from each other but both increased $D.\ frontalis$ catches significantly more than did the (-)-$\alpha$-pinene lure. The single racemic lure did not differ significantly from any of the other $\alpha$-pinene lure treatments in $D.\ frontalis$ catches. There was no main effect for season by treatment.
interaction for *D. frontalis* ($F=1.04; d.f.=4, 162.2; P=0.388$) indicating that their responses to the enantiomers did not vary significantly between the Spring and Fall trapping periods (Table 2.3); however, Fall catches were very low resulting in low power for this test.

More male ($N=817$) than female ($N=625$) *D. frontalis* were captured and the main effect of sex was marginally significant ($F=3.74; d.f.=1,22.44; P=0.066$, Table 2.1). However, there was not a treatment by sex interaction ($F=0.98; d.f.=4, 357.9; P=0.417$), indicating that the two sexes of *D. frontalis* did not differ significantly in their discrimination of the enantiomers. With sexes considered separately, responses of both males and females were significantly enhanced by the presence of any $\alpha$-pinene lure (Fig. 2.2). Males were more responsive to any $\alpha$-pinene lures which included the (+)-enantiomer than to the (-)-$\alpha$-pinene lure [i.e., (-)-$\alpha$-pinene vs (+)-$\alpha$-pinene, $t=3.60$; $d.f.=1,165.9; P=0.004$; vs racemic $\alpha$-pinene, $t=-2.97$; $d.f.=1, 166.1; P=0.028$; vs two racemic $\alpha$-pinene, $F=-5.67; d.f.=1,165.8; P<0.001$] (Fig. 2.2). Traps which included two racemic $\alpha$-pinene lures were significantly more attractive to female *D. frontalis* than traps with a (-)$\alpha$-pinene lure ($t=-3.03; d.f.=4, 172.8; P=0.023$), but otherwise females did not differ significantly in responses to the $\alpha$-pinene lures.

Captures of *T. dubius* differed significantly by treatment ($F=13.19; d.f.=4, 171.2; P<0.001$) but not season ($F=0.35; d.f.=1, 8.09; P=0.572$), and there was not an interaction between treatment and season ($F=1.53; d.f.=4, 170.2; P=0.196$). All $\alpha$-pinene lure treatments significantly increased catches of *T. dubius* (Fig. 2.1b). *Thanasimus dubius* was significantly more attracted to two racemic $\alpha$-pinene lures than any other lure treatment [i.e., vs (+)-$\alpha$-pinene, $t=-3.07$; $d.f.=1, 172.6; P=0.021$; vs (-)-$\alpha$-
pinene, \( t = -3.79; d.f. = 1, 173.7; P = 0.002; \) vs one racemic \( \alpha \)-pinene lure, \( t = -4.37; d.f. = 1, 172; P < 0.001, \) Fig. 2.1], but did not distinguish lures differing merely in the enantiomeric composition of \( \alpha \)-pinene.

2.3.2 Electroantennogram (EAG) Assays

The mixed model ANOVA of EAG responses to a dilution sequence of the two enantiomers of \( \alpha \)-pinene indicated that the fixed effect dose (\( F = 751.3; d.f. = 5, 132; P < 0.001 \)), and the interaction between dose and dilution enantiomer (\( F = 6.74; d.f. = 5, 132; P < 0.001 \)) were highly significant (Fig. 2.3). Dilution enantiomer was not significant as a main effect (\( P = 0.53 \)). Factors sex, live-trap enantiomer, and all possible interactions involving these two factors (i.e., their interactions with each other and with any of the other factors in the ANOVA) were not significant (\( P > 0.14 \)). When data were pooled by sex and trap enantiomer (as justified by the lack of significant interactions for these factors), all doses for both stimulus enantiomers exceeded the threshold of detection of the EAG (i.e., were significantly greater than zero) except the \( 10^{-6} \) dose for both enantiomers and the \( 10^{-5} \) dose for the (-) enantiomer (Fig. 2.3). Two significant pairwise contrasts were detected between the enantiomers at single doses: 1) response to (-)-\( \alpha \)-pinene was significantly greater than to (+)-\( \alpha \)-pinene at the highest tested dose of \( 10^{-1} \) (\( F = 11.38; d.f. = 1, 132; P = 0.006 \)); and 2) response to (+)-\( \alpha \)-pinene was significantly greater than to (-)-\( \alpha \)-pinene at a dose of \( 10^{-4} \) (\( F = 8.91; d.f. = 1, 132; P < 0.02 \)) which was the lowest dose at which both enantiomers exceeded the threshold of detection (Fig. 2.3).

The arithmetic difference between EAG amplitudes produced by puffs of either the (+) or (-) enantiomers of \( \alpha \)-pinene (at a dilution of approximately \( 2.5 \times 10^{-2} \)) depended upon the enantiomer used to adapt the antennal preparation prior to and during the puffs.
$t = -4.01; d.f. = 9; P = 0.003)$. When the preparation was adapted to the (+)-enantiomer, EAG amplitude was greater to (-) than to (+) puffs (mean difference = 0.118 mv; SE=0.034; $t = 3.44; \text{d.f. } = 9; P = 0.007$), but the reverse occurred when the preparation was adapted to the (-) enantiomer (mean difference = -0.060 mv; SE=0.019; $t = -3.22; \text{d.f. } = 9; P = 0.010$). These differences were relatively small, averaging 26% of the voltage amplitude of the EAG response to test stimuli.

### 2.4 Discussion

Our results demonstrate that $\alpha$-pinene can enhance the response of $D.\ frontalis$ and $T.\ dubius$ to traps baited with frontalin and endo-brevicomin. Catches of $D.\ frontalis$ and its predator, $T.\ dubius$, were on average 2-5 times greater when baited traps included the host monoterpene $\alpha$-pinene rather than frontalin and endo-brevicomin alone. Previous studies have similarly shown that $\alpha$-pinene and turpentine derived from $P.\ taeda$ (which has a high $\alpha$-pinene content; Mirov 1961) can enhance $D.\ frontalis$ and $T.\ dubius$ responses to trap lures which include frontalin (Renwick and Vité 1969, Billings 1985, Sullivan et al. 2007, Hofstetter et al. 2008). Our data show that attraction enhancement can occur across a broad range of enantiomeric ratios of $\alpha$-pinene [i.e., 7.0-97.5% (+)-$\alpha$-pinene] but that sensitivity of lures for detecting $D.\ frontalis$ might be improved by increasing the (+)-composition of the $\alpha$-pinene component. The apparent preference of $D.\ frontalis$ for (+)-$\alpha$-pinene implies that alterations to the enantiomeric composition of $\alpha$-pinene in lures used for monitoring $D.\ frontalis$ population levels (Billings 2011) could affect survey results and the forecasting of outbreaks.

Chiral specificity in behavioral responses by $Dendroctonus$ bark beetles to $\alpha$-pinene has been studied extensively only in $D.\ valens$ (LeConte) for which $\alpha$-pinene
apparently serves as a primary host location kairomone and is attractive in the absence of other semiochemicals (Erbilgin et al. 2007). *Dendroctonus valens* has alternately displayed significant preference for (+), (-), or neither enantiomer of α-pinene when trapping studies were conducted in different locations within North America and China, and when α-pinene was presented either alone or in combination with other semiochemicals (Hobson et al. 1993, Erbilgin and Raffa 2001, Erbilgin et al. 2001, Erbilgin et al. 2007). Our results with *D. frontalis* involved a single population, and future studies should address the possibility of regional variation in this species’ responses to α-pinene enantiomers.

Pine species vary in the enantiomeric ratio of α-pinene present in their oleoresin (Hobson et al. 1993, Marques et al. 2012) although a systematic study of α-pinene chirality in *D. frontalis* host pines has not been published. Thus the preference of *D. frontalis* for (+)-α-pinene could potentially play a role in host discrimination. This is suggested by the observation that α-pinene in the oleoresin of *P. taeda*, a preferred host of *D. frontalis* (Hain et al. 2011), is predominantly plus (Gambriel et al. 1985, Marques et al. 2012) whereas the resin of an apparently less susceptible species, *P. elliotti* Engelm. (Hodges et al. 1979), is reported to be nearly racemic (Marques et al. 2012). There is little evidence that α-pinene or host odors in general are attractive alone to *D. frontalis* and thus play a role in primary host location by this species (Payne and Coulson 1985, Sullivan 2011). Thus it is unlikely that *D. frontalis’* preferential attraction to (+)-α-pinene could directly mediate initial selection or location of a host by pioneering females. However, once an attack is initiated on a host, our results suggest that hosts with relatively higher (+)-α-pinene content will be more attractive and presumably more
aggressively attacked than others. The chirality of the host’s α-pinene can influence the composition of the monoterpene pheromone components produced by bark beetles (Klimetzek and Francke 1980), and this could play a role in determining reproductive fitness and thus selectivity of beetles for trees with specific ratios of α-pinene enantiomers.

Enantiomeric specificity in bark beetle responses to semiochemicals is common and well documented (Seybold 1993), and, in the case of D. frontalis, differing behavioral or olfactory responses have been demonstrated with regard to its pheromone components frontalin (Payne et al. 1982), endo-brevicomin (Vité et al. 1985, Sullivan et al. 2007, Sullivan and Mori 2009, Sullivan et al. 2011) and verbenone (Salom et al. 1992).

Our electrophysiological experiments showed that the magnitude of voltage deflections produced by D. frontalis antennae differed for the two enantiomers of α-pinene and that this difference depended on the concentration of the α-pinene stimulus. The (-)-enantiomer stimulated a stronger response than (+) at high concentrations but the reverse occurred at low concentrations. Furthermore, the threshold of detection of the (+) enantiomer (i.e., $10^{-5}$) was lower than for the (-)-enantiomer (i.e., $10^{-4}$). Relatively stronger antennal responses at low doses and lower response thresholds ostensibly signify greater capacity of an insect to detect a given compound. Since the two enantiomers of α-pinene have identical vapor pressures and other physical characteristics, the implication is that D. frontalis can respond to (+)-α-pinene at greater distances from a source than (-) when these are released at identical rates. A capacity to sense the (+) enantiomer at greater distances than (-) might in part explain the greater responses of flying D. frontalis.
to traps baited with lures enriched with the (+)-α-pinene enantiomer. The stronger antennal responses to the (-)-enantiomer at high concentrations suggests that the relative stimulatory capacity and ecological significance of α-pinene enantiomers may change when the beetle is close to a source of α-pinene, such as when approaching a gallery entrance.

Furthermore, we observed that partial olfactory adaptation to either enantiomer reduced EAG responses to the adapted enantiomer more than the non-adapted enantiomer. This implies the existence of olfactory receptors with differing affinities for the two enantiomers of α-pinene, which is a prerequisite for the capacity of D. frontalis to distinguish the enantiomers. Our antennogram results with D. frontalis parallel those of an EAG study of enantiomeric specificity of D. valens (White and Hobson 1993). As with D. frontalis in our study, D. valens did not exhibit sexual dimorphism in EAG responses to α-pinene enantiomers, produced larger EAG voltages in response to (-)- than (+)-α-pinene at high concentrations of exposure, and exhibited enantiomer-specific adaptation.

Bark beetle predators commonly seek their prey by responding to beetle aggregation pheromones often in combination with tree volatiles released by beetle damage (Payne 1989). Furthermore chirality of host tree monoterpenes can influence responses by bark beetle predators to the pheromones of their prey (Erbilgin and Raffa 2001). However, in our trapping study we failed to detect a significant behavioral preference by T. dubius for either enantiomer of α-pinene. Thus, we saw no evidence that chirality of α-pinene from D. frontalis’ host pines influences the prey-seeking
behavior of *T. dubius* when they use the *D. frontalis* pheromone as a host location kairomone.

We caught 93% of trapped *D. frontalis* in the spring replicates, and higher trap catches in the spring relative to other times of the year is a typical pattern for this species (B.T. Sullivan, unpublished data). In the springtime, *D. frontalis* enter mass dispersal during which they abandon dead trees where they have overwintered and seek new hosts (Sullivan 2011). In contrast, 72% of *T. dubius* catches were in the fall replicates. Populations of *T. dubius* vary as a function of *D. frontalis* populations, and there is typically a predator-prey lag (Reeve 1997, Stephen et al. 2011). The apparent seasonal asynchrony in catches observed in our study may reflect this lag.

### 2.5 Acknowledgements

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Table 2.1. Semiochemical lure components used in trapping trials for *D. frontalis* and *T. dubius* in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>Semiochemical</th>
<th>Chemical Purity*</th>
<th>Enantiomeric Purity*</th>
<th>Quantity</th>
<th>Release Rate</th>
<th>Release Device</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-α-Pinene</td>
<td>99%</td>
<td>97.5%</td>
<td>6 ml</td>
<td>~450 mg/d at 21°C</td>
<td>Sealed LDPE sachet</td>
</tr>
<tr>
<td>(-)-α-Pinene</td>
<td>99%</td>
<td>93.0%</td>
<td>6 ml</td>
<td>~450 mg/d at 21°C</td>
<td>Sealed LDPE sachet</td>
</tr>
<tr>
<td>(+/-)-α-Pinene</td>
<td>99%</td>
<td>(1:1 blend of above)</td>
<td>6 ml</td>
<td>~450 mg/d at 21°C</td>
<td>Sealed LDPE sachet</td>
</tr>
<tr>
<td>(+/-)-α-Pinene</td>
<td>99%</td>
<td>(1:1 blend of above)</td>
<td>2 x 6 ml</td>
<td>~900 mg/d at 21°C</td>
<td>Two sealed LDPE sachets</td>
</tr>
<tr>
<td>(+/-)-Frontalin</td>
<td>98%</td>
<td>Racemic</td>
<td>2 x 300 mg</td>
<td>5 mg/d at 20°C*</td>
<td>Two LDPE microcentrifuge tubes</td>
</tr>
<tr>
<td>(+/-)-endo-Brevicomin</td>
<td>96%</td>
<td>Racemic</td>
<td>40 mg</td>
<td>0.3 mg/d at 20°C*</td>
<td>Bubble cap</td>
</tr>
</tbody>
</table>

* Data provided by supplier. Supplier names given in text.
Table 2.2. Total trap catches of *D. frontalis* and *T. dubius* during spring and fall 2011 field trials in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>Beetle Species</th>
<th>Season</th>
<th>Sex</th>
<th>Without α-Pinene</th>
<th>(+) α-Pinene</th>
<th>(-) α-Pinene</th>
<th>(+/-) α-Pinene</th>
<th>(+/-) α-Pinene (two devices)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dendroctonus frontalis</em></td>
<td>Spring</td>
<td>Both</td>
<td>73</td>
<td>426</td>
<td>207</td>
<td>325</td>
<td>513</td>
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<tr>
<td></td>
<td></td>
<td>Female</td>
<td>36</td>
<td>218</td>
<td>108</td>
<td>135</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>37</td>
<td>208</td>
<td>99</td>
<td>190</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>Both</td>
<td>1</td>
<td>20</td>
<td>15</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>0</td>
<td>15</td>
<td>13</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td><em>Thanasimus dubius</em></td>
<td>Spring</td>
<td>Both</td>
<td>19</td>
<td>43</td>
<td>41</td>
<td>38</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>Both</td>
<td>14</td>
<td>136</td>
<td>107</td>
<td>61</td>
<td>209</td>
</tr>
</tbody>
</table>
Figure Legend

**Fig. 2.1.** Mean (± SE) number of A) *Dendroctonus frontalis* and B) *Thanasimus dubius* captured per treatment during spring (April 19-May 4, 2011) and fall (October 12-27, 2011) in the Oconee National Forest, Georgia. All traps were baited with frontalin and *endo*-brevicomin, and either a single device of (+)-α-pinene, (-)-α-pinene, or (+/-)-α-pinene; two devices of (+/-)-α-pinene; or no additional lure.

**Fig. 2.2.** Mean (± SE) number of male and female *Dendroctonus frontalis* captured per treatment during field trials in the spring (April 19-May 4, 2011) and fall (October 12-27, 2011) in the Oconee National Forest, Georgia. All traps were baited with frontalin and *endo*-brevicomin, and either a single device of (+)-α-pinene, (-)-α-pinene, or (+/-)-α-pinene; two devices of (+/-)-α-pinene; or no additional lure.

**Fig. 2.3.** Electroantennogram amplitudes of *D. frontalis* antenna in response to six concentrations of either (+) or (-) α-pinene (n = 16). The X-axis labels indicate the proportion of α-pinene dissolved in mineral oil that was used as the odor stimulus. Amplitudes were normalized relative to those generated with a standard consisting of the pheromone frontalin (amplitude = 1.0) diluted 1/100 in mineral oil. Data for both sexes (n = 8 for each) were combined in this single figure because no sex effects or interactions were detected by ANOVA. Dots associated with treatment means indicate that their associated EAG voltage amplitudes were significantly greater than those of the solvent blank (i.e., a response of zero). Asterisks indicate that antennal response differed significantly between the two enantiomers at a particular dose.
Figure 2.1.
Figure 2.2.

Mean (± SE) *Dendroctonus frontalis* per Treatment

- No α-pinene
- (+)-α-pinene
- (-)-α-pinene
- (±)-α-pinene
- 2 x (±)-α-pinene

Males

- b, d
- b

Females

- a
- b
- c
- b, c
- c
Figure 2.3.
CHAPTER THREE

MULTI-TROPHIC INTERACTIONS MEDIATED BY SOUTHERN PINE
BARK BEETLE GUILD SEMIOCHEMICALS

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Abstract

We determined the semiochemical interactions and the role of tree volatiles on the aggregation behaviors among the southern pine bark beetle guild (SPBBG) guild members [*Dendroctonus frontalis* Zimmermann, *D. terebrans* (Olivier), *Ips avulsus* (Eichhoff), *I. calligraphus* (Germar) and *I. grandicollis* (Eichhoff)] and associated predatory beetles in southern pine stands. In 2010-2011, three experiments were conducted when *D. frontalis* and *Ips* beetle populations were low to assess: 1) the interspecies cross-attraction between *D. frontalis* and *Ips* semiochemicals; 2) the relative role of α-pinene and frontalin in the attraction of SPBBG members and associated predatory beetles to *Ips* lures (ipsdienol and ipsenol); and 3) whether attraction to α-pinene is synergized or inhibited by *Ips* lures (ipsdienol and/or ipsenol). A total 47,582 beetles in 14 families and 41 species were captured in the study. In Experiment 1, *D. frontalis*, *D. terebrans*, *Pycnomerus sulcicollis* LeConte, and *Thanasimus dubius* (Fabricius) were more attracted to α-pinene and frontalin lures, while *I. avulsus* and *I. grandicollis* were predominantly attracted to *Ips* lures. Significantly more *Lasconotus pusillus* LeConte, *Namunaria guttulata* (LeConte), *Platysoma cylindrica* (Paykull), and *Temnochila virescens* (Fabricius) were captured in traps baited with α-pinene, frontalin, ipsdienol and ipsenol. In Experiment 2, *D. terebrans*, *Dryophthorus americanus* Bedel, *Hylastes tenius* Eichoff, *I. avulsus*, *L. pusillus*, *Monochamus* spp., and *Pycnomerus sulcicollis* LeConte were captured in greater numbers in traps baited with α-pinene. Traps baited with α-pinene and frontalin captured significantly more *D. frontalis*, *T. virescens*, and *T. dubius*. In Experiment 3, only *Namunaria guttulata* (LeConte) was less attracted to traps baited with α-pinene and both *Ips* lures. Our results suggest when *D. frontalis* and
Ips populations are low, Ips species do not exploit D. frontalis pheromones and do not differentiate between ipsdienol and ipsenol. Further, I. avulsus may be less attracted to the release of α-pinene, and D. frontalis and T. dubius do not exploit Ips pheromones when D. frontalis and Ips populations are low. Our results also suggest H. tenius is attracted to α-pinene and Ips pheromones, and Acanthus obsoletus Olivier, H. baltimoresis (Gravenhorst), L. pusillus Monochamus spp., N. guttulata, P. flavicorns (Fabricius), P. cylindrica, P. sulcicollis, T. virescens, T. dubius, and T. marginatus exploit semiochemicals released by SPBBG. These findings may lead to improved biological control strategies or bark beetle monitoring programs when D. frontalis and Ips populations are low in pine stands.

**Keywords:** Dendroctonus spp., Ips spp., monoterpenes, pheromones, Pinus spp., southeastern United States
3.1 Introduction

Endophytic herbivorous insects use an array of semiochemical cues to locate optimal hosts to colonize, and to attract their conspecifics for reproduction (Mitton and Sturgeon 1982). Conifer trees have a high and diverse load of endophytic insects that form distinct phloem, xylem, seed and cone, and root feeding guilds. For example, bark beetles (Coleoptera: Curculionidae: Scolytinae) colonize phloem tissue, whereas ambrosia (Coleoptera: Curculionidae: Scolytinae) and woodboring (Coleoptera: Buprestidae and Cerambycidae) beetles colonize xylem tissue (Mitton and Sturgeon 1982). Host plant cues, such as terpenes and ethanol are released when trees are under stress, and used by endophytic insects to varying degrees for communication (Raffa et al. 2005, Miller 2006). Synthesized pheromones of bark beetles are distinct enough to avoid interspecies mating, and orchestrate colonization to overcome host defenses by conspecifics (Mitton and Sturgeon 1982). An evolutionary advantage may exist for multiple and closely related species within a guild to respond to similar chemical cues. This may allow more efficient host location, competitive advantage for the same resource, and perhaps alleviate predation and parasitism pressures across species (Mitton and Sturgeon 1982, Payne et al. 1984). However, tradeoffs may exist for inter- and intra-species where there is a fine balance between the sensitivity and attraction to similar semiochemicals.

Five sympatric bark beetle species, referred to as the southern pine bark beetle guild (SPBBG) constitute the major phloem-feeding endophytic guild on yellow pines (Pinus spp.) trees in southeastern U.S. forests (Nebeker 2011). Members of the guild include southern pine beetle, Dendroctonus frontalis Zimmermann, black turpentine
beetle, *D. terebrans* (Olivier), small southern pine engraver, *Ips avulsus* (Eichhoff), eastern five-spined engraver beetle, *I. grandicollis* (Eichhoff), and eastern six-spined engraver beetle, *I. calligraphus* (Germar) (Payne et al. 1984, Smith et al. 1990). Feeding on pines by the SPBBG cause significant ecosystem disturbance and millions of dollars of timber lost each year as trees are colonized and killed in mass numbers (Georgia Forestry Commission 2013).

*Dendroctonus frontalis* use visual and olfactory signals (e.g., inter- and intraspecies semiochemicals and monoterpenes) to find suitable hosts. Female *D. frontalis* and *D. terebrans* initially bore into the phloem and emit conspecific aggregate pheromone, frontalin that attracts females and males to a tree (Pitman et al. 1969, Renwick and Vité 1970, Mitton and Sturgeon 1982, Berisford et al. 1990, Billings 2011, Hain 2011, Sullivan 2011). In contrast, males are the colonizing sex for *Ips* beetles that burrow into the phloem, and attract mates and conspecifics by releasing ipsdienol (by *I. avulsus, I. calligraphus*) or ipsenol (by *I. grandicollis*) (Vité et al. 1972, Hughes 1974, Švihra 1982).

Economic losses caused by the SPBBG members have prompted numerous studies of the inter-and-intraspecies semiochemical interaction among bark beetle species, tree monoterpenes (Renwick and Vité 1969, Camors and Payne 1973, Birch et al. 1980, Byers 1984, 1989, Smith et al. 1990, Miller et al. 2005), and predators and/or competitors (Payne et al. 1978, Raffa and Klpezig 1989, Miller and Asaro 2005). However, these studies were often conducted during *D. frontalis* population outbreaks, outside the geographic range of *D. frontalis*, and *Ips* semiochemical experiments often ignore semiochemical interactions with predators within the SPBBG (Raffa and Klepzig
1989, Erbilgin and Raffa 2001a, b, Raffa et al. 2007). Hence, the semiochemical ecology of SPBBG during low D. frontalis populations in the southeastern U.S. has largely remained understudied.

Using synthetic pheromone lures, we performed three field trapping experiments to determine the attraction of SPBBG members, their predators, and competitors to varying combinations of known aggregation semiochemicals [(-)-α-pinene, (+)-frontalin, (+)-ipsdienol and/or (+)-ipsenol] when local D. frontalis and Ips populations were low in forest stands. Information from these experiments may improve our understanding of the biological interactions mediating population dynamics of the SPBBG which in turn may enhance bark beetle monitoring programs, natural predation, and interspecies competition for pest management.

3.2 Methods
3.2.1 Study Sites

During 2010-2011, three experiments were conducted in the Piedmont Region of the Oconee National Forest in Putnum County, Georgia. The soils in this area are primarily clayey to sandy Udults (Griffith et al. 2001, National Resources Conservation Service 2013). Local annual mean temperature was 16.33 ± 0.9 °C and precipitation was 191.32 ± 0.44 cm during our sampling period (Georgia Automated Environmental Monitoring Network 2013). Insect sampling occurred in mature loblolly (P. taeda L.) and shortleaf pine (P. echinata Mill.) stands that had not been prescribed burned or thinned for over ten years. The understory trees composed of mixed hardwood trees (Liquidambar styraciflua L. and Quercus spp.) (National Resources Conservation Service 2013).
3.2.2 Beetle Sampling

Beetles were sampled using 12-unit multiple-funnel traps (ConTech Enterprises Inc., British Columbia). Collection cups of funnel traps were filled with 250-350 ml of water and propylene glycol (1:3 mixture) (Low Tox, Prestone® Products Corp., New Jersey) to retain and preserve captured insects. Traps were emptied in 1-3 week intervals, and all captured beetles were recorded and identified to species-level (Wood 1986, Ivie 2002, Lingafelter 2007). For bark beetles, we focused on the Family Curculionidae, Subfamily Scolytinae; for woodboring beetles, we focused on the Family Cerambycidae; and for beetle predators, we focused on the Families Cerambycidae, Cleridae, Curculionidae, Erotylidae, Histeridae, Passandridae, Staphylinidae, Trogossitidae, and Zopheridae. Voucher specimens have been deposited at the University of Georgia, Georgia Museum of Natural History in Athens, Georgia.

3.2.3 Experiment 1: Is there an interspecies cross-attraction of semiochemicals among SPBBG members and their beetle associates?

During 9 July-23 September, 2010, beetles were sampled using six replicate transects, each containing four funnel traps in the Oconee National Forest, Georgia (33° 21´ N, 83° 28´ W). Each funnel trap was suspended by a rope 1-2 m above ground and ≥ 3 m from any pine tree. Traps were placed ≥ 100 m apart within transects that were installed > 150 m apart from each other. We focused on the main semiochemicals for D. frontalis and Ips species. One of four semiochemical lure combinations were randomly assigned to a funnel trap within each transect: 1) unbaited; 2) (-)-α-pinene and (+)-frontalin (for D. frontalis); 3) (+)-ipsdienol and (+)-ipsenol (for Ips spp.); and 4) (-)-α-
pinene, (+)-frontalin, (+)-ipsdienol, and (+)-ipsenol (for both *D. frontalis* and *Ips* spp.) (Table 3.1).

### 3.2.4 Experiment 2: What is the role of host-kairomone, α-pinene, and *D. frontalis* attractant, frontalin, in the attraction of SPBBG members and their beetle associates to *Ips* species pheromones, ipsdienol and ipsenol?

During 13 June-29 July, 2011, beetles were sampled using ten replicate transects (≥ 150 m apart) within the Oconee National Forest (33° 44´ N, 83° 14´ W). Within each transect, four funnel traps were suspended from free standing metal poles and placed ≥ 150 m apart. We focused on the interactions of *Ips* species semiochemicals (ipsdienol and ipsenol) with one or both of the *D. frontalis* semiochemicals (α-pinene and frontalin). One of four treatments were randomly assigned to each funnel trap within a transect: 1) (+)-ipsdienol and (+)-ipsenol; 2) (+)-ipsdienol, (+)-ipsenol, and (-)-α-pinene; 3) (+)-ipsdienol, (+)-ipsenol, and (+)-frontalin; and 4) (+)-ipsdienol, (+)-ipsenol, (-)-α-pinene, and (+)-frontalin (Table 3.1).

### 3.2.5 Experiment 3: What is the role of host-kairomone, α-pinene, in the attraction of SPBBG members and their beetle associates to each of the two *Ips* species pheromones, ipsdienol and ipsenol?

During 29 July-26 August, 2011, beetles were sampled using ten replicate transects (≥ 150 m apart) in the Oconee National Forest (33° 44´ N, 83° 14´ W). Within each transect, four funnel traps were suspended from free standing metal poles and placed ≥ 100 m apart. We focused on the responses of SPBBG members and their beetle associates to combinations of α-pinene, ipsdienol, and ipsenol. One of four treatments were randomly assigned to each funnel trap within each transect: 1) (-)-α-pinene; 2) (-)-α-
pinene and (+)-ipsdienol; 3) (-)-α-pinene and (+)-ipsenol; and 4) (-)-α-pinene, (+)-ipsdienol, and (+)-ipsenol (Table 3.1).

3.2.6 Statistical Analyses

Poisson regression analyses were used to assess differences in beetle catches among semiochemical treatments in all experiments. Transformation procedures associated with general linear models (such as Analysis of Variance) could not temper heteroscedasticity and normalize the distribution of error within our data sets caused by a high number of recorded zero values and overdispersion. Further, the "count" nature of our data made using general linear models (such as ANOVA or linear regression) with a transformation inappropriate (Maindonald and Braun 2007, O’Hara and Kotze 2010). Hence, the beetle catches were not transformed and overdispersion was tempered by the natural log-link function inherent to Poisson regression (O’Hara and Kotze 2010).

Poisson regression was performed on weekly beetle catches per trap within each treatment type of the most abundant beetle species (≥100 adults captured in an experiment). Analyses were performed separately for each beetle species. For each experiment, an offset for the Poisson regression was created to adjust for trap disturbance by pooling beetle catches for all traps per treatment and standardized catches to seven trap-day periods. Hence, the average number of beetles captured per trap per treatment on a weekly basis was assessed for each experiment. After significance of the main model, differences among treatments were assessed using Tukey-Kramer tests.

3.3 Results

3.3.1 Experiment 1: Is there an interspecies cross-attraction of semiochemicals within the SPBBG members and their beetle associates?
In Experiment 1, a total of 10,544 beetles representing 10 families, 27 genera, and 35 species were captured (Appendix A). The most abundant species (with total number of individuals) included in our analyses were as follows: 1) bark beetles, *D. terebrans* (144), *I. avulsus* (1,051), *I. grandicollis* (2,442), and *D. frontalis* (1,098); 2) wood borers, *Monochamus* species including *titillator* and *carolinensis* complex (1,164) and *Pycnомерus sulcicollis* LeConte (1,010); and 3) predators, *Lasconotus pusillus* LeConte (217); *Namunaria guttulata* (LeConte) (107), *Platysoma cylindrica* (Paykull) (259), *Temnochila virescens* (Fabricius) (1,168), and *Thanasimus dubius* (Fabricius) (953) (Appendix A).

The following major trends were evident for beetle species where they were respectively trapped in greatest numbers with the following lure combinations: 1) α-pinene and frontalin; *D. frontalis, D. terebrans, P. sulcicollis, and T. dubius*. Similar numbers of these species were also trapped when ipsdienol and ipsenol were paired with α-pinene and frontalin; 2) ipsdienol and ipsenol; *I. avulsus* and *I. grandicollis*. Further, *I. grandicollis* was captured in similar numbers and fewer *I. avulsus* captured when ipsdienol and ipsenol were paired with α-pinene and frontalin; and 3) beetle species trapped in greatest numbers when α-pinene and frontalin were paired with ipsdienol and ipsenol included *L. pusillus, N. guttulata, P. cylindrica, and T. virescens* (Tables 3.2; Figs. 3.1, 3.2). Few beetles were captured in unbaited traps. Lure-treatment was a significant factor for *Monochamus* spp. \( F = 5.15, \ d.f. = 3, \ 20, \ P = 0.008 \), however conservative Tukey-Kramer test indicated no significant difference among treatments.
3.3.2 Experiment 2: What is the relative role of host-kairomone (α-pinene) and *D. frontalis* attractant, frontalin, in the attraction of SPBBG members and their beetle associates to *Ips* species pheromones, ipsdienol and ipsenol?

In Experiment 2, a total of 26,500 beetles representing 13 families, 28 genera, and 28 species were captured (Appendix B). The most abundant species (with total number of individuals) included in our analyses were as follows: 1) bark beetles; *D. terebrans* (177), *Dryophthorus americanus* Bedel (241), *Hesperus baltimorensis* (Gravenhorst) (163), *Hylastes tenius* Eichoff (112), *I. avulsus* (8,704), *I. grandicollis* (10,807), and *D. frontalis* (1,923); 2) woodborers- *Monochamus* spp. (1,396) and *P. sulcicollis*, (101), and 3) predators; *Anobium punctatum* (DeGeer) (297), *L. pusillus*, (106), *P. cylindrica* (161), *T. virescens* (947), *Tenebroides marginatus* (Palisot de Beauvois) (165), and *T. dubius* (496).

The following major trends were evident for beetle species where they were respectively trapped in greatest numbers when lure combinations included ipsdienol and ipsenol and either: 1) α-pinene; *D. terebrans, D. americanus, H. tenius, Monochamus* spp., *P. sulcicollis, and T. virescens*; 2) α-pinene and frontalin; *D. frontalis and T. dubius* (Table 3.3; Figs. 3.3, 3.4). *Temnochila virescens* was significantly less attracted to *Ips* lures and α-pinene when frontalin was included (Fig. 3.3). *Ips avulsus* was significantly less attracted to lure combinations which included α-pinene (Fig. 3.3). There were no significant differences among treatments for *I. calligraphus, I. grandicollis, P. cylindrica*, and *T. marginatus*. 
3.3.3 Experiment 3: What is the role of host-kairomone, α-pinene, in the attraction of SPBBG members and their beetle associates to each of the two *Ips* species pheromones, ipsdienol and ipsenol?

In Experiment 3, a total of 10,488 beetles representing nine families, 24 genera, and 34 species were captured (Appendix C). The most abundant species (with total number of individuals) included in our analyses were as follows: 1) bark beetles; *D. terebrans* (169), *H. tenius* (260), *I. avulsus* (408), and *I. grandicollis* (6,352); 2) woodborers; *Acanthocinus obsoletus* Olivier, (455) and *Monochamus* spp. (941); and 3) predators; *L. pusillus* LeConte (239), *Namunaria guttulata* (LeConte) (153), *Platypus flavicornis* Fabricius (167), *P. cylindrica* (269), *P. sulcicollis* (117), *T. marginatus* (208), and *T. dubius* (953).

*Namunaria guttulata* was the only species whose capture significantly differed among treatments. Captures of *N. guttulata* were significantly lower in traps baited with α-pinene, ipsdienol, and ipsenol (*F* = 5.64, d.f. = 3, 27, *P* = 0.004). Captures of other beetle species did not significantly differ among lure combinations (p-values ranged from 0.140 to 0.886) (Tables 3.4; Figs. 3.5, 3.6).

3.4 Discussion

3.4.1 Bark Beetle Response to Lure Combinations

Studies have indicated that *D. frontalis* may coexist on *Ips*-infested trees when they are in non-outbreak population levels (Nebeker 2011). Hence, *D. frontalis* may use *Ips* pheromones or volatiles released from pines being colonized by *Ips* beetles to locate suitable hosts. In contrast to this hypothesis, we found (Experiment 1) *D. frontalis* attraction to α-pinene and frontalin was interrupted by the addition of *Ips* lures. Thus,
kairomones released by the tree itself may be a greater attractant synergist for *D. frontalis* than pheromones released by other colonizing bark beetle species. Since *Ips* spp. pheromones appear to be deterrents to *D. frontalis*, they may be using this chemical as a guide to colonize other parts of the bole thus, reducing competition for phloem habitat.

Previous studies of conspecific and cross-attraction among *Ips* spp. have reported mixed results. For example, *I. calligraphus* infested logs (likely releasing ipsdienol) have either been found to either enhance or deter *I. avulsus* colonization (Birch et al. 1980, Byers 1989). *Ips avulsus* have been either unresponsive or have colonized logs infested with *I. grandicollis* (likely releasing ipsenol) (Hedden et al. 1976, Birch et al. 1980, Švihra et al. 1980). Further, *I. avulsus* have either responded to or have been unresponsive to ipsdienol (Vité et al. 1964, Smith et al. 1990) and ipsenol (Hedden et al. 1976, Smith et al. 1990, Payne et al. 1984), and attraction to ipsdienol may decrease with increasing volumes in turpentine baits (Billings 1985). Further, *I. calligraphus* is typically not attracted to *I. avulsus* and *I. grandicollis* aggregation pheromone, ipsenol, but reportedly is attracted to logs infested with *I. grandicollis* (Birch et al. 1980, Švihra 1982, Payne et al. 1984). *Ips calligraphus* is also typically not attracted to logs infested by *D. frontalis* (Vité et al. 1964, Birch et al. 1980, Švihra 1982), but has also been observed colonizing logs infested with female *D. frontalis* (Dixon and Payne 1980).

*Ips avulsus* is typically characterized as the most cross-attractive *Ips* species and is reportedly not attracted to *D. frontalis* aggregation pheromones (Payne et al. 1984) and logs infested with *D. frontalis* (Vité et al. 1964, Švihra et al. 1980, Švihra 1982, Birch et al. 1980). However, the volatiles released from logs infested with bark beetles are often not analyzed, so the emitted semiochemical mixtures often remain unidentified in such
studies (Payne et al. 1984). In our studies, *I. avulsus* was similarly attracted to ipsdienol and ipsenol (Figs. 2.10), their captures increased when frontalin is added to *Ips* lures, but they were deterred by the presence of α-pinene (Figs. 2.1 and 2.6). This suggests *I. avulsus* does not differentiate between ipsdienol and ipsenol, and may exploit *D. frontalis* pheromones when *D. frontalis* and *Ips* populations are low. In this instance, host attractants may have lower attraction than interspecific pheromones to the beetles.

Our findings also support previous work identifying α-pinene and *Ips* lures as *I. grandicollis* attractants. For example, *I. grandicollis* is typically attracted to traps baited with loblolly pine turpentine in Texas (Billings 1985). In the Great Lakes region, tree volatiles (+)-α-pinene, β-pinene, myrcene, and 3-carene did not synergize or interrupt *I. grandicollis* attraction to (-)-α-pinene lures (Erbilgin and Raffa 2000). However, Miller and Rabaglia (2009) found adding α-pinene, ethanol, and ipsenol interrupted attraction of *I. avulsus* and *I. grandicollis* to funnel traps in Georgia, Florida, Louisiana, and Virginia.

In our experiments, *I. grandicollis* was responsive to lure combinations which included (-)-α-pinene, and did not differentiate between ipsdienol and ipsenol. Hence, *I. grandicollis* likely exploits conspecific and interspecies pheromones of *Ips* beetle for host location and selection when *D. frontalis* and *Ips* populations are low.

Little is known about the biology or behavior of *H. tenuis* (Eckhardt et al. 2004). They are small, root-feeding beetles which breed and reproduce within dead tissue layers of dying pines (Furniss et al. 1992, Eckhardt et al. 2004). This likely explains *H. tenuis* attraction to damaged Virginia pine (*P. virginiana* Mill.) trees (Howden and Vogt 1951, Hines and Heikkenen 1977), loblolly billets (Flechtmann et al. 1999), and (-)-α-pinene (Miller and Rabaglia 2009). Our results demonstrate *H. tenuis* attraction to (-)-α-pinene,
and suggest *H. tenuis* is attracted to and does not differentiate between *Ips* pheromones. Thus *H. tenuis* may utilize α-pinene and *Ips* pheromones to locate and select hosts.

### 3.4.2 SPBBG Predators Response to Lure Combinations

In the Great Lakes region, *T. dubius* is reportedly attracted to (+)-ipsdienol (Raffa et al. 2007, Gandhi et al. 2009) and is the primary predator of *I. pini* (Say) (Aukema and Raffa 2004). However, in the southeastern U.S., *T. dubius* is described as the primary predator of *D. frontalis* (Thatcher and Pickard 1966, Dixon and Payne 1979, Bunt et al. 1980, Billings and Cameron 1984, Payne et al. 1984, Reeve 1997, Sullivan 2011), and is responsive to *Dendroctonus* lures (frontalin and α-pinene) alone (Hain 2011, Sullivan 2011) or paired with *Ips* lures (Billings and Cameron 1984). Further, responsiveness of *T. dubius* to *Dendroctonus* or *Ips* pheromones is reportedly dependent upon the relative abundance of bark beetle species (prey) and, therefore, often changes from year to year (Billings and Cameron 1984, Herms et al. 1991, Reeve 1997). However in our experiment, *T. dubius* was unresponsive to *Ips* lures unless paired with *Dendroctonus* lures (frontalin and α-pinene) although *D. frontalis* populations were low. Thus attraction of *T. dubius* may have a stronger coevolution history with *Dendroctonus* than *Ips* spp., but in the absence of *D. frontalis*, may exploit *Ips* spp. as prey.

*Platysoma* spp. are described as predators of *Ips*. For example, catches of *Platysoma* spp. often reflect the seasonal abundance of *Ips* spp. in Louisiana (Shepherd and Goyer 2003). *Platysoma* spp. may make up to 65% of *Ips* (Kulhavy et al. 1989) and 7% of *D. frontalis* (Berisford 1980) predator abundance within *D. frontalis* communities. The attraction of *P. cylindrica* to *Ips* lures and *I. pini* infested logs (Raffa and Dahlsten 1995, Aukema et al. 2000b) indicates *P. cylindrica* may decrease the survival and
reproductive success of bark beetle infestations in the Great Lakes region (Aukema and Raffa 2002, Aukema and Raffa 2004). In our experiments, *P. cylindrica* was more attracted to traps baited with *Ips* lures, frontalin, and α-pinene, and responded similarly to *Ips* lure combinations in Experiments 2 and 3. We found *P. cylindrica* was predominantly attracted to lure combinations with *D. frontalis* and *Ips* attractants. Hence, *P. cylindrica* may be characterized as a generalist predator of the SPBBG in the southeastern U.S. (Dixon and Payne 1979, Shepherd and Goyer 2003).

Reportedly, *T. virescens* responds almost exclusively to *Ips* pheromones (ipsdienol and ipsenol with *cis*-verbenol) compared to pheromones associated with *D. frontalis* (frontalin, *trans*-verbenol, and turpentine) (Billings and Cameron 1984). However, we found *T. virescens* captures increased when α-pinene was included in lure combinations (Figs. 3.2 and 3.8). Tree volatiles may synergize *T. virescens* attraction to *Ips* lures (Billlings 1985), thus α-pinene may mediate *T. virescens* attraction to bark beetle pheromones.

### 3.4.3 SPBBG Competitors Response to Lure Combinations

In this study, *Monochamus* spp. refers to two specific species, *M. titillator* and *M. caroliensis*. Originally believed to have a commensal (Flamm et al. 1989) or competitive (Coulson et al. 1980) relationship with the SPBBG members, *Monochamus* spp. are currently considered facultative intraguild predators (Dodds et al. 2002, Schoeller et al. 2012). Adults are highly attracted to bark beetle infested, wind-thrown, lightning struck, or otherwise damaged *Pinus, Picea*, or *Abies* spp. (Lingafelter 2007, Gandhi et al. 2009), where they feed on and compete with established bark beetle communities (Coulson et al. 1976, 1980, Allison et al. 2001, Raffa et al. 2005). *Monochamus* spp. have been reported
to be attracted to α-pinene in the Great Lakes region (Erbilgin and Raffa 2000) and Southeast U.S. (Miller 2006). Significantly more Monochamus spp. are attracted to traps baited with ipsdienol, ipsenol, α-pinene, and ethanol than traps baited with α-pinene and ethanol (Miller et al. 2011). Similar results were found for traps baited with ipsdienol, ipsenol, cis-verbenol, and turpentine compared to traps baited with either turpentine alone or ipsdienol and ipsenol without a tree volatile lure (Billings 1985). Further, α-pinene may synergize Monochamus spp. attraction to (+)-frontalin (Coulson et al. 1980; Sullivan 2011).

We found Monochamus spp. are attracted to ipsdienol and ipsenol but did not observe a decrease in captures due to the addition of frontalin (Billings and Cameron 1984, Miller and Asaro 2005). However, our result suggest α-pinene synergizes Monochamus spp. attraction to frontalin and Ips pheromones in Georgia. It is, therefore likely Monochamus spp. exploit a combination of bark beetle pheromones and tree volatiles (if not simply volatiles alone) to find prey and phloem conditions ideal for oviposition and reproduction activities (Miller et al. 2011).

Seven zopherid species have been described as predator-scavenger associates of D. frontalis infestations (Overgaard 1968, Dixon and Payne 1980); two of which (L. pusillus and P. sulcicollis) were captured in large enough numbers to conduct statistical analyses. Adult L. pusillus have received interest as biological control agents for Ips beetles because they are specialist predators which feed on Ips (not Dendroctonus) spp. Adult L. pusillus feed on eggs and newly emerged Ips beetles while larvae feed on Ips late-instar larvae and pupae (Rohlf and Hyche 1983). Further, adult activity often parallels the colonizaton and growth of within-tree Ips infestations (Overgaard 1968,
Rohlfs and Hyche 1983). For example, in Alabama, *L. pusillus* was not collected from *D. frontalis* infested trees until 15-25 days following the secondary colonization of *Ips* beetles (Rohlfs and Hyche 1981).

Results from our Experiments 1 and 2 suggest that frontalin interrupts *L. pusillus* attraction (Figs. 3.3, 3.9, 3.12). However, α-pinene may also prove to be an important synergist for *L. pusillus* attraction to *Ips* lures because pairing α-pinene with *Ips* lures with α-pinene in Experiment 2, captured more *L. pusillus* than *Ips* lures alone or *Ips* and *Dendroctonus* lures combined. Further, *L. pusillus* did not differentiate between ipsdienol and ipsenol treatments within Experiment 3, demonstrating *L. pusillus* is attracted to all *Ips* species. Hence, *L. pusillus* may exploit *Ips*, not *Dendroctonus* pheromones to find suitable hosts and likely do not differentiate among *Ips* species for prey.

*Pycnomerus sulcicollis* colonize the crown of decaying pines or standing trees infested with *Ips* beetles or will synchronize colonization with *Ips* beetles attacking felled pines (Rohlfs and Hyche 1981, Ivie 2002). In our experiments, *P. sulcicollis* captures increased when lure combinations included *Ips* lures with or without α-pinene (Figs. 3.4, 3.9), and lure combinations including *Dendroctonus* lures interrupted *P. sulcicollis* attraction (Figs. 3.4, 3.9). Each lure combination in Experiment 3 included α-pinene, so the lack of significant treatment effect in Experiment 3 further suggests *P. sulcicollis* attraction was mediated by the inclusion of α-pinene. It is likely that *P. sulcicollis* is attracted to *Ips* beetle pheromones and volatiles signaling decreasing tree health.

Little is known about the biology and life history of *N. guttalata*. Adult *N. guttalata* were more attracted to traps baited with *Dendroctonus* and *Ips* lures in Experiment 1 (Fig.3.4). However, in Experiment 2, too few *N. guttalata* were captured...
for statistical analysis \((N = 25)\); with notably low captures in traps baited with *Dendroctonus* and *Ips* lures \((N = 5\), Table 3.3). Also, *N. guttalata* captures were lower in traps baited with both ipsdienol and ipsenol (Figs. 3.4, 3.13). Hence, *N. guttalata* attraction was synergized by *Dendroctonus* with *Ips* lures in Experiment 1 and *Ips* pheromones appear to have interrupted attraction in Experiments 2 and 3. These mixed results suggest the response of *N. guttalata* to bark beetle pheromones should be further investigated.

Our results suggest complex semiochemical communication occur among SPBBG members and their predators. At a time of low bark beetle activity, we found that: 1) *D. frontalis* and *T. dubius* did not exploit *Ips* pheromones; 2) *Ips* spp. did not exploit *D. frontalis* pheromones; 3) *Ips* spp. did not differentiate between ipsdienol and ipsenol; 4) *I. avulsus* attraction to *Ips* pheromones maybe interrupted by \(\alpha\)-pinene; 5) *D. terebrans* cross-attraction to *Ips* lures support characterizing *D. terebrans* as a secondary colonizer; and 6) *H. tenuis* attraction was synergized by \(\alpha\)-pinene and *Ips* pheromones. Our findings also indicate that *T. virescens*, *T. dubius*, and *P. cylindrica* are generalist predators. Our results suggest beetles associated with SPBBG (including *A. obsoletus*, *H. baltimorensis*, *L. pusillus Monochamus* spp., *N. guttalata*, *P. flavicornis*, *P. sulcicollis*, and *T. marginatus*) exploit semiochemicals released by SPBBG. Our study facilitates an improved understanding of the semiochemical interactions among SPBBG members and their predators, which may be used to create biological control strategies or further improve monitoring programs of local SPBBG populations.

3.5 Acknowledgements
We thank Wayne C. Berisford and Mark J. Dalusky (Department of Entomology, University of Georgia), and the USDA-Forest Service, Forest Health Protection for trapping materials. Members of the Gandhi Forest Entomology Laboratory (Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia) contributed to field and lab work. Species identifications of beetles were verified by Rick Hoebeke (Georgia Museum of Natural History, University of Georgia). Funding for the project was provided by USDA-Forest Service, Forest Health Protection, and Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia.
3.7 Literature Cited


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Monochamus titillator (Cerambycidae), Thanasimus dubius (Cleridae), and Temnochila virescens (Trogositidae), to behavioral chemicals of southern pine bark beetles (Coleoptera: Scolytidae). Environmental Entomology, 13: 1542-1548.


Retrieved from:


Miller, D. R., and C. Asaro. 2005. Ipsonol and ipsdienol attract *Monochamus titillator*


Overgaard, N. A. 1968. Insects associated with the southern pine beetle in Texas,


Table 3.1. List of semiochemicals, their enantiomeric purity, volume, and release devices. All semiochemical devices were provided by Synergy Semiochemical Corporation (Burnaby, British Columbia).

<table>
<thead>
<tr>
<th>Semiochemical</th>
<th>Enantiomeric Purity</th>
<th>Volume</th>
<th>Release Device</th>
<th>Release Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-α-pinene</td>
<td>75%</td>
<td>170 g</td>
<td>polyethylene sleeve</td>
<td>2.0 g/day at 20°C</td>
</tr>
<tr>
<td>(+)-Frontalin</td>
<td>98%</td>
<td>300 mg</td>
<td>Centrifuge tube device</td>
<td>0.14 mg/day at 24°C</td>
</tr>
<tr>
<td>(+)-Ipsdienol</td>
<td>50:50%</td>
<td>100 mg</td>
<td>Bubble cap</td>
<td>0.2 mg/day at 22-24°C</td>
</tr>
<tr>
<td>(+)-Ipsenol</td>
<td>50:50%</td>
<td>100 mg</td>
<td>Bubble cap</td>
<td>0.2 mg/day at 22-24°C</td>
</tr>
</tbody>
</table>
Table 3.2. Statistical analyses comparing captured subcortical beetle species among lure combinations including: α-pinene, frontalin, ipsdienol and ipsenol in 2010 in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>Lure Combinations</th>
<th>Family</th>
<th>Beetle Species</th>
<th>F-statistic</th>
<th>D.F.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significantly more attracted to α-pinene with frontalin</td>
<td>Cleridae</td>
<td><em>Thanaisimus dubius</em> (Fabricius)</td>
<td>21.35</td>
<td>3, 14.93</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td></td>
<td>Curculionidae</td>
<td><em>Dendroctonus frontalis</em> Zimmermann</td>
<td>8.77</td>
<td>3, 15.36</td>
<td>0.001</td>
</tr>
<tr>
<td>Significantly more attracted to ipsdienol and ipsenol</td>
<td>Curculionidae</td>
<td><em>Ips avulsus</em> (Eichhoff)</td>
<td>18.92</td>
<td>3, 15.27</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Significantly more attracted to α-pinene, frontalin, ipsdienol, and ipsenol</td>
<td>Histeridae</td>
<td><em>Platysoma cylindrica</em> (Paykull)</td>
<td>7.01</td>
<td>3, 15.49</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Trogossitidae</td>
<td><em>Temnochila virescens</em> (Fabricius)</td>
<td>10.1</td>
<td>3, 15.23</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Zopheridae</td>
<td><em>Lasconotus pusillus</em> LeConte</td>
<td>6.73</td>
<td>3, 14.34</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Zopheridae</td>
<td><em>Namunaria guttulata</em> (LeConte)</td>
<td>12.34</td>
<td>3, 20</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Significantly attracted to α-pinene and frontalin or α-pinene, frontalin, ipsdienol, and ipsenol</td>
<td>Curculionidae</td>
<td><em>Dendroctonus terebrans</em> (Olivier)</td>
<td>5.43</td>
<td>2, 10.08</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>Zopheridae</td>
<td><em>Pycnomenus sulcicollis</em> LeConte</td>
<td>15.76</td>
<td>3, 14.9</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Significantly attracted to ipsdienol and ipsenol or α-pinene, frontalin, ipsdienol, and ipsenol</td>
<td>Curculionidae</td>
<td><em>Ips grandicollis</em> (Eichhoff)</td>
<td>13.71</td>
<td>3, 13</td>
<td>0.000</td>
</tr>
<tr>
<td>No significant difference among treatments</td>
<td>Cerampyceidae</td>
<td><em>Monochamus</em> Species</td>
<td>5.15</td>
<td>3, 20</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Table 3.3. Statistical analyses comparing captured subcortical beetle species among lure combinations including: ipsdienol and ipsenol with α-pinene and/or frontalin in 2011 in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>Ipsdienol and Ipsenol Lure Combinations</th>
<th>Family</th>
<th>Beetle Species</th>
<th>F-statistic</th>
<th>D.F.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significantly more attracted to Ips lures with α-pinene only</td>
<td>Trogossitidae</td>
<td><em>Tenebroides marginatus</em> (Palsot de Beauvais)</td>
<td>0.43</td>
<td>3, 27</td>
<td>0.734</td>
</tr>
<tr>
<td>Significantly more attracted to Ips lures with frontalin only</td>
<td>Zopheridae</td>
<td><em>Lasconotus pussillus</em> LeConte</td>
<td>7.73</td>
<td>3, 27</td>
<td>0.001</td>
</tr>
<tr>
<td>Significantly more attracted to Ips lures with α-pinene and frontalin</td>
<td>Curculionidae</td>
<td><em>Ips avulsus</em> (Eichhoff)</td>
<td>10.06</td>
<td>3, 27</td>
<td>0.000</td>
</tr>
<tr>
<td>Significantly more attracted to Ips lures with α-pinene and frontalin</td>
<td>Trogossitidae</td>
<td><em>Temnoscheila virescens</em> (Fabricius)</td>
<td>12.46</td>
<td>3, 27</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td><em>Thanasimus dubius</em> (Fabricius)</td>
<td></td>
<td>39.29</td>
<td>3, 27</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Dendroctonus frontalis</em> Zimmermann</td>
<td></td>
<td>24.21</td>
<td>2, 18</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td>Monochamus Species</td>
<td></td>
<td>13.91</td>
<td>3, 27</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Dendroctonus terebra</em> (Olivier)</td>
<td></td>
<td>24.41</td>
<td>3, 27</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Dryophthor us americanus</em> (Bedel)</td>
<td></td>
<td>7.28</td>
<td>3, 27</td>
<td>0.001</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Hylastes tenius</em> (Eichhoff)</td>
<td></td>
<td>8.56</td>
<td>3, 27</td>
<td>0.000</td>
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<tr>
<td>Curculionidae</td>
<td><em>Pycnoderus sulcicollis</em> LeConte</td>
<td></td>
<td>11.3</td>
<td>3, 27</td>
<td>&lt; 0.000</td>
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<tr>
<td>No significant difference among treatments</td>
<td>Curculionidae</td>
<td><em>Ips calligraphus</em> (Germar)</td>
<td>1.87</td>
<td>3, 27</td>
<td>0.158</td>
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<tr>
<td>No significant difference among treatments</td>
<td>Curculionidae</td>
<td><em>Ips grandicollis</em> (Eichhoff)</td>
<td>1.94</td>
<td>3, 27</td>
<td>0.147</td>
</tr>
<tr>
<td>Histeridae</td>
<td><em>Platysoma cylindrica</em> (Paykull)</td>
<td></td>
<td>1.3</td>
<td>3, 27</td>
<td>0.295</td>
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<td>Staphylinidae</td>
<td><em>Hesperus balitmorensis</em> (Gravenhorst)</td>
<td></td>
<td>1.34</td>
<td>3, 27</td>
<td>0.284</td>
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</table>
Table 3.4. Statistical analyses comparing captured subcortical beetle species among lure combinations including: α-pinene with ipsdienol and/or ipsenol in 2011 in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>α-pinene Lure Combinations</th>
<th>Family</th>
<th>Beetle Species</th>
<th>F-statistic</th>
<th>D.F.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significantly less attracted to</td>
<td>Zopheridae</td>
<td><em>Namunaria guttulata</em> (LeConte)</td>
<td>5.64</td>
<td>3, 27</td>
<td>0.004</td>
</tr>
<tr>
<td>α-pinene, ipsdienol, and ipsenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No significant difference among treatments</td>
<td>Cerambycidae</td>
<td><em>Acanthocinus obsoletus</em> Olivier</td>
<td>1.98</td>
<td>3, 27</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Cerambycidae</td>
<td><em>Monochamus</em> Species</td>
<td>0.42</td>
<td>3, 27</td>
<td>0.743</td>
</tr>
<tr>
<td></td>
<td>Curtulionidae</td>
<td><em>Dendroctonus terebrans</em> (Olivier)</td>
<td>2.35</td>
<td>3, 27</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>Curtulionidae</td>
<td><em>Hylastes tenius</em> (Eichhoff)</td>
<td>1.94</td>
<td>3, 27</td>
<td>0.148</td>
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<td></td>
<td>Curtulionidae</td>
<td><em>Ips avulsus</em> (Eichhoff)</td>
<td>0.36</td>
<td>3, 27</td>
<td>0.785</td>
</tr>
<tr>
<td></td>
<td>Curtulionidae</td>
<td><em>Ips calligraphus</em> (Germar)</td>
<td>0.36</td>
<td>3, 27</td>
<td>0.785</td>
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<td></td>
<td>Curtulionidae</td>
<td><em>Ips grandicollis</em> (Eichhoff)</td>
<td>1.92</td>
<td>3, 27</td>
<td>0.151</td>
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<td>Histeridae</td>
<td><em>Platysoma cylindrica</em> (Paykull)</td>
<td>3.33</td>
<td>3, 27</td>
<td>0.034</td>
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<td>Platypodidae</td>
<td><em>Platypus flavicornis</em> Fabricius</td>
<td>1.99</td>
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<td>0.139</td>
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<td></td>
<td>Trogossitidae</td>
<td><em>Tenebrionodes marginatus</em> (Palisot de Beauvois)</td>
<td>1.47</td>
<td>3, 27</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>Zopheridae</td>
<td><em>Lasconotus pusillus</em> LeConte</td>
<td>1.15</td>
<td>3, 27</td>
<td>0.345</td>
</tr>
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**Figure Legend**

**Figure 3.1.** Mean (± SE) number of subcortical bark beetles captured caught in multiple funnel traps using traps baited with α-pinene, frontalin, ipsdienol and ipsenol in 2010 in the Oconee National Forest, Georgia.

**Figure 3.2.** Mean (± SE) number of subcortical beetle species captured caught in multiple funnel traps using traps baited with α-pinene, frontalin, ipsdienol and ipsenol in 2010 in the Oconee National Forest, Georgia.

**Figure 3.3.** Mean (± SE) number of subcortical bark beetles captured caught in multiple funnel traps using traps baited with ipsdienol and ipsenol, α-pinene and/or frontalin in 2011 in the Oconee National Forest, Georgia.

**Figure 3.4.** Mean (± SE) number of subcortical beetle species captured caught in multiple funnel traps using traps baited with ipsdienol and ipsenol, α-pinene and/or frontalin in 2011 in the Oconee National Forest, Georgia.

**Figure 3.5.** Mean (± SE) number of subcortical bark beetles captured caught in multiple funnel traps using traps baited with α-pinene with ipsdienol and/or ipsenol in 2011 in the Oconee National Forest, Georgia.
Figure 3.1.
Figure 3.2.
Figure 3.3.
Figure 3.4.
Figure 3.5.
CHAPTER FOUR
CORRELATIONS BETWEEN STAND INFESTATIONS OF SOUTHERN PINE
BEETLE (DENDROCTONUS FRONTALIS ZIMMERMAN) AND LIGHTNING
STRIKES IN THE HOMOCHITTO NATIONAL FOREST, MISSISSIPPI

1 Jenny C. Staeben, Nathan P. Nibbelink, and Kamal J. K. Gandhi. To be
submitted to Forest Science
Abstract

Southern pine beetle (*Dendroctonus frontalis* Zimmermann) (SPB) outbreak populations typically move quickly through forested landscapes, creating “spots” of dead or dying pines (*Pinus* spp.) in the southeastern United States. Previous studies suggest pines struck by lightning serve as resource reservoirs for endemic SPB populations, however such relationships between abiotic disturbance and SPB populations remain understudied. Using multiple logistic regression we examined the relationship between SPB spot formation and lightning strikes in 2012 during a SPB outbreak in the Homochitto National Forest, Mississippi. We modeled the occurrence of SPB infestations (presence/absence) as a function of several variables including distance (250 and 100 km), strike polarity and magnitude, the number of trees infested by SPB, and the average basal area of pine and hardwood trees. Our results indicate that SPB spot formation is significantly related to prior lightning strike. SPB infested stands are more likely to occur within 100 and 200 m distance from lightning strikes. All lightning strikes affiliated with spot formations were of negative polarity and a magnitude of > 150 kilo amps. Surprisingly, there was no relationship between SPB spots and basal area of pine trees and all of the tree species within the lightning strike frequency or proximity. Results from this study may be used for better prediction of SPB spot formations using real-time lightning data in other pine-dominated areas of southeastern U.S.

Keywords: *Dendroctonus frontalis*, lightning strike, Homochitto
4.1 Introduction

The southern pine beetle (SPB) (*Dendroctonus frontalis* Zimmermann) is one of the most economically important forest insect pests in the southeastern United States. Outbreak SPB populations typically move quickly through the forest, killing groups of pine species in a characteristic “spot” formation (Peacher 2011). Typically, low SPB populations are active within pine forests for prolonged time periods without causing noticeable damage. SPB dispersal is limited due to their small size (2-4mm), tendency to desiccate in warm ambient temperatures, and numerous predators and parasites within their ecosystem. Suitable hosts are located visually and by semiochemical cues emitted conspecific and intraspecific organisms, and stressed pines (Sullivan 2011). Adult SPB burrow through the outer bark, creating galleries within the phloem layer of mature pines. Within the galleries they reproduce and introduce fungi which act as a food source for developing SPB larvae. Eventually infested trees die due to the girdling effect of gallery construction within the phloem and the movement of fungal gardens into the xylem (Sullivan 2011).

The thick phloem layer of large, mature pines provide optimal SPB habitat (Coulson et al. 1983). However, healthy mature pines thwart infestation by releasing copious amounts of oleoresin that “push out” or kill invading beetles. Healthy pines retain high water content in xylem tissue which maintains high oleoresin exudation pressure so copious amounts of oleoresin are easily released (Hodges and Pickard 1971) and within phloem tissues which inhibit the establishment of fungus. However, epithelial cells within the phloem and xylem lose the ability to retain water and become conducive
to fungal establishment and oleoresin production slows (if not stops) when a pine is
physiologically stressed or damaged (Hodges and Pickard 1971, Coulson et al. 1983).

Researchers have postulated a close relationship of SPB epidemiology to
lightning where resource reservoirs created by lightning strikes may act as loci for SPB
identified lightning as an important component in sustaining SPB populations in the Gulf
Coastal Plain because it creates habitat in low risk stands for reservoir populations during
endemic population phases (Hodges and Pickard 1971). Miller (1983) conducted ground-
checks and determined that 29% of active SPB spots, over a four year period, were
initiated by lightning strikes.

Lightning-struck pines are vulnerable to bark beetle for a number of reasons.
First, electrocution slows stops resin production and movement by reducing oleoresin
exudation pressure in the xylem (Coulson et al. 1983, Blanche et al. 1984). Second, bark
beetle attraction may initially increase as monoterpane attractants are released from
fractions of finely damaged bark or slivers of sapwood exposed by lightning strike
(Miller 1983). Third, damaged or stressed pines release host volatiles which synergize
bark beetle activity. Specifically, lightning struck pines release increased levels of SPB
monoterpane attractants, α-pinene and myrcene, and limonene (a highly toxic
monoterpane) becomes limited (Blanche et al. 1984). Monoterpane α-pinene is
recognized as the most attractive volatile released by pine species within the SPB
behavioral complex because it is metabolizes by SPB into two conspecific and
intraspecific attractant pheromones, trans- and cis-verbenol, which synchronizes SPB
colonization. (Blanche et al. 1984, Hain 2011, Sullivan 2011). Fourth, Hodges and
Packard (1971) reared heavier weight (but not size) SPB from lightning struck trees than non-struck trees, and suggested lightning strike alters carbohydrate composition within the phloem which likely improves SPB brood development by increasing reducing sugars and nitrogen factions. Lastly, in summer months when SPB is highly active, warm ambient temperatures and warmer outer-bark increase transpiration and, consequently, water content within the phloem of standing trees. These physiological changes escalate the conductivity of electrical charges that may increase the likelihood of lightning strikes, and consequently SPB colonization of these trees (Coulson et al. 1983). The likelihood of lightning strike occurring is directly related to its height of an object and the severity of electrocution varies with the season of the year and tree species (Coulson et al. 1983). As a negative charged lightning strike builds at the base of a storm cloud, positive electric charges build and flow from the tops of tall objects (such as mature pines). A polar attraction builds between the cloud base and tall objects, creating a ground anchor which attracts lightning to the Earth’s surface (Coder 2007). Non-commercial thinning is a common stand management practice to prevent SPB infestations by reducing within-stand competition and leaving commercially desirable pine species well-spaced from similarly sized trees (Billings 2011). Pines within thinned stands may be more likely to be struck by lightning because they are taller than their surroundings.

In 2012, SPB outbreaks occurred when ≥ 500 active spot infestations developed in the Homochitto National Forest in Mississippi. We used this opportunity to study the relationship between lightning strikes and SPB outbreaks in the southeastern U.S. We hypothesized lightning strikes created loci of subsequent SPB spot formations. We also hypothesized, the spatial distance between strikes and infestations, strike magnitude, and
the basal area of infested stands related to the likelihood of a SPB spot infestation occurring.

4.2 Methods

4.2.1 Study Sites

The Homochitto National Forest is located within the Southern Rolling Plains ecoregion, and is dominated by loblolly and shortleaf pines (\textit{Pinus taeda} L., \textit{Pinus echinata} Mill.). Soils in this region are clayey or loamy with a thin loess layer (Omernik et al. 2008). Annual temperature varies from 10.83 to 14.17 °C, and annual precipitation ranges between 5.74 and 23.9 cm (Southern Regional Climate Center 2013). In 2011, SPB infestations were not detected by aerial or ground surveys. However, in 2012, ≤193 active SPB spots were recorded following aerial or ground surveys (Southern Pine Beetle Information System 2012).

4.2.2 SPB Spot Data

We used the SPBIS database to find SPB spot coordinates, date of initial aerial detection of SPB spots (which were confirmed by ground check), the average basal area of all trees within a spot, and average basal area of pines within a spot. USDA-Forest Service Southern Pine Beetle Information System (SPBIS) is an Oracle® database, developed by the USDA-Forest Service, Southern Region, Forest Health Protection that includes SPB spot formation data in the southeastern U.S. All SPB infestations on federal lands were reported and recorded weekly from April to September 2012 by forest district field crews who conduct ground surveys following aerial detection of SPB infestations. During the summer months, aerial surveys identified SPB spots by a grouping of > 5-10 pine trees whose crown had changed in color from green to yellow, red, or brown.
Foliage which has faded to yellow indicated active SPB infestation (Hain 1980, Billings 2011b, Peacher 2011). Foliage that had faded to brown or red coloration infesting beetles had exhausted phloem resources and vacated the tree (Doggett 1971, Billings and Kibbe 1978, Billings 1979, Hain 1980). Aerial surveyors typically record infestations within 0.8-4.02 km from the plane by traveling within flight lines spaced 1-8 km apart at 300-610 m above ground (Aldrich et al. 1958, Billings 2011).

### 4.2.3 Lightning Strike Data

The US NLDN® monitors over 100 ground-based remote Vaisala IMPACT ESP (Enhanced Sensitivity and Performance) Lightning Sensors® (Vaisala-GAI Inc., Tucson, Arizona, USA), which annually record > 20 million cloud-to-ground lightning flashes 200-300 km from the coastline and throughout the U.S. Data access is limited to partnerships associated with the NASA Marshall Space Flight Center (MSFC) Lightning Group. Lightning strikes are recorded by IMPACT sensors, which record the sound of electromagnetic signals released by each stroke. Typically 6-8 time-of-arrival antennae record and map a hyperbola of electromagnetic signals (using GPS timing), and direction finding creates an azimuth information to the point of contact. Coordinates, time, polarity, and peak signal amplitude of the first return stroke and the number of successive strokes (multiplicity) are documented in the database (Global Hydrology Resource Center 2013). The precision of lightning strike coordinates was assured by only using strike coordinates recorded with a semi major axis value > 0.05.

Lightning strike is the electrostatic discharge between two electrically charged objects. A single strike is the compilation of 2-3 electric strokes (but maybe as high as 20), often occurring within a sequence of 20-100 ms and lasting 20-50 ms. Stroke
discharges may occur within a cloud or between clouds, or may escape and move cloud-to-air or cloud-to-ground. A series of cloud-to-ground strokes typically contact the earth’s surface at the same point, thus being observed as a single lightning strike. Close proximity of stroke contact occurs because subsequent strokes typically follow an ionized air channel created by the initial stroke. Hence, a lightning strike is defined as the total number of ground-to-cloud strokes (multiplicity) which occur within a one second time period in a 10 km radius (Coder 2007).

4.2.4 Statistical Analyses

4.2.4.1 Comparison of Uninfested and Infested SPB Stands

Multiple logistic regression models were used to assess whether SPB spot formations were more likely to occur closer in spatial proximity and time to lightning strikes than arbitrarily in 2012 within the Homochitto National Forest. Logistic regression is commonly used to assess the relationship between one or more predictive independent variables and categorical dependent variables within large data sets.

Independent variables within models included the status of a location as uninfested (0) or infested (1). ARC-GIS was used to randomly generate an equal number of coordinates of uninfested stands as SPB spots within the geographic boundary of the Homochitto National Forest (ESRI 2011). Coordinates of uninfested stands and spots did not coincide. A shape file, polygon of the Homochitto National Forest was provided by the USDA-Forest Service, FSGeo Data Clearinghouse (2013).

The time period prior to observation of a spot or uninfested location was included as a second independent variable because if lightning strikes and formation of SPB spots are correlated, it is likely trees are struck prior to and not after a successful SPB
infestation has occurred. Thus, the time at which a strike was recorded and the time at which a spot was detected are likely correlated. So in our models, reasonable comparisons of time among multiple locations were made by limiting time parameters for both SPB spots and uninfested stands. Time parameters for spot were 14 days prior to initial aerial detection of SPB because SPB spots are typically visible by aerial surveillance within 14 days following successful infestation (Hain 1980, Billings 2011). However lightning strikes occur year round, and coordinates for uninfested stands were arbitrary, so time parameters for uninfested coordinates extended from the first date of SPB surveillance (2 April, 2012) to 14 days prior to the final SPB aerial survey date (22 August, 2012) within the national forest.

The relationship between spot formation and the distance at which a lightning strike occurred was assessed by comparing independent variables, SPB spots to uninfested areas, including time as a second independent variable, and distance as the dependent variable. However, the maximum distance parameter was 500 m because the median dispersal distance of SPB in summer months is typically 530 m, so distances > 500 m lacked biological significance (Turchin and Thoeny 1993), and the median strike location accuracy of the lighting detection sensors was 500 m (Global Hydrology Resource Center 2013).

The relationship between lightning characteristics on spot formations was assessed by comparing independent variables, SPB spots to uninfested areas, including time as an independent variable and magnitude and/or polarity as independent variables. Magnitude values ranged from 5-300 kilo amps and polarity was either positive (transfer of a positive charge from ground to cloud) or negative (transfer of a negative charge from
cloud to ground) (Macgorman and Burgess 1994). Linear regression was used to assess the relationship between the distance of prior lighting strikes and the number of trees infested by SPB at the time of the first ground check with the distance between lightning strikes to the spots as independent variables and the log function of the total number of infested trees within spots as dependent variables.

4.2.4.2 Correlation between Lightning Strike and Stand Basal Area

Logistic regression was also used to assess whether the total average basal area (BA) or average BA of pines within infested stands correlated with the frequency or distance at which lightning strikes had occurred. Independent variables were total average BA or average BA of pines within spots, and the 14 day time parameter. Dependent variables included the distance between strike and spot coordinates and the number of strikes which occurred within previously specified 14 day time period.

4.2.4.3 Model Validation

Chi Square Tests and Wald Tests were used to assess significance findings within models. The Chi Square Test examines goodness of fit by calculating an approximation of the sampling distribution of test statistics which are equal to theoretical Chi Square distributions within large data sets (Lemeshow and Hosmer 1982). The Wald Test assesses the relationship between independent and dependent variables through the through sample estimates of the independent parameters (Bergerud 1996). Model calibration was assessed by the Hosmer-Lemeshow Test, which determined the similarity between expected and observed rates of risk values within data subgroups (Hosmer and Lemeshow 1989).

4.3 Results
In this study, the response variable was whether lightning strikes had (1) or had not (0) occurred. Predictive variables in viable models included the infestation status of “spot” (infested (1) or uninfested (0) location), distance (between lightning strike coordinates and coordinates or either the infested or uninfested locations, and magnitude (the amount of energy released by a strike in kilo amps). Magnitude alone was not a predictive variable ($\chi^2(1, N=414) = 0.000, P=0.723$), unless it exceeded > 150 kilo amps ($\chi^2(1, N=414) = 0.1087, P=0.001$). Strike polarity was a poor response variable because all of the lightning strikes (100%) recorded in our data had negative polarity. Thus negative polarity occurred too frequently prior to SPB spot detection for the calculation of the maximum likelihood estimate.

Our data set included only SPB spots which were detectable by aerial survey due to the red coloration of the upper crown. Within a two week time period, SPB usually colonize and consume enough phloem to cause tree death. In infested trees, upper crown needles turn yellow to red in color which indicates a SPB infestation. Thus time was a biologically significant predictive parameter associated with beetle infested spots. So for each location, time was a significant prediction variables. So the time variable for each infested spot location extended from 14 days before the date each spot was detected to the date the spot was detected by aerial survey. The coordinates of uninfested locations were generated at random. Therefore, there was no inherent biological reason to individually restrict a time periods for each uninfested location. So time variables for uninfested locations extended from the first recorded lightning strike to 14 days prior to the last date of the sample period (2 April 2012). More restrictive time parameters for
either infested or uninfested locations excluded ≥ 43% of the infested and uninfested location coordinates.

Results from this model indicate lightning strike was similarly likely to occur within 500 m of infested and uninfested locations ($\chi^2 (1, N=414) = 0.125, P=0.723$). However, lightning strikes within 250 m ($\chi^2 (1, N=414) = 0.125, P=0.005$) and 100 m ($\chi^2 (1, N=414) = 6.677, P=0.009$) were significantly more likely to occur 14 days prior to spot formation near SPB spots than uninfested locations.

Using logistic regression, we did not find a relationship between average stand BA or the average BA of pines within SPB spots and the likelihood of lightning strike. Specifically, we did not find a relationship between the frequency (number of lightning strikes) and the proximity of lightning strikes to the average stand BA or the average BA of pines within SPB spots.

### 4.4 Discussion

Our findings suggest that SPB spot formation was indicative of previous strikes within at least 250 m in the Homochitto National in 2012. Lightning is the only natural disturbance associated with SPB population dynamics (Coulson et al. 1983). It is apparent SPB successfully colonize lighting struck trees (Hain et al. 2011), and likely that lightning struck trees are susceptible hosts that support SPB populations (Lorio 1986). Researchers suggest that although lightning struck trees may be exploited by SPB during low population phases, they remain underutilized when outbreak populations are large enough so conspecifics may coordinate colonization of healthy trees (Rykiel et al. 1988, Lovelady et al. 1991). Our results support previous work proposing temporal and/or
spatial lightning regimes connect pine stand dynamics to SPB biological requirements, which in turn may drives SPB population cycles (Coulson et al. 1983).

Identifying lightning struck trees is difficult because tree species, seasonality, electrical conductivity, and water content within vascular tissues determine whether a tree is damaged, killed, or unaffected by lightning strike. If enough energy is transmitted to the tree and the outer bark is dry, while the sap wood is wet, the tree itself won’t be affected but the electricity is transmitted, and neighboring trees are electrocuted (Coulson et al. 1983). Lightning struck trees are typically identified by a downward spiraling pattern of removed bark around the bole, running from the crown to the ground. This pattern occurs when the phloem retains high water content. However, lightning may also cause indeterminable patches of removed bark, an exposed root system, or not display any physical signs of damage if the outer bark was sufficiently soaked with water (Hodges and Pickard 1971, Coulson et al. 1983, Miller 1983, Blanche et al. 1984). If the sapwood has high water content a struck tree is likely explode (Coulson et al. 1983, Miller 1983, Coder 2007). Furthermore, approximately only 20% of lightning struck trees are visibly damaged with the spiral shaped scar indicating where an electric charged had traveled through cambium-xylem initiation cells and the phloem. Lightning damage is more often indicated by wilting or death of a single limb or twig over a period of months or up to three years (Coder 2007). Difficulty in identifying lightning struck trees probably downplays the importance of lightning struck trees in SPB ecology and population dynamics.

Lightning strikes also occur frequently within forested landscapes. At least 90% of all ground lightning strikes are characterized as cloud-to-ground electric charges and at
least one tree is struck by lightning within each square kilometer in a Southeastern forest (Flamm et al. 1993, Coulson et al. 1999). Even our study sites were located in a geographic area where approximately 35 cloud-to-ground lightning strikes occur per 1.6 km annually (Coder 2007) and in Louisiana, Hodges and Pickard (1971) attributed 75% of new SPB spots to trees struck by lightning. Hence, it is likely that observers are unaware of both the number of lightning struck trees within a forested landscape and the frequency of the cloud-to-ground electric charges which likely play a greater role in SPB spot development or population outbreak than currently acknowledged.

Estimating a time parameters was confounded with observation time because SPB spots could not be detected or recorded within the SPBIS data set until enough time has passed for ≥ 5 trees to attract and become sufficiently infested with SPB (and the bark beetle guild associated) to cause crown death. Before a group of trees exhibit crown death, flying beetles must first find a susceptible host, and then synchronize their attack in large enough numbers to successfully infest a tree (Hain 2011, Sullivan 2011). Then, colonizing beetles must create galleries expansive enough to effectively girdle nutrient flow within the phloem and passive transport of fungi to slow or stop moisture within the phloem. Further, trees may successfully thwart infesting beetles for a time, depending on tree species, within tree moisture content, and prolonged weather conditions (Sullivan 2011). Additionally, there was no biological process or known time limit (similar to the development of spots) to measure time in the uninfested locations. Hence, the underlying assumption of our modeling technique is that the same relationship between time-period and the probability of certain lightning strikes having occurred will hold true for the uninfested sites as for the infested sites. Limiting time parameters for spots to 14 days
(due to biological significance) and the entire sampling period of spots for uninfested locations was a reasonable comparison of time among multiple locations.

Polarity of lightning strikes was an insignificant model parameter because the occurrence of cloud-to-ground lightning strike was too frequent prior to SPB spot detection (100%) and within the sampled time period of uninfested areas (93.9%). This was expected, as most trees are damaged by cloud-to-ground charges, and typically 90% of cloud-to-ground charges begin as a negative charge at the base of storm clouds (Coder 2007). Our findings indicate negative polarity (cloud-to-ground lighting strike) is an important lightning strike characteristic for SPB spot formation (Coulson et al 1983).

Overall, we found that SPB infested stands are more likely to occur within 100 and 250 m distance from lightning strikes with a negative polarity and magnitude > 150 kilo amps. There was no relationship between the likelihood of lightning strike occurring and the mean total BA or mean BA of pine species within an infested stand. Thus the average stand BA or BA of pines within a stand is not a significant parameter in predicting the likelihood of a lightning strike occurrence.

4.5 Acknowledgements

We thank Valli Peacher (USDA-Forest Service, Forest Health Protection) for access and guidance in using SPBIS data sets, and James Meeker and Brian Sullivan (USDA-Forest Service, Forest Health Protection) for their input and guidance on the project. We appreciate collaborating with the Geography Department for partnership with the NASA Marshall Space Flight Center (MSFC) Lightning Group. We also thank Kimberly Love-Myers (University of Georgia) for additional data analyses and technical
support. Funding was provided by USDA-Forest Service, Forest Health Protection, and Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia.
4.6 Literature Cited


Table 4.1. Logistic regression model parameters and fit statistics using negative polarity and electrical current strength of > 150 kilo amps as predictors of lightning strikes occurring prior to SPB spot formations.

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Table 4.2. Logistic regression model parameters and fit statistics using time and < 0.05 semi-major axis as a predictors of lightning strikes occurring within ≥ 500 m, ≥ 250 m, and ≥ 100 m, prior to SPB spot formations.

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CHAPTER FIVE
RESPONSES OF GROUND-FORAGING ANTS (HYMENOPTERA: FORMICIDAE)
TO FOREST MANAGEMENT TECHNIQUES IN PINE FORESTS IN THE
SOUTHEASTERN UNITED STATES

1 Jenny C. Staeben and Kamal J. K. Gandhi. To be submitted to the
Journal of Economic Entomology.
Abstract

Pine plantations constituting primarily of loblolly pines (*Pinus taeda* L.) dominate 117,360 ha of forests in the southeastern U.S. Since loblolly pines are highly susceptible to insect and diseases, forest managers attempt to actively manage these forests through pre-commercial thinning and prescribed-burning. Further, these areas may be replanted by longleaf pines (*Pinus palustris* Mill.) that have historically dominated these landscapes. We assessed the effects of three management techniques: prescribed burning, non-commercial thinning, restorative planting of longleaf pine following clearcutting of loblolly pine stands, and unmanaged (control) stands, on ground-active ant assemblages in central Georgia. During the summer of 2010, a total of 21,441 ants represented by 15 species were caught in unbaited pitfall traps. Catches were dominated by *Formica integra* Nylander (70%), *Pachycondyla chinensis* (Emery) (25%) and *Solenopsis invicta* Buren (21%); the last two species are non-native to North America. Ant captures (as based on the proportion of occurrences) did not differ among the managed and unmanaged stands. Rarefaction analyses indicated that the unmanaged stands harbored the highest ant species diversity with the lowest diversity in longleaf stands, planted following a clearcut. There were no unique species found within any sampled stands. Ordination analyses suggested ant assemblages within the stands replanted with longleaf pine were dissimilar from those in unmanaged stands. Overall, forest management activities may alter the species composition and diversity of ground-foraging ant species for a short- time period within the Piedmont region of southeastern U.S.
Keywords: *Formica integra, Pachycondyla chinensis*, prescribed burn, *Solenopsis invicta*, thinning
5.1 Introduction

Forests in the Southeast U.S. have been dubbed as the “wood-baskets” of the world, spanning over 30 million hectares of pine (*Pinus* spp.) plantations with < 3% of the original longleaf pine (*P. palustris* Mill.) habitat (Frost 1993). However, prior to the European colonization, longleaf pine was the primary pine species found in the Atlantic and Gulf Coastal plain (Richter 1994). As European colonization extended into the southeastern U.S., settlers quickly removed longleaf pine for firewood, export, building materials, and cleared land for agricultural crops (Richter 1994). Due to the collapse of agriculture in the region in late 1800s, abandoned agricultural lands quickly developed into eroding “old fields”; where surrounding pine species released seed which developed into second growth stands. With time, a timber industry developed as local and federal government agencies intensively planted pine species to control the erosion of abandoned agricultural lands. Longleaf pine is the single most resistant pine species to southern pine beetle (*Dendroctonus frontalis* Zimmermann) and other pests and pathogens, and it was not commonly planted (Spring 1974). Longleaf pine requires extensive site preparation, offers little seed dispersal, and grows slowly so it typically offers only long-term economic returns (Markewitz et al. 1994). Hence, abandoned agricultural lands were more often replanted with the faster maturing loblolly pine (*P. taeda* L.) because it requires little site preparation, can grow in poor soil, and offers short-term economic returns (Markewitz et al. 1994, Schultz 1997).

Consequently, loblolly pine has been extensively planted in the region, and with it there has been a greater incidence of insect and disease issues. For example, southern pine beetle is an important pest of loblolly pines, and is considered the most
economically damaging southeastern forest insect (Birt 2011, Peacher 2011). In contrast, southern pine beetle rarely colonizes longleaf pines. Over a 30 year period, southern pine beetle outbreak populations have created “spots” of dead or dying pine stands causing approximately $900 million dollars of direct economic loss to landowners (Price et al. 1997). To control southern pine beetle outbreaks, prescribed burning, non-commercial thinning, and restorative planting of longleaf pine have been recommended. Prescribed burning and non-commercial thinning prevent southern pine beetle outbreak by creating low-density pine stands that increases tree vigor and resistance (Guldin 2011). Non-commercial thinning techniques also provide remaining trees with adequate lateral area and decreased tree competition. Prescribed burning controls competing vegetation and promotes nutrient cycling. Low-level prescribed burns typically remove fire intolerant plant species and woody debris, causing insignificant damage to larger or fire-tolerant pine species (Hermann et al. 1998, Haywood et al. 2001, Sayer and Haywood 2006). Restorative longleaf planting renews economic value to the property, reduces risk of future southern pine beetle infestations, and reintroduces longleaf pine to its historic range within the Southeast (Schowalter et al. 1981, Georgia Forestry Commission 2005).

Although forest management practices are effective and widely used (Billings 2011, Guldin 2011), the ecological impacts of these strategies on faunal taxa are not well understood. Ants (Hymenoptera: Formicidae) are ideal ecological indicators for disturbance studies because they occur within every terrestrial ecosystem, constitute a large portion of the invertebrate biomass, perform important ecological functions, are easily captured, and taxonomically well described (Lynch et al. 1988, Hölldobler and Wilson 1990, Bolton 1995). Sampling ant assemblages may provide insight to ecosystem
conditions by indicating: 1) changes in endangered species populations; 2) the presence of invasive species; and 3) temperature, spatial, and temporal variations within the physical environment (Underwood and Fisher 2006). Our research objective was to understand the impact of three management techniques: prescribed burning, non-commercial thinning, and restorative planting of longleaf pine on ground-active ant assemblages in central Georgia.

5.2 Methods

5.2.1 Study Sites

The study sites are located within the Piedmont Region in Greene, Hancock, Jefferson, Newton, Walton, and Wilkes counties in central Georgia (Table 5.1). Soils in this area are clayey-Udults (Griffith et al. 2001, National Resources Conservation Service 2013). The local annual mean temperature in 2010 was $16.33 \pm 0.9$ °C and precipitation was $191.32 \pm 0.44$ cm (Georgia Automated Environmental Monitoring Network 2013). Study sites had $\geq 4.05$ ha of contiguous loblolly or longleaf pines at risk, infested with, over previously cleared due to southern pine beetle infestation prior to treatment application. Within stand, stocking rates of trees are typically $< 700$ trees per 0.05 hectare or $\leq 37$ m$^2$ of basal area.

5.2.2 Forest Management Activities

This study assessed four forest management techniques applied to loblolly pine stands: 1) control or unmanaged stands (which had not been burned, thinned, or treated with herbicide for $\geq 20$ years prior to sampling); 2) prescribed-burn stands (sampled 1-7 years following a burn application); 3) non-commercially thinned stands (sampled 2-5 years following the removal of non-merchantable sized trees); and 4) clearcut loblolly
stands (sampled 4 – 5 years following the planting of longleaf pine seedlings) (Fig. 5.1). There were three control, four prescribed burned, and four thinned stands. Three loblolly stands were clearcut and replanted with longleaf pine. Understory vegetation was identified as wiregrass (Aristida spp.), mixed wiregrass (Rubus and Smilax spp.), and mixed herbaceous-woody which included oak (Quercus spp.) and sweetgum (Liquidambar styraciflua L.) species (Table 5.1).

5.2.3 Ant Sampling

Ants were sampled during 1 May-16 September, 2010. Within each sampled stand, three circular 30 x 30 m plots were installed ≥ 50 m apart. Circular plots were further divided into four quadrants, and a single unbaited pitfall trap was installed within each quadrant so that no trap was ≤ 15 m in proximity to another trap. Hence, a total of four pitfall traps were used to sample ground-active ants within each plot, and three plots were installed within each sampled stand, for a total of 12 traps per stand, and 168 traps for the study. Pitfall sampling was appropriate in this study because catches within a single season were compared and our sampling technique was standardized across all sampled stands (Spence and Niemelä 1994). Pitfall traps are commonly used to capture ground-dwelling ant species despite sampling biases criticisms, (Andersen 1991a, Peet and Allard 1993, York 2000, Stuble et al. 2011, Woinarski et al. 2002, Lubertazzi and Tschinkel 2003, Lassau and Hochuli 2004, Stephens and Wagner 2006, Rodriguez-Cabal et al. 2012). These traps are easily constructed from a variety of materials and continually sample active invertebrate communities without investigator assistance that likely leads to better estimates of species richness (Luff 1975, Andersen 1997, Bestelmeyer et al. 2000, Ward et al. 2001, Steiner et al. 2005). However, captures by smaller pitfall traps
may be biased towards larger bodied ants, and species composition and number 
individuals may be affected by the contents (propylene glycol or water) within collection 
cups (Abensperg-Traun and Steven 1995, Calixto et al. 2007). Furthermore, comparisons 
of pitfall trap-types within a single habitat suggests that trap design significantly 
determines which ant species are captured (Andersen 1991b, Longino and Colwell 1997). 
Despite these limitations, pitfall trapping is a superior capturing method to capture ants 
as compared to hand-sampling or sweep netting), and remains an optimal tool in 
assessing ecological impacts of forest management practices when ants are utilized as 
2004).

Pitfall traps were constructed with ~470 ml cup (Solo© Cup Company, Urbana, 
Illinois) buried with rim flush with the soil surface. This first cup maintained the 
structural integrity of the hole. A second interior ~120 ml plastic non-sterile collection 
cup (Amsino International Incorporated, Pomona, California) was fitted with an 8.64 cm 
diameter plastic funnel to facilitate trap capture of insects. The 120 ml interior cup was 
filled with 60 ml of (1: 3) water and propylene glycol (Low Tox, Prestone® Products 
Corp.) to kill and preserve captured ants. A protective 10.2 x 10.2 cm plywood roof 
supported by four ~9 cm nails were used to keep rain and leaf litter from entering the 
trap. To prevent trap disturbance by small mammals, each pitfall trap was additionally 
covered with a 31 x 31 cm piece of chicken wire, secured by four 31 cm long galvanized 
spikes. Overestimation of diversity was constrained by installing pitfall traps far apart (> 
20 m) and collecting samples at multiple time periods throughout the summer (Gotelli et 
al. 2011). Traps were emptied every 3-4 weeks, and all ants were identified to species-
level using taxonomic key provided by MacGown (2011). Voucher specimens have been deposited at the University of Georgia, Museum of Natural History, Collection of Arthropods in Athens, Georgia.

5.2.4 Statistical Analyses

Ant colonies are super organisms, made of eusocial animals existing and acting within larger social units (colonies). Hence, the abundance of captured foraging ants indicates foraging activity, ant biomass, or proximity of active colonies to pitfall traps (Schlick-Steiner 2006, Gotelli et al. 2011). We assessed the effects of forest management practices on ant communities by using the proportion of occurrence of each captured ant species instead of absolute numbers caught in traps (Shingleton and Foster 2000, Vasconcelos et al. 2008, Higgins and Lindgren 2012). Catches from each pitfall trap were pooled over the summer, and standardized to 100 trapping days (number of ants captured/trap/treatment/100 days) to account for trap disturbances. The proportion of each ant species captured within each pitfall trap was calculated for analyses.

Analyses of variance tests (ANOVA) were used to analyze differences of ant captures among land-area treatments. Data were normalized using log transformations (Higgins and Lindgren 2012). Tukey’s Standardized Range Tests (HSD) were used for posthoc analyses (Zar 1999, SAS 2008). ANOVAs were conducted for both total catches (all ants and only native species separately), and at species-level for the five most abundant ant species.

Venn diagrams were created to demonstrate species richness by illustrating shared and unique species among stands. Sample-based rarefaction was used to estimate ant species diversity based on the number of samples within which each species was
captured, not the total number of captured individuals (Holland 2003, Goletti and Colwell 2001, Gotelli et al. 2011). Rarefaction analysis accounts and adjusts for species abundance and richness (Holland 2003, Magurran 2004), and discrepancies in sampling effort due to trap disturbances by randomly, repeatedly, resampling a collection of individuals (Gotelli and Colwell 2001).

Nonmetric multidimensional scaling (NMS) using the Bray-Curtis similarity index was used to quantify the compositional dissimilarity between land-area treatment types, based on standardized proportional-count data from each sampled site (McCune and Mefford 2011). Data were arcsine-transformed before analyses to reduce the coefficient of variation to <100% (Shingleton and Foster 2000, Vasconcelos et al. 2008). Bray-Curtis distance measure was used to conduct preliminary NMS ordination. Initial distance measures of all captured ant species occurred on three axes using random coordinates, with stability criterion of 0.000010, 78 iterations to evaluate stability, 250 iterations at 0.2 step lengths, and 50 runs for real data. The same values excluding counts of non-native ant species were used for the initial distance measures of endemic species, except 68 iterations were required to evaluate stability. Plot stress versus number of iterations was used to verify the solution. Correct number of dimensions was determined by plotting the final stress versus number of dimensions. In the final NMS ordination analysis, stress was within the intermediate range for community data sets (10-20) (Vasconcelos et al. 2008). Final stress for all ant species was 2.78333 and final stability was 0.00001. Final stress for only native ant species (excluding exotic species) was 4.080646 and final stability was 0.00001. Ordination graphs of all captured ant species and native species were created using two environmental axes which expressed the
greatest variation among ant species composition among treatments (i.e., the highest $R^2$ value).

5.3 Results

A total of 21,441 ants were captured of which 70% of catches were *Formica integra* Nylander, 12% *Crematogaster lineolata* (Say), and 4% *Crematogaster lineolata* (Say); all native ant species. Two non-native and highly invasive species, *Pachycondyla chinensis* (Emery) and *Solenopsis invicta* Buren were captured and accounted for 25% and 21% of the total catches, respectively (Table 5.2).

Total ant captures (including all species) significantly differed among the four land-area treatments ($F = 0.64$; d.f. = 3, 14; $P = 0.044$) (Fig. 5.2). However, there was no significant difference in pairwise comparisons of forest management techniques using the conservative Tukey’s Standardized Range Test. Trap catches of native ant species (i.e., excluding *P. chinensis* and *S. invicta*) also did not significantly differ among treatments ($P = 0.342$). Captures of the most abundant ant species, *C. chromaiodes*, *C. lineolata*, *F. integra*, *P. chinensis*, and *S. invicta* did not significantly differ among forest treatments ($P$-value ranged from 0.453 to 0.606.) Further, catches of the three most abundant native ant species, *C. chromaiodes*, *C. lineolata*, and *F. integra* did not differ among forest treatments ($P$-value ranged from 0.177 to 0.24).

Venn diagrams for comparisons of species richness among treated stands indicated ant richness was ~ 93% similar among burned, thinned, and clearcut-replanted stands (Fig. 5.3). There were no unique species to either of the forest stands (Fig. 5.3). Rarefaction curves at the lowest subsample size suggested that unmanaged loblolly pine stands harbored the greatest ant species diversity followed by prescribed burned, thinned,
and clearcut-replanted stands (Fig. 5.4). Species accumulation curves eventually converged with increasing sample size for both thinned and prescribed burned stands. Further, curves for clearcut-replanted stands kept on accumulating more species with sample size, and did not level off in our study suggesting that those sites could harbor more species than reported in our study (Fig. 5.4).

Ordination plot of all captured ant species generated from the NMS analyses showed that replanted longleaf pine and the unmanaged stands had the most different ant assemblages from each other, as they were present on the opposite end of the gradient \( R^2 \) for axis 1 = 61\%, \( R^2 \) for axis 2 = 35\%, \( r = 0.101 \) (Fig. 5.5). Thinned and prescribed burned forest stands had similar ant assemblages. *Formica integra* and *S. invicta* were closely associated to prescribed burned stands, and *P. chinensis* with clearcut-replanted stands (Fig. 5.5). Ordination plot for only native ant species indicated that species composition within the unmanaged and clearcut stands, replanted with longleaf pine were more similar to each other \( R^2 \) for axis 1 = 15\%, \( R^2 \) for axis 2 = 77\%, \( r = -0.143 \) (Fig. 5.6). *Camponotus chromaiodes* was more closely associated with clearcut-replanted stands, and *C. lineolata* and *F. integra* with burned stands (Fig. 5.6).

### 5.4 Discussion

We found ant species abundance did not significantly differ among forest treatments. These results differ from previous studies which reported a decrease in ant species numbers within prescribed burned and thinned stands (Majer 1977, Andersen 1986, York 2000). Typically the density of ant populations and species richness depends upon nest and ground surface temperatures associated with vegetation density and diversity, soil type and moisture, foraging behavior of species, and the diameter of the
pitfall trap used for sampling (Greaves 1979, Goldstein 1975, Marsh 1985, Andersen 1986, Olson 1991, Bestelmeyer et al. 2000). Decreases in ant species abundance caused by burning or thinning are typically attributed to reductions of canopy coverage, litter density, soil moisture levels, and structural complexity of ground vegetation (Anderson 1991, Andersen 1997, Melbourne 1999, York 1999, 2000, Hoffmann and Andersen 2003, Graham 2004). In our study, certain ant species appear to be resilient to these forest disturbances, and as such to changes in the soil-litter interphase in these areas.

Pitfall trap catches within managed pine stands were dominated by two non-native (P. chinensis and S. invicta) and one native (F. integra) ant species. Native to Asia, P. chinensis, and South America, S. invicta, these species have been established in the southeastern United States since the 1930’s (Zungoli and Benson 2008, Guénard and Dunn 2010, Yashiro et al. 2010). Formica integra, P. chinensis, and S. invicta are aggressive, omnivorous, and polygynous species which demonstrate sociotomy (Kloft et al. 1973, Porter and Savignano 1990, Leal and Oliveira 1998, Yashiro et al. 2010). Once established, these species create multiple colonies with reproductive castes throughout the landscape. Territorial aggression towards other ant species, a broad diet, and prolific reproductive strategy has enabled them to competitively replace local ant populations (Hölldobler 1990, Porter and Savignano 1990, Vinson 1994, Leal and Oliveira 1998). For example, when insecticide was applied to longleaf pine stands, captures of S. invicta and native ant species did not differ between untreated and treated stands. Solenopsis invicta may also invade disturbed areas where native ant populations are already low due to a disturbance as it has documented in a longleaf pine stand in Georgia (Carroll and Hoffman 2000, Wojcik et al. 2001, Stuble et al. 2009, 2010).
Rarefaction analysis indicated that ant species diversity was greater in unmanaged stands followed by prescribed burned, thinned, and clearcut-replanted stands. Thus, species diversity declined in a linear manner with increasing disturbance intensity. Our results are in contrast to that of the Intermediate Disturbance Hypothesis where highest diversity is in the intermediate stages of disturbance which would have been the thinned and prescribed-burned stands in our study (Beckage and Stout 2000). Similar results have been found for ants across a disturbance gradient where either greater ant diversity or richness was found in relatively undisturbed sites (Vasconselos 1999, Dunn 2004, Schonberg et al. 2004). Ants (particularly native species) in the disturbed sites may have experienced either direct or indirect mortality due to management disturbances through compaction of soil, loss of prey, and burning as previously reported (Beckage and Stout 2000, Ratchford et al. 2005).

NMS analyses indicated that species composition was unique in unmanaged stands, compared to managed stands which retained invasive ant species, *P. chinensis* and *S. invicta*. When invasive species were excluded from the analyses, the unmanaged stands remained dissimilar to the managed stands. These results indicate intermediate stand disturbances alter herbaceous cover or soil attributes which may adversely affect native ant species assemblages and facilitate colonization and dominance by invasive species overtime (Lubertazzi and Tschinkel 2003, Cumberland and Kirkman 2012).

Burned and thinned stands were similar to each other, and the clearcut-replant stands were dissimilar to them at least along one gradient. *Pachycondyla chinensis* and *S. invicta* were primarily associated with the clearcut-replanted and prescribed-burned stands, respectively instead of the unmanaged stands. Lubertazzi and Tschinkel (2003)
and Graham et al. (2004) reported *S. invicta* was associated with wiregrass in pine stands in Florida and Georgia. Graham et al. (2004) postulated *S. invicta* may either prefer an understory of wiregrass or the high frequency burning which is indicative of wiregrass landscapes. Since wiregrass was essentially absent in our prescribed burned stands, we conclude that *S. invicta* may be adapted to burning cycles more so than the presence of wiregrass.

Furthermore, the presence and high numbers of invasive ant species in forest stands may cause ecological consequences. For example, in North Carolina, *P. chinensis* reduced native ant populations, and ant species diversity to 30-40% in hardwood stands (Guénard and Dunn 2010). The broad diet, territorial behavior, and establishment of > 50% polygynous colonies of *S. invicta* was found to reduce the number of ant species by 70% and native ant species by 90% in Texas (Porter and Savignano 1990). *Solenopsis invicta* also typically colonizes and displace native ant species quickly in longleaf pine stands in Georgia (Carroll and Hoffman 2000, Wojcik et al. 2001, Stubel et al. 2009).

Overall, we conclude that while there were little differences in numbers of ant individuals, there were differences in species diversity and composition between unmanaged and managed forest stands. Non-native species especially numerically dominated the managed stands. Our results could be confounded by a number of factors e.g., the variability in time since treatment in stands (1-7 years), use of pitfall traps instead of hand-sampling and soil extractions that provide density information, and sampling only for one growing season with questions about whether these patterns would persist or disappear over long periods in the Piedmont Georgia.
5.5 Acknowledgements

Many members of the Forest Entomology Laboratory at the University of Georgia contributed to the fieldwork and sorting trap catches. Thanks to Jim Hanula and Scott Horn, USDA-Forest Service, Southern Research Station, Athens, Georgia for trapping materials. Species identification of ants was verified by Doug Booher, Department of Ecology and Evolutionary Biology, University of California, Los Angeles and members of the Georgia Museum of Natural History. Funding was provided by USDA-Forest Service, Forest Health Protection, Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, and McIntire-Stennis Grant.
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Table 5.1. Site characteristics of sampling areas located in six counties within the Piedmont region of north-central Georgia.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Years Since</th>
<th>Georgia</th>
<th>Latitude &amp; Longitude</th>
<th>Overstory</th>
<th><em>Pinus</em> Species</th>
<th>Average DBH (± SE)</th>
<th>Dominant Understory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmanaged</td>
<td>&gt; 20</td>
<td>A Greene</td>
<td>33.57° N, -83.16° W</td>
<td>Loblolly / Mixed Deciduous</td>
<td>33.4 ± 0.54</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 20</td>
<td>B Newton</td>
<td>33.54° N, -83.85° W</td>
<td>Loblolly / Mixed Deciduous</td>
<td>34.1 ± 0.57</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 20</td>
<td>C Walton</td>
<td>33.78° N, -83.73° W</td>
<td>Loblolly / Mixed Deciduous</td>
<td>34.1 ± 0.65</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td>Non-commercial Thin</td>
<td>2</td>
<td>A Wilkes</td>
<td>33.77° N, -82.74° W</td>
<td>Loblolly Pine</td>
<td>30.9 ± 0.56</td>
<td>Mixed wiregrass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>B Hancock</td>
<td>33.26° N, -83.00° W</td>
<td>Loblolly Pine</td>
<td>34.6 ± 0.63</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>C Hancock</td>
<td>33.26° N, -83.00° W</td>
<td>Loblolly Pine</td>
<td>32.3 ± 0.61</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D Walton</td>
<td>33.78° N, -83.73° W</td>
<td>Loblolly Pine</td>
<td>33.4 ± 0.54</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td>Prescribed Burn</td>
<td>1</td>
<td>A Wilkes</td>
<td>33.77° N, -82.74° W</td>
<td>Loblolly Pine</td>
<td>29.6 ± 0.55</td>
<td>Mixed herbaceous-woody &amp; wiregrass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>B Hancock</td>
<td>33.26° N, -83.00° W</td>
<td>Loblolly Pine</td>
<td>25.2 ± 1.64</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>C Hancock</td>
<td>33.78° N, -83.73° W</td>
<td>Loblolly Pine</td>
<td>26.7 ± 0.49</td>
<td>Mixed herbaceous-woody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>D Greene</td>
<td>33.57° N, -83.16° W</td>
<td>Loblolly Pine</td>
<td>28.8 ± 0.47</td>
<td>Mixed herbaceous-woody</td>
<td></td>
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<tr>
<td>Clearcut &amp; Longleaf Planted</td>
<td>5</td>
<td>A Newton</td>
<td>33.54° N, -83.85° W</td>
<td>Longleaf Pine</td>
<td>15.3 ± 0.33</td>
<td>Mixed wiregrass</td>
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<tr>
<td></td>
<td>5</td>
<td>B Walton</td>
<td>33.78° N, -83.73° W</td>
<td>Longleaf Pine</td>
<td>14 ± 0.37</td>
<td>Wiregrass</td>
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<td></td>
<td>4</td>
<td>C Jefferson</td>
<td>33.05° N, -82.42° W</td>
<td>Longleaf Pine</td>
<td>17.2 ± 0.46</td>
<td>Mixed wiregrass</td>
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</table>
Table 5.2. Captured ant species in 2010 within sampled stands in six counties in the Piedmont Georgia.

<table>
<thead>
<tr>
<th>Species Names</th>
<th>Unmanaged</th>
<th>Thinned</th>
<th>Prescribed</th>
<th>Clear-cut,</th>
<th>Totals</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Burn</td>
<td>Replanted</td>
<td></td>
</tr>
<tr>
<td><em>Aphaenogaster fulva</em> Roger</td>
<td>149</td>
<td>171</td>
<td>115</td>
<td>9</td>
<td>444</td>
</tr>
<tr>
<td><em>Aphaenogaster treatae</em> Forel</td>
<td>23</td>
<td>60</td>
<td>133</td>
<td>0</td>
<td>216</td>
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<tr>
<td><em>Camponotus castaneus</em> Latreille</td>
<td>36</td>
<td>83</td>
<td>100</td>
<td>8</td>
<td>227</td>
</tr>
<tr>
<td><em>Camponotus chromaiodes</em> Bolton</td>
<td>51</td>
<td>35</td>
<td>285</td>
<td>123</td>
<td>494</td>
</tr>
<tr>
<td><em>Crematogaster lineolata</em> Say</td>
<td>81</td>
<td>744</td>
<td>526</td>
<td>71</td>
<td>1,422</td>
</tr>
<tr>
<td><em>Forelius mccooki</em> (McCook)</td>
<td>0</td>
<td>2</td>
<td>18</td>
<td>22</td>
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<tr>
<td><em>Formica integra</em> Nylander</td>
<td>126</td>
<td>2,502</td>
<td>3,365</td>
<td>2,077</td>
<td>8,070</td>
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<tr>
<td><em>Hypoponera opacior</em> (Forel)</td>
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<td>12</td>
<td>26</td>
<td>3</td>
<td>42</td>
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<tr>
<td><em>Myrmecina americana</em> Emery</td>
<td>28</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>51</td>
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<td><em>Neivamyrmex opacithorax</em> (Emery)</td>
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<td>2</td>
<td>7</td>
<td>2</td>
<td>14</td>
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<td><em>Nylanderia consina</em> (Trager)</td>
<td>33</td>
<td>261</td>
<td>145</td>
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<td><em>Nylanderia vividula</em> (Nylander)</td>
<td>13</td>
<td>7</td>
<td>28</td>
<td>4</td>
<td>52</td>
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<tr>
<td><em>Pachycondyla chinensis</em> (Emery)</td>
<td>7</td>
<td>14</td>
<td>293</td>
<td>4,995</td>
<td>5,309</td>
</tr>
<tr>
<td><em>Pheidole crassicornis group</em> Emery</td>
<td>33</td>
<td>62</td>
<td>45</td>
<td>3</td>
<td>143</td>
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<tr>
<td><em>Solenopsis invicta</em> Buren</td>
<td>36</td>
<td>2,679</td>
<td>751</td>
<td>1,038</td>
<td>4,504</td>
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<tr>
<td>Total Number of Individuals</td>
<td>626</td>
<td>6,645</td>
<td>6,348</td>
<td>8,369</td>
<td>21,480</td>
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<td>Total Number of Species</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>16</td>
</tr>
</tbody>
</table>

* Non-native ant species.
**Figure Legend**

**Figure 5.1:** Land-area treatments included merchantable-sized loblolly (*Pinus taeda* L.) stands which were either: (A) unmanaged (control); (B) had non-commercial thinning, (C) prescribed burning, or (D) clearcut and replanted with longleaf pine (*Pinus palustris* Mill.).

**Figure 5.2:** Mean (± SE) of captured ant species, standardized to 100 trap-catch days in loblolly pine stands that were either unmanaged, non-commercially thinned, prescribed burned, or clear-cut and replanted with longleaf pine.

**Figure 5.3:** Venn diagram of ant species unique to or shared by each forest management technique. Values in parenthesis are the total number of species captured.

**Figure 5.4:** Rarefaction diversity estimates of ant species captured by pitfall traps in loblolly pine stands that were either unmanaged, non-commercially thinned, prescribed burned, or clear-cut and replanted with longleaf pine.

**Figure 5.5:** NMS ordination analysis of species composition of both native and exotic ant species in loblolly stands which were either unmanaged, non-commercially thinned, prescribed burned, or clear-cut and replanted with longleaf pine. Abbreviations of species names are: Cline: *Crematogaster lineolata* (Say); Finte: *Formica integra* Nylander; Pchin: *Pachycondyla chinensis* (Emery); and Sinvi: *Solenopsis invicta* Buren.
Figure 5.6: NMS ordination analysis of species composition of native ant species in loblolly stands which were either unmanaged, non-commercially thinned, prescribed burned, or clear-cut and replanted with longleaf pine. Abbreviations of species names are: Cline: *Crematogaster lineolata* (Say); Finte: and *Formica integra* Nylander.
Figure 5.1

A. Unmanaged Stand

B. Thinned Stand

C. Prescribed Burned Stand

D. Clearcut, Replanted with Longleaf Pine Stand
Figure 5.2

Mean (± SE) Number of Captured Ants

SPB Prevention Techniques

Figure 5.2
Total Number of Species = 15

![Venn Diagrams showing species distribution across different management practices.](image)

Figure 5.3
Figure 5.4

Expected Number of Species vs. Rarefied Sample Size

- Unmanaged Stand
- Thinned
- Prescribed Burned
- Clearcut, Replanted
Figure 5.5

- Unmanaged
- Thinned
- Prescribed Burned
- Clearcut, Replanted

Unmanaged, thinned, prescribed burned, and clearcut-replanted points are plotted along the x-axis and y-axis. The graph shows the impact of different management practices on a specific variable.
6.1 Dissertation Conclusions

My research was focused on the population and chemical ecology of southern pine beetle (*Dendroctonus frontalis* Zimmermann), pine engraver beetles (*Ips* spp.), and their associated bark, woodboring, and predaceous beetles. In Chapter Two, I evaluated whether *D. frontalis* and its major predator, *Thanasimus dubius* (Fabricius) can differentiate between enantiomers of α-pinene. Results indicated that male *D. frontalis* were somewhat more responsive to (+)-α-pinene enantiomer and female *D. frontalis* did not differentiate between enantiomers. *Thanasimus dubius* did not differentiate between α-pinene enantiomers, but *T. dubius* captures increased with greater volumes of α-pinene. *Dendroctonus frontalis* infestations begin when female beetles burrow into the bole and release frontalin to attract males. Thus, male beetles are likely more exposed to predation by *T. dubius*. By evolving an enantiomeric specificity, male *D. frontalis* may have evolved a partial escape mechanism from predation to counteract *T. dubius* exploitation of host volatiles (Raffa and Klepzig 1989).

The majority of *D. frontalis* captures were in the spring (93%) and *T. dubius* captures were 38% greater in the fall. In the spring-time, *D. frontalis* emerge from overwintering within host whose resources have been exhausted, and these adults are adapted for flying long distances to find vulnerable hosts. Searching for new hosts is
powered by high lipid content in larger bodied beetles and a heightened sensitivity to interspecific pheromones and host volatiles (Sullivan 2011). In contrast, populations of the predator, T. dubius, typically follow a predator-prey lag phase as a function of the D. frontalis populations (Stephen et al. 2011). So greater captures of T. dubius and lower captures of D. frontalis in the fall likely indicates the predatory pressure of T. dubius on D. frontalis and heightened sensitively to host kairomones in the fall.

In Chapter Three, I assessed the response of southern pine bark beetle guild (SPBBG), and their competitors and predators in three separate experiments using varying combinations of SPBBG semiochemical lures including (-)-α-pinene, (+)-fronalin, (+)–ipsdienol and/or (+)-ipsenol. Dendroctonus frontalis attractants are α-pinene and frontal, and Ips spp. lures are ipsdienol and ipsenol. In Experiment 1, a total of 10,544 beetles representing 10 families, 27 genera, and 35 species were captured. The most abundant beetle species were: 1) bark beetles, D. frontalis, D. terebrans (Olivier), I. avulsus (Eichhoff), I. grandicollis (Eichhoff), and D. frontalis; 2) woodborers, Monochamus complex (which includes Monochamus titillator (F.) and Monochamus carolinensis (Olivier) species), Pycnomenus sulcicollis LeConte; and 3) predators, Lasconotus pusillus LeConte; Namunaria guttulata (LeConte), Platysoma cylindrica (Paykull), Temnochila virescens (Fabricius), and Thanasimus dubius (Fabricius). The following lure combinations were associated with large catches (> 100) of the following species: 1) α-pinene and frontal or α-pinene, frontal, ipsdienol, and ipsenol: D. frontalis, D. terebrans, P. sulcicollis, and T. dubius; 2) ipsdienol and ipsenol: I. avulsus and I. grandicollis; 3) α-pinene, frontal, ipsdienol, and ipsenol: L. pusillus, N. guttulata, P. cylindrica, and T. virescens; 4) and few beetles were captured unbaited traps.
In the second experiment, a total of 26,500 beetles representing 13 families, 28 genera, and 28 species were captured. The most abundant species were: 1) bark beetles, *D. frontalis*, *D. terebrans*, *Dryophthorus americanus* Bedel, *Hesperus baltimoresensis* (Gravenhorst), *Hylastes tenius* Eichoff, *I. avulsus*, and *I. grandicollis*; 2) woodborers, *Monochamus* complex and *P. sulcicollis*; 3) and predators, *Anobium punctatum* (DeGeer), *L. pusillus*, *P. cylindrica*, *T. virescens*, *Tenebroides marginatus* (Palisot de Beauvois), and *T. dubius*. The following lure combinations attracted large catches (> 100) of listed species: 1) α-pinene: *D. terebrans*, *D. americanus*, *H. tenius*, *I. avulsus*, *L. pusillus*, *Monochamus* complex, and *P. sulcicollis* and ; 2) α-pinene and frontal: *D. frontalis*, *T. virescens*, and *T. dubius*. There were no significant differences among treatments for *H. baltimoresensis*, *I. calligraphus*, *I. grandicollis*, *P. cylindrica*, and *T. marginatus*. Few beetles responded when *Ips* lures were paired with frontal.

In the third experiment, I captured 10,488 beetles representing nine families, 24 genera, and 34 species. The most abundant species were: 1) bark beetles, *D. terebrans*, *H. tenius*, *I. avulsus*, *I. grandicollis*; 2) woodborers, *Acanthocinus obsoletus* Olivier, *Monochamus* complex; 3) and predators, *L. pusillus* LeConte, *N. guttulata* (LeConte), *Platypus flavicornis* Fabricius, *P. cylindrica*, *P. sulcicollis*, *T. marginatus*, and *T. dubius*.

Overall, *D. frontalis* and its predator *T. dubius* were primarily attracted to *D. frontalis* attractants, α-pinene and frontal, and attraction to α-pinene and frontal was interrupted by the addition of ipsdienol and ipsenol although local *D. frontalis* populations were low. Our results also suggest *D. frontalis* and *T. dubius* remain attracted to *D. frontalis* attractants, α-pinene and frontal, and do not exploit *Ips* pheromones when local *D. frontalis* populations are low. Our results agree with previous research
identifying α-pinene, ipsdienol, and ipsenol as *D. terebrans* and *I. grandicollis* attractants. *Ips avulsus* attraction to ipsdienol and ipsenol increased when frontalin was included in lure combinations and decreased when α-pinene was included. Thus, *I. avulsus* likely exploit both *Ips* and *D. frontalis* pheromones when *D. frontalis* and *Ips* populations are low. Host kairomone, α-pinene, synergized the response of SPBBG predators including: *H. tenius, L. pusillus* and *T. virescens* to ipsdienol and ipsenol. The *Monochamus* complex captures were greatest when traps were baited with α-pinene to frontalin and *Ips* lures.

*Pycnomerus sulcicollis* were primarily attracted to *Ips* lures; and frontalin interrupted their response to ipsdienol and ipsenol. *Platysoma cylindrica* appear to be a generalist predator because it was attracted to both *Ips* and *D. frontalis* lure combinations. Overall, our results indicate a complex semiochemical communication occurs among SPBBG members, their predators and competitors.

In Chapter Four, I explored the relationship between the formation of *D. frontalis* infestations and occurrence of lightning strike during a *D. frontalis* outbreak in the Homochitto National Forest, Mississippi in 2012. A relationship between *D. frontalis* infestations and lightning strikes was determined using linear regression. Independent variables were whether a *D. frontalis* infestations did (1) or did not (0) occur. A relationship between *D. frontalis* infestations and lightning strike were determined using the following dependent variables: (1) distance between *D. frontalis* infested stands and lightning strike coordinates limited to 100 or 250 m; (2) lightning strike polarity > 150 kilo amps; and (3) time between the occurrence of a lightning strike and spot formation. Time parameters for *D. frontalis* infestations were restricted to the date lightning strike
was first recorded (2 April 2012) to 14 days prior to the first aerial detection date of each infestation. Time parameters for uninfested stands were limited to the first recorded lightning survey date to 14 days prior to the last date of the sample period (2 April to 22 August, 2012). More restrictive time parameters excluded ≥ 43% of the coordinates within the uninfested or spot data sets. I did not find a relationship between the number of trees infested with *D. frontalis* and the spatial proximity or frequency of lightning strikes. Further, there was not relationship between the frequency and proximity of lightning strike and the mean basal area (BA) of pine trees or all trees within infested stands.

In chapter five, I used ground-foraging ant species to assess the ecological impacts of forest management techniques used to control or suppress SPB populations. In my study, loblolly pine (*Pinus taeda* L.) stands were either burned, thinned, clear-cut and replanted with longleaf pine (*Pinus echinata* Mill.) or left unmanaged (as control replicates). Ground-foraging ant communities were sampled using pitfall traps which were installed: 1) in unmanaged stands that had not been managed for ≥ 20 years prior to sampling; 2) 2-5 years following the removal of non-merchantable sized trees in thinned stands; 3) 1-7 years following a burn applications; and 4) four to five years after longleaf pine seedlings were planted following a clear-cut.

Pairwise comparisons indicated ant species assemblages did not significantly differ among treated and unmanaged stands. Venn diagrams indicated species richness was ~ 93% similar among thinned, burned, and clearcut-replanted stands. Species accumulation curves indicated unmanaged stands support the greatest ant diversity; followed by prescribed burned, thinned, and clearcut-replanted stands. However,
clearcut-replanted stands appear to support more species than were captured in our study. NMS analyses including native and invasive ant species suggests species assemblages were similar in unmanaged and clearcut-replanted stands. However, NMS analyses of only native species indicated species assemblages were extremely dissimilar between unmanaged and clearcut-replanted stands and similar in burned and thinned stands.

6.2 Implications for Forest Management

*Dendroctonus frontalis* and *Ips* species are considered the most economically damaging forest pests in eastern North America. Their location and selection of hosts and conspecifics is mediated by a dynamic semiochemical communication system among these species, and their competitors and predators. Hence, controlling, monitoring, and suppressing *D. frontalis* and *Ips* species infestations requires an improved understanding of the semiochemical interactions among beetle species associated with members within the SPBBG and the host kairomone, α-pinene. Further, we do not fully understand the ecological impacts of the forest management practices currently used to control or suppress *D. frontalis* and *Ips* species infestations. Standard control and suppression methods (such as prescribed burning, thinning, and replanting clearcut areas with longleaf pine) may negatively affect important ecosystem functions through disturbance of native arthropod and plant communities.

Overall, my studies provide information which supports the following forest management recommendations:

1. The prediction accuracy of *D. frontalis* monitoring programs may be improved by using (+)-α-pinene in lure combinations to improve the capture of *D. frontalis* when beetle populations are low. Current *D. frontalis*
monitoring programs utilize frontalin (D. frontalis attractant pheromone) and (+)-α-pinene (host monoterpane). The ratio of the number of D. frontalis and its predator, T. dubius, are used to predict forthcoming beetle infestations with ~ 73% accuracy. My study suggests, more D. frontalis are attracted to lure combinations which include (+)-α-pinene.

(2) Results from my research indicate attraction to semiochemicals released by SPBBG members is species specific. However, addition of α-pinene to either frontalin, ipsdienol, or ipsenol increased the attraction of SPBBG members and predatory beetles. Thus, the addition of α-pinene to trees already infested with bark beetles are likely to suppress small bark beetle infestations by increasing competition among SPBBG members and attracting predatory beetles. Predators attracted to these trap trees may include: A. obsoletus, H. baltimoresensis, L. pusillus, Monochamus species, N. guttulata, P. flavicornis, P. cylindrica, P. sulcicollis, T. virescens, T. dubius, and T. marginatus.

(3) There was a significant relationship between lighting strike and development of D. frontalis infestations. Thus, forest managers may reduce the risk of severe D. frontalis infestations by closely monitoring stands damaged by lightning strike.

(4) Forest management techniques that cause ground surface disturbance may increase or limit the spread of invasive ant species in the southeastern United States. Specifically, prescribing burning or thinning may limit distribution of the Asian needle nose ant [Pachycondyla chinensis (Emery)].
6.3 Directions for Future Research

My studies should be considered a starting point towards better understanding the importance and complexity of semiochemical communication and lightning strike in *D. frontalis* population dynamics and ecological impacts of *D. frontalis* suppression and control. The following are viable research initiatives which may be undertaken in the future:

(1) Effects of both host kairomones and their enantiomers on the attraction of bark beetles and their predators should be further explored. Improved understanding of the driving forces behind bark beetle attraction may improve monitoring programs and may lead to exploitation of deterrent kairomones which may slow the growth of bark beetle infestations.

(2) The semiochemical system among SPBBG members and their predators should be expanded towards improving bark beetle monitoring programs and creating biological control strategies. This may lead to crashing bark beetle populations by manipulating semiochemicals to increase the attraction of predators or competitors to bark beetle infestations.

(3) Lightning struck pines provide optimal habitat for *D. frontalis* populations when local beetle populations are too low to mass attack healthy pines. Forest managers may take advantage of the relationship between the development of *D. frontalis* infestations and lightning strike by improving the accuracy of detecting and recording cloud-to-ground lightning strike coordinates. Improvement in lightning strike detection would provide an opportunity to lower the risk of severe bark beetle outbreaks by giving forest managers an
opportunity to more closely monitor points of potential bark beetle infestations across the landscape.

(4) Finally, the ecological impacts of forest management practices which control or suppress bark beetle infestations remains understudied. Further understanding the impacts of prescribed burning, thinning, and replanting clearcut areas with longleaf pine, may provide alternative management techniques geared towards control of aggressive, invasive ant species as well as bark beetle infestations.
Appendix A

List of subcortical beetle species and their numbers caught in multiple funnel traps using traps with following lures: α-pinene, frontalin, ipsdenol and ipsenol in 2010 in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>Family</th>
<th>Beetle Species</th>
<th>Unlured</th>
<th>α-pinene + Frontalin</th>
<th>Ipsdenol + Ipesol</th>
<th>α-pinene + Frontalin + Ipesol</th>
<th>Total Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprestidae</td>
<td><em>Saperda leucotricha Fabricius</em></td>
<td>0</td>
<td>13</td>
<td>9</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Buprestidae</td>
<td>Chalcolepidius virgatus (Dittrich)</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td><em>Anoplophora phloea</em> (Olivier)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td><em>Anoplophora vestalis</em> (Fabricius)</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td><em>Anoplophora obscurata</em> ( Olivier)</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>33</td>
<td>41</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td><em>Corymbia sartorius</em> Annand</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<td>2</td>
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<tr>
<td>Cerambycidae</td>
<td><em>Melicertus stenopterus</em></td>
<td>20</td>
<td>20</td>
<td>146</td>
<td>789</td>
<td>1,164</td>
</tr>
<tr>
<td>Cerambycidae</td>
<td><em>Xylotrupes sphyrus</em> (Goliath)</td>
<td>0</td>
<td>56</td>
<td>0</td>
<td>94</td>
<td>94</td>
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<tr>
<td>Cerambycidae</td>
<td>Thanasimus dubius (Fabricius)</td>
<td>10</td>
<td>570</td>
<td>9</td>
<td>364</td>
<td>953</td>
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<td>Curculionidae</td>
<td>Otiorhynchus spiculatus Say</td>
<td>1</td>
<td>14</td>
<td>2</td>
<td>39</td>
<td>56</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Ctenotus frontalis</em> Zimmerman</td>
<td>8</td>
<td>345</td>
<td>45</td>
<td>489</td>
<td>1,099</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Ctenotus frontalis</em> (Olivier)</td>
<td>0</td>
<td>75</td>
<td>8</td>
<td>61</td>
<td>144</td>
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<td>Curculionidae</td>
<td><em>Enemborus americana</em> Redel</td>
<td>3</td>
<td>11</td>
<td>4</td>
<td>41</td>
<td>59</td>
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<td><em>Hylotrupes piceae</em> Breuning</td>
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<td>0</td>
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<td>34</td>
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<td>Curculionidae</td>
<td><em>Hylastes sexdens</em> Schoutte</td>
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<td>43</td>
<td>0</td>
<td>34</td>
<td>76</td>
</tr>
<tr>
<td>Curculionidae</td>
<td><em>Ips ovalis</em> (Eichhoff)</td>
<td>17</td>
<td>34</td>
<td>731</td>
<td>249</td>
<td>1,031</td>
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<td>43</td>
<td>54</td>
<td>26</td>
<td>93</td>
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<td><em>Ips grandicollis</em> (Eichhoff)</td>
<td>21</td>
<td>131</td>
<td>963</td>
<td>1,434</td>
<td>2,642</td>
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<td>5</td>
<td>5</td>
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<td>9</td>
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<td><em>Triex attractivas</em> Lacordaire</td>
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<td>3</td>
<td>12</td>
<td>5</td>
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<td><em>Triex frontalis</em> Horn</td>
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<td>1</td>
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<td>8</td>
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<td><em>Triex frontalis</em> Say</td>
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<td>0</td>
<td>1</td>
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<td>4</td>
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<td>Platynota affinis* (LeConte)</td>
<td>0</td>
<td>5</td>
<td>15</td>
<td>18</td>
<td>38</td>
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<td>Histeridae</td>
<td><em>Platynota scalaris</em> (Eichhoff)</td>
<td>3</td>
<td>16</td>
<td>43</td>
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<td>Cortogonus nigricornis (Fabricius)</td>
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<td>24</td>
<td>9</td>
<td>22</td>
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<td><em>Ampius punctulatus</em> (Dittrich)</td>
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<td>4</td>
<td>15</td>
<td>7</td>
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<td><em>Buprestes rufus</em> (Linnaeus)</td>
<td>4</td>
<td>2</td>
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<td>12</td>
<td>3</td>
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<td>10</td>
<td>42</td>
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<td>Ctenotus collaris* (Eichhoff)</td>
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<td>15</td>
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<td><em>Terminus scribani</em> (Fabricius)</td>
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<td><em>Terminus irminius</em> (Fabricius)</td>
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<td><em>Asomus subhabitus</em> LeConte</td>
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<td>0</td>
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<tr>
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<td><em>Lacromela gunnarii</em> LeConte</td>
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<td>98</td>
<td>146</td>
<td>217</td>
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<td>69</td>
<td>69</td>
<td>197</td>
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<td>96</td>
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<td>1</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>2</td>
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</tbody>
</table>

Total Number of Individuals: 203, 2,774, 2,165, 5,402, 10,544
Total Number of Species: 33, 33, 21, 28, 41
Appendix B

List of subcortical beetle species and their numbers caught in multiple funnel traps using traps with following lures: α-pinene, frontalin, ipsdienol and ipsenol in 2011 in the Oconee National Forest, Georgia.

<table>
<thead>
<tr>
<th>Family</th>
<th>Beetle Species</th>
<th>Ispedienol + Ispenol</th>
<th>Ispedienol + α-pinene</th>
<th>Ispedienol + Frontalin</th>
<th>Ispedienol + α-pinene</th>
<th>Ispedienol + Frontalin</th>
<th>Total Individuals</th>
</tr>
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<td>Buprestis lineata Fabricius</td>
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<td>Zopherus hemiferus LeConte</td>
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<td>58</td>
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<td>7,206</td>
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<td>30</td>
<td>21</td>
<td>49</td>
<td>95</td>
<td>95</td>
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</tbody>
</table>
Appendix C

List of subcortical beetle species and their numbers caught in multiple funnel traps using traps with following lures: α-pinene, ipsdienol and ipsenol in 2011 in the Oconee National Forest, Georgia.

<table>
<thead>
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<th>Family</th>
<th>Beetle Species</th>
<th>α-pinene</th>
<th>α-pinene + Ipsdienol</th>
<th>α-pinene + Ipsenol</th>
<th>α-pinene + Ipsdienol + Ipsenol</th>
<th>Total Individuals per Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprestidae</td>
<td>Buprestis lineata (Fabri)</td>
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<td>0</td>
<td>4</td>
<td>12</td>
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<td>0</td>
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<td>Acanthocinus modestus (Fabricius)</td>
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<td>15</td>
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<td>58</td>
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<td>45</td>
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<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Total Number of Individuals: 2,400
Total Number of Species: 85