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Southeastern *Monochamus* and Their Interactions with Healthy Shortleaf Pine Trees and
Associated *Ips grandicollis* Bark Beetles

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Entomology

by

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Bachelor of Science in Biology, 2013

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This thesis is approved for recommendation to the Graduate Council

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Abstract

Insects in the genus *Monochamus* are medium to large-sized, wood-boring beetles whose primary hosts in the Northern Hemisphere are pine trees. These beetles interact with both conifer hosts and associated insects throughout their life history. Past research has demonstrated that *Monochamus* are saprophagic, but recent findings show that they may colonize healthy pine trees. To determine if southeastern *Monochamus* could colonize healthy pines, adult *Monochamus* were attracted to healthy shortleaf pine trees from May to September, 2014, using host volatiles, *Ips* bark beetle kairomones, and congeneric pheromones. Subsequent development of oviposited eggs from 18 host trees was monitored. The results demonstrate that southeastern *Monochamus* species may oviposit into healthy shortleaf pines, but host resin flow inhibits egg survival and larval development.

Southeastern *Monochamus* are also associated with the southern pine beetle feeding guild, which includes three species of *Ips* bark beetles that inhabit pine hosts. Species of *Monochamus* and *Ips* share host trees and many aspects of their life history making sub-cortical interaction highly likely. In Arkansas *M. titillator* and *I. grandicollis* are the most abundant of each genera. When hosts are shared *I. grandicollis* first colonize hosts while *M. titillator* land and colonize some days afterwards. To determine if sub-cortical interactions between both species occur and lead to decreased survival and emergence of *I. grandicollis*, shortleaf pine bolts were exposed to both beetles under controlled laboratory conditions and *I. grandicollis* emergence was monitored. Both number of *M. titillator* females introduced and the time between *I. grandicollis* and *M. titillator* colonization was varied. My results demonstrate that sub-cortical interaction between *M. titillator* and *I. grandicollis* does occur and that *I. grandicollis* survival and emergence is reduced by their interactions. Results also show that

these interactions do not depend on the difference in colonization time between the two species, at least within the first six days after *I. grandicollis* have started colonization.

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Dedication

This work is dedicated to my parents, Mark and Natalie Ethington, who have provided me with a myriad of opportunities to continually learn and progress, as well as the support and encouragement needed to succeed.

Table of Contents

1.	Literature review	
	Introduction	1
	<i>Monochamus</i>	1
	Life History	2
	Chemical Ecology	6
	Economic Importance	9
	Ecological Roles	11
	Southeastern <i>Monochamus</i>	12
	Shortleaf Pine	13
	<i>Ips grandicollis</i>	13
	Competition	15
	References	16
2.	Colonization of healthy shortleaf pines by southeastern <i>Monochamus</i> beetles	
	Introduction	27
	Materials & Methods	30
	Results	37
	Discussion & Conclusions	40
	References	43
	Tables & Figures	50
3.	Sub-cortical interaction of <i>Monochamus titillator</i> and <i>Ips grandicollis</i> in a laboratory setting	
	Introduction	59
	Materials & Methods	62
	Results	67
	Discussion & Conclusions	68
	References	70
	Tables & Figures	75

Chapter 1: Literature review

Introduction

Insects in the genus *Monochamus* Dejean (Cerambycidae: Lamiinae) are medium- to large-sized, wood-boring beetles native to every continent except Antarctica. The majority of *Monochamus* species are native to tropical regions, but, owing to economic concerns, scientific research has focused on the species found in temperate ecosystems. While researchers have explored aspects of *Monochamus* life history, economic damage, and ecological roles, many details regarding their biology, behavior, and interspecific interactions remain poorly known. Following is a broad review of available literature concerning *Monochamus*, with specific focus on those most commonly found in the southeastern United States.

Monochamus

The genus *Monochamus* consists of approximately 180 species of medium to large, wood-boring beetles (Roguet, 2015). This genus is in the subfamily Lamiinae, the flat-faced longhorns, within the family Cerambycidae, the longhorn beetles. The majority of *Monochamus* species are found in the tropics with only fourteen species found in temperate North America (Linsley & Chemsak, 1984; Roguet, 2015).

Morphology

The eggs of *Monochamus* are similar to those of many other cerambycids. Eggs are white and cylindrical, measuring 2.5 to 4.0 mm in length, with somewhat flattened ends (Webb, 1909; Raske, 1972).

The larval forms are legless grubs that can grow to as large as 60 mm in length and 9 mm in width. The mandibles are especially strong, to enable mastication of wood, and while the

head capsule is initially creamy colored, it darkens as the larvae mature (Webb, 1909; Raske, 1972).

Pupal *Monochamus* resemble adults in form, but are completely white with all the appendages folded or curled up near the body. As the pupae mature they also become a darker color (Webb, 1909; Raske, 1972).

Adult beetles are medium- to large-sized insects with long antennae and acute tubercles on the pronotum. Antennae can be two to three times the body length in males while measuring close to body length in females (Linsley & Chemsak, 1984). Their eyes are large and deeply emarginate. Large hypognathous mandibles allow for chewing through bark and wood. Elytra are twice as long as wide, and in many species have a mottled or bark-like coloration. Males also have elongated prothoracic legs that support contact with females during copulation (Linsley & Chemsak, 1984).

Life History

The life history of *Monochamus* beetles involves a cycle of oviposition, larval development, adult emergence, sexual maturation, and host discovery. Throughout this cycle the beetles are intimately involved with their host trees and interact with many associated insects.

Oviposition

The events prior to oviposition in *Monochamus* appear similar to the sequence suggested by Ginzel & Hanks (2005) for the Cerambycinae, a closely related subfamily. The sequence includes: (1) attraction to suitable hosts by host volatiles, (2) short-range attraction of females to males mediated by male-produced pheromones, and (3) identification of females by male conspecifics using palpation of cuticular hydrocarbons followed by copulation (Ginzel & Hanks, 2005).

Attraction of sexually mature *Monochamus* to suitable host trees is mediated by host volatiles, but also by associated insect kairomones (Allison et al., 2001; Miller et al., 2013). The preferred host of all North American *Monochamus* is pine, but they may also colonize fir (*Abies*) and spruce (*Picea*) (Craighead, 1923; Linsley & Chemsak, 1984). Males, due to earlier emergence, arrive at hosts prior to females and protect mating territory in areas well suited for oviposition (Hughes & Hughes, 1982; Naves et al., 2008). As females arrive on host trees they are attracted to short-range male-produced pheromones (Allison et al., 2012; Fierke et al., 2012; Macias-Samano et al., 2012). When congeners are encountered, male antennal and tarsal palpation of cuticular hydrocarbons on the elytra determines if species and sex are proper for mating (Ibeas et al., 2009). Subsequently, males use their long prothoracic tarsi to pull themselves into a mounted position where they will remain during copulation and often for some time afterwards (Hughes, 1979). Males riding on females use their long antennae to sense approaching males and fighting may occur if other males attempt to approach or copulate with the joined female (Hughes & Hughes, 1982). This tandem position may ensure that the female will only oviposit eggs fertilized by the protecting male. Copulation may be repeated by males with the same females after each oviposition (Hughes, 1979; Fauziah et al., 1987).

After copulation females walk along the host surface until they locate a suitable oviposition site. Females, using their sharp mandibles, then excavate oviposition pits through the outer and into the inner bark. Pits can take the form of small slits in thin bark or large, deep pits in thicker bark (Alya & Hain, 1985; Edwards & Linit, 1991). Single pit creation may take up to one-half hour depending on bark thickness (Edwards & Linit, 1991). Pit creation occurs preferentially on the lateral sides of horizontal logs, which may help avoid rapid desiccation on top of the log or slower development in the shaded underside (Rose, 1957; Allison & Borden,

2001). Pits are rarely created in the thickest bark of the trunk or in the smaller branches and twigs. These preferences may avoid areas where bark is too thick or where sub-cortical phloem and cambium is insufficient for development (Reagel et al., 2012). Once pits have been created, females rotate their bodies 180 degrees and insert their ovipositors into pits to deposit eggs. Egg placement usually occurs in a circular fashion surrounding the oviposition opening with 1 to 9 eggs placed in each pit (Webb, 1909; Alya & Hain, 1985).

Larval development

Eggs hatch within 5 to 17 days and larvae commence feeding on inner bark, cambium, and phloem (Webb, 1909; Rose, 1957; Alya & Hain, 1985). The number of larval instars can be variable, but ranges from three to six instars (Pershing & Linit, 1988). Within three to six weeks the larvae begin to tunnel into the xylem, but continue to return to the nitrogen-rich sub-cortical cambium to feed (Webb, 1909; Rose, 1957). Larval *Monochamus* are unable to produce the enzymes necessary to digest cellulose, but ingestion of fungal-permeated wood allows for some digestion of cellulose and hemicellulose (Kukor & Martin, 1986). Larval galleries extend through the sapwood and can score the heartwood before turning upwards and starting to tunnel outwards, creating a U-shaped gallery (Webb, 1909). The time necessary for larval development is variable, but eventually larvae block the larvae with frass and create a pupation chamber. After pupation, adult eclosion is associated with creation of round emergence holes out through the sapwood and bark (Webb, 1909; Alya & Hain, 1985). The time period of egg to adult development can be variable, with some individuals emerging within 60 days after egg deposition while others overwinter and emerge the following spring (Webb, 1909; Cerezke, 1977). Due to the variable time as eggs, larvae, and pupae, emergence is somewhat staggered within the same cohort (Webb, 1909).

Sexual Maturation

Newly emerged adults fly to host canopies and feed on small shoots, needles, tender twigs, and pine cones for 7 to 12 days to complete sexual maturity (Walsh & Linit, 1985). When densities of emerging *Monochamus* are high this feeding can injure surrounding trees. Feeding on spruce near logging operations can be sufficient at times to kill limbs and severely damage trees (Linsley & Chemsak, 1984). Maturation feeding has also been correlated with balsam fir dieback (Linsley, 1959). During maturation feeding attraction to hosts may depend almost solely on alpha-pinene, while after sexual maturation adults are attracted to combinations including other host volatiles such as (+)-juniperol and (+)-pimiral (Sakai et al., 1992).

Population Dynamics

The number of generations per year depends primarily on local climate. There may be one or two generations per year in the warmer southeastern United States, while in northern climates most *Monochamus* require two or even three years to develop (Webb, 1909; Cerezke, 1977; Alya & Hain, 1985). Although some areas may have bi-voltine populations, most individuals will still overwinter and emerge the next spring (Cerezke, 1977; Alya & Hain, 1985).

Phenological studies in North America have found that *Monochamus* are usually encountered flying, mating, and ovipositing during summer months with adult populations tapering off into the fall (Hanks et al., 2014). Cumulative survival from eggs to adults is quite low in *Monochamus*, from 71 to 85 percent mortality of each generation, in both free and laboratory populations (Cerezke, 1977; Alya & Hain, 1985; Linit, 1985). The low survival rate may result from scramble competition for ephemeral host resources, which can lead to high mortality from intraspecific competition and resource depletion by other guild members (Hanks,

1999). Intraspecific cannibalism among larvae occurs in several species of *Monochamus* (Rose, 1957; Graber, 2000).

Several factors influence *Monochamus* fecundity including species and phenology. Mean fecundity in *M. alternatus* ranges from 86 to 91 eggs (Togashi et al., 2009; Jikumaru et al., 1994) while mean fecundity in *M. carolinensis* ranged between 127 to 179 eggs (Akbulut & Linit, 1999a, 1999b; Akbulut et al., 2004). The time of female emergence influences fecundity with *M. carolinensis* that emerge during spring significantly more fecund than those that emerge in the summer or fall (Akbulut & Linit, 1999b). Longevity is not commonly recorded for males, but *M. alternatus* males kept in an indoor laboratory lived for up to 110 d (Togashi, 1981) while field caged *M. carolinensis* lived for 51 d (Togashi et al., 2009). Longevity for females ranges from 120 d for laboratory females (Togashi, 1981) to between 46 to 57 d for field caged females (Jikumaru et al., 1994; Akbulut & Linit, 1999a, 1999b).

Chemical Ecology

Adult *Monochamus* are attracted to a variety of chemicals which play an important role in finding host material for mating and oviposition. Anatomical studies have shown that many chemoreceptors are present on the long antennae of both sexes which may allow for detection of long-range attractants as well as short-range pheromones and cuticular hydrocarbons (Dyer & Seabrook, 1975). *Monochamus* are attracted to hosts and then use short range pheromones and cuticular hydrocarbons to find mates (Hanks, 1999). Attraction to a single chemical may occur, but is more likely when several synergistic chemicals are present. Attractants can be grouped into host volatiles, bark beetle kairomones, and conspecific pheromones. Chemical signals also play a role in deterring or promoting oviposition pit creation as well as egg deposition.

Host volatiles

Host volatiles are chemicals produced by wounded or stressed trees, as part of their defense and healing mechanism, or chemicals present in and around these trees due to the biochemical breakdown of tree mass by other organisms (Graham, 1968; Kimmerer & Kozlowski, 1982). Trees may be wounded or stressed by a variety of factors including drought, flooding, freezing, insect attack, nutrient deficiency, or mechanical wounding during logging operations. Because *Monochamus* utilize stressed or weakened hosts for larval development, the ability to sense chemical signatures for these trees is essential (Hanks, 1999). Ethanol, a volatile produced when trees are under stress, is attractive to *Monochamus*, both by itself and in combination with turpentine, a tree wound volatile (Fatzinger, 1985; Phillips et al., 1988). Alpha- and beta-pinenes, signals of tree responses to stress and wounds, are also attractive, by themselves or as synergists, to *Monochamus* species (Fan et al., 2007). Trapping studies have shown that ethanol and monoterpenes (including alpha- and beta-pinenes) are synergistic, leading to greater attraction when combined (Ikeda et al., 1980). *Monochamus alternatus* males are also attracted to (+)-cis-3-pinen-2-ol, a bi-product of induced oleoresin flow in pine sapwood (Sakai & Yamasaki, 1991).

Kairomones

“Eavesdropping” on the long-range pheromones of associated species allows *Monochamus* to find hosts which are stressed or weakened without having to rely solely on ubiquitous host volatiles. Because saprophagous bark beetles have similar nutritional requirements, develop in the same sub-cortical space, and communicate host status and species aggregation using chemical pheromones they are ideal species for *Monochamus* to track. The majority of kairomones that attract *Monochamus* are produced by *Ips* bark beetles, which

produce aggregation pheromones to mass attack weakened and stressed trees. Gardiner (1957) found that *Monochamus* are usually present in trees that had been infested by *Ips* beetles. *Ips* pheromones that attract *Monochamus* include 2-methyl-6-methylene-7-octen-4-ol (hereafter Ipsenol) and (4*S*)-2-methyl-6-methylideneocta-2,7-dien-4-ol (hereafter Ipsdienol) (Miller & Asaro, 2005; Ibeas et al., 2007). While both of these attract *Monochamus*, the most attractive component is Ipsenol (Allison et al., 2001, 2003; Miller & Asaro, 2005). These kairomones have also been shown to be synergistic with host volatiles (Ibeas et al., 2007).

Male-produced pheromones

While long-range attraction to host volatiles and kairomones leads adult *Monochamus* to suitable hosts, attraction over short distances can be mediated by male-produced pheromones (Wang et al., 1991). *Monochamus* species are attracted to a male-produced pheromone, 2-undecyloxy-1-ethanol (commercially known as Monochamol), in North America (Fierke et al., 2012), Europe (Pajares et al., 2010), and Asia (Teale et al., 2011). This male-produced pheromone attracts *Monochamus*, but not other closely related lamiines, thus appearing to be a recently evolved trait. This pheromone also was found to be synergistic with host volatiles ethanol and alpha-pinene (Teale et al., 2011; Macias-Samano et al., 2012) as well as with bark beetles kairomones, especially Ipsenol (Macias-Samano et al., 2012). Because this pheromone attracts both sexes it can be classified as an aggregation pheromone rather than a sex pheromone (which only attracts the opposite sex). After approaching males producing the pheromone, palpation of the elytra takes place and copulation may occur if the male recognizes the conspecific as female (Kim et al., 1992).

Egg deposition

In addition to host discovery, chemical cues may also play a role in the choice to deposit eggs within oviposition pits. After eggs are deposited, a jelly-like substance is placed over openings and the females move on. This jelly-like substance appears to deter both the same female as well as other females of the same species from creating oviposition pits and depositing eggs nearby (Anbutsu & Togashi, 2000; Peddle et al., 2002). Larval frass also repels conspecific oviposition in *M. alternatus* (Anbutsu & Togashi, 2002). This may be an adaptation to decrease intraspecific competition among larvae of the same cohort. Female *Monochamus* also do not oviposit into every oviposition pit created, although pits may consume a significant amount of time to excavate. In several studies only 70 - 85% of the excavated pits contained eggs (Walsh & Linit, 1985; Peddle et al., 2002). This behavior appears to stem from the need for a proper amount of free moisture to be present in inner bark which stimulates oviposition behavior (Yamasaki et al., 1989). Excavated pits not used for egg deposition may be areas where sufficient free moisture is absent. Other chemicals may also play a role in the oviposition decision. Islam (1997) reported that a chemical isolated from the inner bark, D-catechin, was at least one of the chemicals responsible for oviposition response. Several other compounds, including flavanonol glucoside and proanthocyanidins, from the inner bark also stimulate oviposition in *M. alternatus* (Sato et al., 1999).

Economic Importance

Lumber loss

Colonization of pines by *Monochamus* species in North America can be a source of economic loss for lumber mills and other manufacturers of forest products. Large populations of adult *Monochamus* are attracted to host volatiles released when trees are harvested or injured by

natural processes, such as lightning, wind, or ice (Safranyik & Raske, 1970; Gardiner, 1975; Fan et al., 2007). Females frequently oviposit into recently downed timber if logs are not placed in water for a holding period or treated with chemicals (Dunn, 1931). As colonizing larvae develop they create galleries throughout the sapwood of the logs. These large galleries, termed “worm-holes”, can lead to degradation of lumber from Grade 1 to Grade 3, a much less valuable lumber grade. Degradation can also occur due to wood-rotting fungi which invade the wood by way of *Monochamus* galleries (Wilson, 1962). Reported losses due to degrading of wood range between 14 to 35 percent of possible lumber value (Safranyik & Raske, 1970; Gardiner, 1975). One study found that a conservative estimate of loss to an average lumber mill in British Columbia was \$600,000 each year due to *Monochamus* boring (Carlson, 1997). Losses in salvage logging may be even greater due to increased exposure of timber to oviposition before harvest and milling (Webb, 1909).

Vector of pine wilt nematode

Another negative economic aspect of *Monochamus* biology is their ability to vector pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle 1970, the cause of pine wilt disease (Linit, 1988). Pinewood nematodes feed on cells lining resin canals as well as fungi present in conifers (Futai, 2013). As *Monochamus* galleries can lead to the introduction of a number of fungi, pinewood nematode are especially prevalent in the sapwood surrounding larval galleries (Futai, 2013). After *Monochamus* pupate, dauer (dispersal stage) nematodes enter the callow adult beetle through the spiracles and proceed to the tracheae (Kondo, 1986; Necibi & Linit, 1998). As the lipid reserves of the nematodes decrease over time they become more attracted to pine host volatiles than to *Monochamus* cuticle hydrocarbons, and consequently will leave the beetle and infest trees when host volatiles are present (Stamps &

Linit, 1998). The nematodes infest trees principally through oviposition pits created by females, but wounds created during maturation and adult feeding of both sexes also permit infection (Wingfield & Blanchette, 1983; Luzzi et al., 1984; Edwards & Linit, 1992; Linit, 1990). The nematodes reproduce quickly in susceptible trees and their large numbers clog the xylem channels transporting water to upper sections of the tree, leading to red and then gray needles, and eventually the death of main tree limbs and the entire tree (Mamiya, 1983). Pine trees indigenous to North America, pinewood nematode's native habitat, appear to be resistant to pine wilt while pines native to other regions of the Northern hemisphere are susceptible to the disease (M. J. Linit & Tamura, 1987). Exotic pines planted in North America are also susceptible to pine wilt, with Scotch pine (*P. sylvestris*) and Austrian pine (*P. nigra*) being very susceptible (Gleason, 2000). Significant pine loss in Asia and Europe has occurred due to pine wilt disease (Mamiya, 1988; Sousa et al., 2001). In each of these areas the nematodes are currently vectored by native *Monochamus* species, but initial introduction is believed to originate from North America, where pine wilt nematode is indigenous (Mamiya, 1988). Because of the risk of pinewood nematode being imported, many European countries have enacted embargoes on untreated wood chips and lumber coming from North America (Vallentgoed, 1991).

Ecological Roles

Many cerambycids, including *Monochamus*, accelerate the process of decomposition and mineralization of wood. Through larval feeding they decompose wood, reducing numbers of dead and dying trees, broken branches, and slash in forests (Linsley, 1959; Dajoz, 1998). This becomes especially important in forested areas where a majority of the available biomass is trapped in tree wood. Wood biomass decomposition functions to maintain nutrient and mineral cycles leading to vigorous plant growth within forest ecosystems (Dajoz, 1998). Larval galleries

are also used as entry points for other insects, fungi, and bacteria which can digest and further decompose woody material thus returning nutrients to the soil (Parmelee, 1941). The deep galleries of *Monochamus* can be the first point of introduction for fungi capable of efficiently utilizing sapwood and heartwood material (Leach et al., 1937). Both *Monochamus* fecal pellets and chewing dust contain high amounts of undigested nitrogen which is returned to the soil. This has been linked to increased soil microbial activity as well as increased germination and growth of new plants on a local scale (Cobb et al., 2010).

Monochamus species also function as interspecific competitors and intraguild predators of bark beetles. *Monochamus titillator* (F.) competes for phloem with the southern pine beetle, *Dendroctonus frontalis* Zimmerman, and negatively influence survival of the bark beetles (Coulson et al., 1976, 1980). *Monochamus titillator* is also a facultative predator of *Ips calligraphus* Eichoff, as well as other bark beetles in the southern pine beetle guild (Miller, 1986; Dodds et al., 2001; Schoeller et al., 2012). These interactions may play an important role in regulation of bark beetle densities and triggering population crashes (Stephen, 2011).

Southeastern *Monochamus*

While the native range of four *Monochamus* species includes the southeastern United States, the most common species detected are *Monochamus titillator* and *Monochamus carolinensis* (Olivier) (Linsley & Chemsak, 1984; Alya & Hain, 1985). The similar appearance of these two species can result in misidentification, leading several authors to describe them as a complex rather than trying to discern between species (Miller et al., 2013). Dillon & Dillon (1941), as well as Lingafelter (2007), separate the two species on the basis of more or less defined patches of color and dissimilar apical ends of the elytra, with that of *M. titillator* being more "spine-like". While this can differentiate the species in some cases, many individuals

display intermediate features which confounds identification. Males of *M. carolinensis* and *M. titillator* can be differentiated on the basis of genitalia, but this does not provide a method of species determination for females or living individuals (Pershing & Linit, 1985). Genetic analyses may be necessary to determine if these are truly distinct species.

Shortleaf Pine

Shortleaf pines (*Pinus echinata* Mill.) are large conifer trees which are both economically and ecologically important. The trees can grow up to 25 to 30 m tall and have a dbh (diameter at breast height) of 0.6 to 0.9 m. The trees are fully mature at 170 years old but may live up to 400 years (Hardin et al., 2001). Native range of shortleaf pine is the widest of all southeastern pines, covering 22 states and a variety of ecosystems and soil types. Best development occurs in drier soils in well drained areas such as in Arkansas, northern Louisiana, and the southern Piedmont (Burns & Honkala, 1990). When shortleaf stands are harvested on wetter sites they are usually replaced with loblolly pine (*Pinus taeda* L.). Shortleaf pine has the interesting ability to sprout vigorously at the root collar if the crown is killed or badly damaged. This allows young trees to renew apical growth after serious fire or mechanical injury (Burns & Honkala, 1990). Shortleaf pine is one of the four most important commercial conifers in the southeastern United States and is used for lumber, plywood, and pulpwood (Hardin et al., 2001). Cone-bearing trees produce large numbers of seeds of which the majority are eaten by birds and small mammals (Burns & Honkala, 1990). This is ecologically important especially where shortleaf is scattered throughout hardwood stands. These trees also provide protection from wind and cold for many animals in hardwood-dominated stands (Burns & Honkala, 1990). Defenses against boring insects in shortleaf pine are similar to other pines and includes both constitutive and induced responses.

Ips grandicollis

Ips is an economically important genus of bark beetles found throughout the Northern Hemisphere. These beetles colonize primarily dead and dying conifers, although during outbreak conditions, or during strong biotic and abiotic stress, live trees may be attacked and killed (Garraway, 1986; Nebeker, 2011). Colonization is initiated by males who are weakly attracted to host volatiles of weakened or stressed trees (Anderson, 1948). Once on hosts males will create entry galleries and a nuptial chamber. Species-specific aggregation pheromones are produced when males tunnel into susceptible hosts. Secondary attraction to pheromones creates aggregation of both sexes (Anderson, 1948). Single or multiple females join the male in the brood chamber, mating occurs, and females oviposit in the sides of galleries. Females which have previously mated may also create galleries and lay eggs in the absence of males, but no brood chamber is created (All & Anderson, 1972). Larvae feed on the nitrogen-rich sub-cortical material throughout development, but may move to the outer bark to complete pupation and eclosion. Newly eclosed adults will seek out new host trees after exiting through round emergence holes. Previously mated adults may also reemerge from trees and infest new trees (Wilkinson, 1964; All & Anderson, 1972).

Ips grandicollis Eichhoff, the eastern five-spined engraver, is one of three *Ips* species which are part of the southern pine beetle (SPB) guild found in the southeastern United States (Nebeker, 2011). Males produce the aggregation pheromone Ipsenol as they create entry galleries into hosts (Vite & Renwick, 1971; Werner, 1972). This not only serves as an aggregation pheromone to both sexes of *I. grandicollis*, but is also highly attractive to *Monochamus* species present in the southeast (Allison et al., 2003; Miller & Asaro, 2005). When found in association with other beetles in its guild, *I. grandicollis* usually colonizes from

the middle of the bole up to the large branches (Paine et al., 1981; Garraway, 1986).

Phenologically, adult *I. grandicollis* are usually found initiating attacks from June to September in the southeastern United States (All & Anderson, 1972). Colonization of hosts by *I. grandicollis* lack the concentrated attack patterns seen in species more likely to kill living trees, such as *Dendroctonus frontalis* or *Ips avulsus* (Berisford & Franklin, 1971).

Competition

Among the interactions that may occur between individuals, species, populations, or communities, competition focuses on the shared requirement of two groups or organisms for the same resources. Research regarding competition has often assumed an antagonistic relationship with negative consequences for all involved, but the extent of negative effects depend on the species and situation (Price et al., 2011). Competition is thought to be highest among conspecifics (intraspecific competition) due to identical resource requirements, but not all studies show that to be true (Price et al., 2011). Competition between different species (interspecific competition) is frequently observed, but the degree to which it influences population growth is quite variable and depends upon many factors (Denno et al., 1995).

Competition may increase among members of feeding guilds that utilize resources in a similar fashion (Root, 1967). This intraguild competition may be lowered in some cases by resource partitioning as well as temporal and phenological differences (Paine et al., 1981; Denno et al., 1995; Ayres et al., 2001). Competition may also increase in organisms, such as sub-cortical insects, which are unable to escape their habitat to find additional resources.

Interspecific competition may be symmetrical, but meta-analysis of competition literature has shown that competition is predominantly asymmetrical (Kaplan & Denno, 2007). In asymmetric competition shared resource utilization produces less of a negative effect in superior competitors

than it does in inferior competitors. In hosts shared by *Monochamus* and bark beetle species, the presence of bark beetles appears to have very little, if any, negative effects on the much larger and more mobile *Monochamus* larvae however the presence of *Monochamus* has a strong, negative effect on bark beetle survival and emergence (Coulson et al., 1976, 1980; Miller, 1986). In this case *Monochamus* appears to be the superior competitor and bark beetles are the inferior competitors. This asymmetry may also be exacerbated by facultative predation of bark beetles by *Monochamus* larvae (Dodds et al., 2001; Schoeller et al., 2012).

Competition may also be viewed by differences in how resources are exploited. If resources are exploited in a chaotic fashion where resources are available to all, but consumed quickly, this is scramble, or exploitative, competition. This "scramble" leads to large population increases when resources are readily available, but corresponding population crashes when required resources and conditions are scarce (Nicholson, 1954). In contrast, if resources are available, but access to the resources is controlled by only one of the species than this is contest, or interference, competition (Nicholson, 1954). Most competition is not strictly one type or the other, but may have aspects of both scramble and interference competition present in different interactions and environmental circumstances (Price et al., 2011). Where *Monochamus* and bark beetles share ephemeral conifer hosts aspects of both types of competition occur, although contest competition may be more applicable due to strong asymmetric utilization of resources.

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Introduction

The genus *Monochamus* Dejean is comprised of large, wood-boring, longhorn beetles primarily found in the tropics, but also represented by a number of species in temperate regions. In North America there are fourteen native *Monochamus* species with partially overlapping geographical distributions (Linsley & Chemsak, 1984; Roguet, 2015). The primary hosts of *Monochamus* in North America are pine trees (*Pinus*), but other hosts may include fir (*Abies*) and spruce (*Picea*) (Linsley & Chemsak, 1984). These beetles can be both economically and ecologically important in forested ecosystems yet many aspects of their behavior, as well as interactions with hosts and associated insects, are poorly understood.

Both sexes of *Monochamus* are attracted to stressed or weakened trees by host volatiles and associated insect kairomones (Hanks, 1999; Allison et al., 2001; Miller et al., 2013). Once present on the host, conspecific encounters are promoted by attraction to male-produced pheromones (Allison et al., 2012; Fierke et al., 2012; Macias-Samano et al., 2012). When congeners are encountered, males palpate elytral hydrocarbons to determine if species and sex are appropriate for copulation (Kim et al., 1992; Ibeas et al., 2009). Following copulation, females use large mandibles to excavate oviposition pits and place 1 to 9 eggs in a circular pattern around the pit (Webb, 1909; Alya & Hain, 1985). After eggs hatch the larvae develop by consuming inner bark, cambium, and phloem (Webb, 1909; Rose, 1957; Alya & Hain, 1985). During development larvae create galleries into the sapwood while continually returning to the sub-cortical material to feed. Galleries may score the heartwood before turning upwards to form a U-shaped gallery in the sapwood in which they create a pupation chamber (Webb, 1909). After pupation and eclosion, adults create a circular emergence hole through the bark, emerge, and fly

to nearby hosts to feed. A period of 5 to 14 days of maturation feeding on needles, twigs, and branches of host trees is required for sexual maturation to occur (Linsley, 1959; Walsh & Linit, 1985). After sexual maturation adults become attracted to hosts suitable for oviposition and the cycle repeats (Sakai et al., 1992).

While *Monochamus* can negatively affect pine lumber production, they are usually secondary colonizers that develop in injured, dead, or recently felled trees (Webb, 1909; Hanks, 1999). Larval galleries facilitate the introduction of wood-destroying fungi as well as creating large “worm holes” in cut timber, both of which can drastically reduce lumber value (Webb, 1909; Wilson, 1962). At times this damage can decrease the value of cut lumber by more than 30 percent (Wilson, 1962; Safranyik & Raske, 1970; Raske, 1972). These beetles are also the primary vectors of *Bursaphelenchus xylophilus* (Steiner and Buhner), a nematode which causes pine wilt disease (PWD) (Kondo et al., 1982; Linit, 1988). In pine forests in Asia and Europe PWD has caused widespread tree death within healthy stands (Yamane & Oda, 1975; Sousa et al., 2001). Exotic pines that are planted in North America, such as Scots (*P. silvestris*) and Austrian (*P. nigra*), are also susceptible to PWD (Robbins, 1982; Linit & Tamura, 1987).

Monochamus beetles play a role in the decomposition of woody material to mineral and organic components (Linsley, 1959; Dajoz, 1998). Larvae not only digest sub-cortical and sapwood material of recently dead or dying hosts, but their galleries directly contribute to colonization by other saprophilic species (Parmelee, 1941). Because *Monochamus* feeding galleries and emergence tunnels traverse from the outer bark to the heartwood, they can serve as an entry point for various saprophagous insects, fungi, and bacteria important to nutrient cycling in forested ecosystems (Leach et al., 1937). Larval feeding and frass accumulation can also lead to separation of bark from the sapwood, thereby allowing invaders to bypass an important tree

defense. *Monochamus* frass also contributes to increased microbial respiration and nitrogen availability (Cobb et al., 2010). Several species of *Monochamus* are competitors of associated bark beetles for phloem resources in their shared sub-cortical residence (Coulson et al., 1976, 1980; Miller, 1986). In Arkansas, *Monochamus* colonize pines synchronously with attacking *D. frontalis* adults (Stephen pers. obs. 1996). *Monochamus* may also act as facultative predators of associated bark beetle larvae and may influence population dynamics within bark beetle guilds (Dodds et al., 2001b; Stephen, 2011; Schoeller et al., 2012a).

The majority of previous research suggests that these beetles are saprophagous; ie., they only attack dead or dying pines (Webb, 1909; Hanks, 1999). Although this is the general assumption regarding the genus, observations of *Monochamus* infesting living trees near logged or even healthy stands has been reported (Clark, 1953; Yang et al., 2014). Recent research in a heavily wind-damaged site in Minnesota found that *Monochamus scutellatus* were not only ovipositing and developing in undamaged *Pinus banksiana* Lamb., but they were doing so as primary colonizers without previous infestation by bark beetles (Gandhi, 2005; Gandhi et al., 2007). Although it was difficult to determine if subsequent tree death was solely due to *Monochamus* colonization or if there were other biotic and abiotic agents creating stress and injury to the trees, Gandhi (2005) concludes that *Monochamus* were primary colonizers and at least contributed to healthy tree death within the stand.

Although species of both pines and *Monochamus* are different in the southeastern United States from those in Minnesota, similar ecological conditions occur. In both areas pine stands are routinely injured and/or stressed by both large weather events and logging activities. In both areas *Monochamus* populations can become very abundant, and sexually mature *Monochamus* in both regions are attracted at long distances to host volatiles and associated insect kairomones,

leading to colonization of suitable hosts. In the southeastern United States shortleaf pine, *Pinus echinata* Mill., shares several characteristics with jack pine in the northern United States. Both are widely distributed in their respective regions, well adapted to fire, and suffer from frequent wind and ice damage (Burns & Honkala, 1990).

While the geographic range of four species of *Monochamus* overlap in the southeastern United States, the most abundant are *M. titillator* (Fabricius) and *M. carolinensis* (Olivier) (Linsley & Chemsak, 1984; Miller et al., 2011). The two species are challenging to separate taxonomically due to morphological similarities and are highly similar in their ecological niches. These two species may appear to act as one population as has been described for other *Monochamus* species which co-occur (Gardiner, 1954).

This study was designed to attract *Monochamus* adults to healthy shortleaf pines to determine if they could successfully colonize these trees. Colonization is considered successful if eggs are able to develop to adults in the host tree. In order to determine if successful colonization is possible *Monochamus* pit creation and oviposition of eggs was monitored on healthy shortleaf pines to determine if egg deposition could lead to insect development within the host.

Materials & Methods

Site selection

Studies were conducted in two geographically distinct sites in the Ozark-St. Francis National Forest. Sites were located near Shores Lake and Lake Wedington, both in northwestern Arkansas. Two plots, each logged within the last year, were selected at each site, totaling four plots. Sufficient downed woody material was found in each plot to support a substantial *Monochamus* population. Shortleaf pine was the dominant tree species found in each plot, and all

plots were surrounded by mixed oak-hickory-pine forest. Pine in each of the plots appeared to be healthy with little to no flagging in the tree crowns or injury to the trees from logging activity.

Pre-treatment trapping

A short trapping study was conducted prior to initiating the experiment to subjectively ascertain if each plot contained a sufficient population of *Monochamus*. Within each plot one black cross-vane panel trap (APTIV™ Intercept) was hung between two non-host trees at a height of approximately 2 m and baited with a combination of ethanol and alpha/beta-pinene. In previous studies this lure combination has been shown to attract *Monochamus* beetles (Fatzinger, 1985; Phillips et al., 1988). A collection cup containing ethylene glycol, a preserving agent, was hung below the panel trap. Trap catch was collected weekly and *Monochamus* beetles were sexed and identified to species. Traps were first placed on March 28th and checked each week until experimental treatments commenced, on May 21st.

Tree selection, initial measurements, and treatment

Selection

Within each plot healthy, medium-sized (20-35 cm diameter at breast height) shortleaf pine trees were selected for each of the two treatment periods. Trees were determined to be healthy if they showed no serious defects in growth or crown condition. Serious defects in growth included a split trunk or abnormal limb growth. Serious defects in crown condition included flagging needles and limbs, split branches, major scarring or weeping wounds, signs of previous insect attack, and any other poor crown condition. All selected trees were located at least 20 m from another selected tree. Before being treated trees were sampled for the presence of pinewood nematode and resin flow was measured.

Nematode sampling

Pinewood nematode has not been observed affecting shortleaf pine, but trees were sampled to determine whether our trees contained nematodes present prior to *Monochamus* attacks. Using a 2.54 cm auger bit, a hole was drilled into the phloem of the tree bole at breast height. As the drill bit was backed out of the bole the phloem shavings were gathered in a plastic bag and taken back to the University of Arkansas Forest Entomology Lab. Each bag of pine shavings was soaked in water for 24 hours and then examined under a 400x stereomicroscope to determine nematode presence or absence. Each tree was again tested for the presence of pinewood nematode at the end of the field season.

Resin sampling

Resin flow on each tree was estimated in order to determine if resin flow changed as a result of *Monochamus* pits and any concurrent beetle development. Resin sampling was accomplished by hammering through the bark and about 0.5 cm into xylem with a 2.54 cm dia. arc punch. A plastic collector, with an attached plastic 15-ml collection tube, was inserted to allow resin to drain (Karsky et al., 2004). The tubes were collected 24 hours after placement. The tubes were held in an upright position and resin was allowed to settle for 24 hours. Resin in each tube was measured. Each sample consisted of two collection tubes at a height of 1.5 m on opposite sides of the tree, whose measurements were summed and averaged. Resin was initially sampled when treatments were applied and subsequently measured again at the end of the field season.

Treatment

There were two full treatment periods, each covering eight weeks in an attempt to mirror time periods of high densities of *Monochamus* adults in the field, and possibly representing two generations of adults (Alya & Hain, 1985). A shortened "test" treatment period covering four

weeks was included during the first full treatment period to evaluate attraction of additional lure combinations. The lures used in all treatment periods were: low-release ethanol (0.1 g/d at 25°C) and 2-undecyloxyethan-1-ol (hereafter Monochamol) (0.7 mg/d at 25°C). Lures used in the test and second treatment periods were: 2-methyl-6-methylene-7-octen-4-ol (hereafter Ipsenol) (0.2 mg/d at 25°C), and cis-3-pinene-2-ol (no elution data available). All treatment lures were supplied by Synergy Semiochemicals, Burnaby, British Columbia.

Trees were treated by placing a nail into the trunk of the tree at approximately 6m high and then hanging a semi-permeable lure bag on the nail. Lures were placed in the bag on the north side of the tree to minimize high elution during hotter months. The lure for the full treatment periods remained on the tree for eight weeks before removal. One tree was selected in each plot to remain an untreated control throughout both treatment periods.

In the first full treatment period, May 23rd to August 1st, three trees were selected in each plot, giving a total of 12 treated trees. Trees were treated with a single lure containing Monochamol + low-release ethanol (ME), a combination of host volatiles and male-produced sex pheromone shown to be attractive to *Monochamus* species (Pajares et al., 2010; Teale et al., 2011) [Table 1]. The results of the first six weeks after treatment showed very little response to this lure combination.

To determine if different lure combinations would be needed to improve attraction we treated trees in a "test" treatment period. Trees were treated for four weeks, July 3 through August 1. Two new trees were treated in each plot giving a total of 8 new trees in the "test" treatment period. One tree was treated with Ipsenol, a bark beetle pheromone known to attract *Monochamus* in trapping studies (Allison et al., 2001; Miller & Asaro, 2005), while the other

was treated with Monochamol + low-release ethanol + Ipsenol (MEI) [Table 1]. The results of this shortened treatment period led us to include these treatments in the 2nd full treatment period.

The second full treatment period was August 1st to September 26th. The treatments included in the second period were: (1) Monochamol + ethanol (ME), (2) Monochamol + ethanol + Ipsenol (MEI), (3) Ipsenol, and (4) cis-3-pinene-2-ol (CIS-OL) [Table 1]. In each plot the treatments were applied to two trees. With four treatment combinations applied to two trees in each plot a total of eight trees were treated per plot, giving a total of 32 treated trees in the second full treatment period. Cis-3-pinene-2-ol is a precursor of alpha-pinenes and has shown to attract male *Monochamus alternatus* in Japan (Sakai & Yamasaki, 1991).

Periodic tree inspection

Treated trees were examined every two weeks for new oviposition pits. Trees were climbed using Swedish sectional tree-climbing ladders placed against the tree and secured with chains. Two sections, measuring 3 m each, were used to allow close observation of the tree bole from ground level to approximately 7 m. After ascending the ladders a researcher would work from 7 m down to ground level observing the entire bole for oviposition pits. A removed and modified truck side-view mirror was used to inspect the side of the tree opposite the ladders. Other than a small amount of resin flow from prongs on the tree-climbing ladders, no injury to the tree was caused by ladder placement and chain attachment.

Monochamus oviposition pits are oblong, bowl-shaped pits about 8 mm long and 3 mm wide excavated out from deep bark, or merely slits measuring 2 mm long with the ovipositor hole in the middle in thinner bark (Alya & Hain, 1985) [Figure 1]. Pits that appeared to be from woodpeckers, which can appear more round with no *Monochamus* mandible “cut” marks on the sides, as well as small slits in the bark without an ovipositor hole, were not counted. Identified

pits were counted and marked with small colored map pins, which were inserted into the middle of pits to avoid damaging eggs.

Trees were also inspected for any change in branch or crown condition, such as flagging in the needles. If no flagging in needles or other signs of tree health change were observed the tree was designated as healthy.

Tree felling and dissection

To determine if any egg development had occurred, 18 trees with pits were destructively sampled at the end of the field season. Trees were felled and portions of the bole that contained *Monochamus* pits were cut into 0.5 m or 1 m bolts. The bolts were transported back to the University of Arkansas Forest Entomology Lab for dissection. Each pit was dissected by centering a 2.54-cm dia. arc punch over the pit and hammering through outer and inner bark. The outer bark within the circular cut was removed and any eggs, larvae, or larval galleries present were counted. The presence, or absence, of resin in each dissected pit was also noted.

2015 trapping study

In 2015 a trapping study was undertaken to determine if the two *Monochamus* species showed a difference in their attraction to the lure combinations. The study took place between May 26th and July 21st. Two plots were selected within the St. Francis-Ozark National Forest near Lake Wedington, in northwest Arkansas, in which shortleaf pine is the most abundant tree and where adult *Monochamus* had been captured earlier in the year. At each site a series of four traps were placed in a rough square pattern approximately 25 m from one another. Traps consisted of the same black cross-vane panel trap (APTIV™ Intercept) setup as previously described, but attached to the lid of a closed 121-liter plastic garbage can. A hole was cut in the garbage can lid to allow entry of attracted insects which fell into the hole at the base of the

panels. The garbage can was modified by cutting ventilation holes, which were covered with mesh screening [Figure 2]. Fresh pine branches were also cut and placed within the can to provide food and a complex habitat surface to minimize interactions among *Monochamus* adults. A small lure bag was placed between the panels of the trap and contained one of four possible lures: (1) Ipsenol, (2) Monochamol + ethanol (ME), (3) Monochamol + ethanol + Ipsenol (MEI), or (4) Monochamol + Ipsenol + UHR alpha-pinene (MIA). All treatment lures were supplied by Synergy Semiochemicals, Burnaby, British Columbia. Each trap top, including the lure, was moved to the next trap in a clockwise direction each week to account for possible differences in trap location. Each trap was checked weekly and collected beetles were identified to species and sex using the key produced by Lingafelter (2007).

Statistical analysis

All data were analyzed using R (R Core Team, 2015). Data were tested for normality using the Shapiro-Wilks test. Where both raw data and subsequent log-transformed data failed to achieve normality, a Kruskal-Wallis rank sum test was used to determine statistical significance. Where normality was achieved ANOVA was used to determine if significant differences were present.

Initial and season end measurements of resin flow were analyzed to see if a significant change had occurred. A Welch two-sample t-test was used to test if a significant difference existed. The change in resin flow between initial treatment and end of the field season was analyzed to determine if it was influenced by number of oviposition pits. Treatments with zero pits were removed (control and cis-3-pinene-2-ol trees) and oviposition pit numbers were log-transformed to conform to normality. A one-way ANOVA was used to determine if correlations existed. All tests were conducted at significance levels of $p = 0.05$.

The raw number of pits by lure treatment failed the test of normality, but when lure treatments with no oviposition pits (control and cis-3-pinene-2-ol) were removed and the data were log-transformed normality was achieved. An ANOVA was used to determine if significant differences in numbers of oviposition pits existed between lure treatments. Tukeys HSD at $p = 0.05$ was used as a post hoc analysis to isolate differences.

Due to a lack of normality in raw and log-transformed numbers, significant differences in the spatial occurrence of oviposition pits between different bole sections was analyzed using the Kruskal-Wallis test by ranks. Post hoc multiple comparison analysis of differences was produced using the "pgirmess" package in R (Giraudoux, 2015).

The 2015 trapping study was analyzed by summing all beetles caught by sex and species and then using a Pearson's chi-squared analysis to determine if species or sex were significantly different among attractant combinations. We also analyzed the presence of significant differences in beetles caught between each lure using the Kruskal-Wallis test by ranks because of failed normality. Post hoc multiple comparison analysis was produced using the "pgirmess" package in R (Giraudoux, 2015).

Results

Pre-treatment trapping

Initial capture of *Monochamus* occurred on May 12th in all plots. Both *M. titillator* and *M. carolinensis* were found in the May 12th trap catches at each plot. The initial number of *Monochamus* beetles captured ranged from 8 to 11 beetles with an average of 9 beetles per plot. The number of beetles caught each week remained approximately the same until treatments were initiated. Common associates captured included *Rhagium inquisitor*, *Temnoscheila virescens*,

Thanasimus dubius, *Platysoma cylindrical*, and a variety of bark and ambrosia beetles. Of note was low numbers of *Ips* bark beetles detected in each of the plots.

Tree measurements; Nematodes; Resin sampling

Selected trees ranged between 19.0 and 47.5 cm in diameter at breast height (dbh) with a mean dbh of 29.0 cm. There were no pinewood nematodes detected in the selected trees either before or after the experiment, although nematodes were visibly noted on several *Monochamus* specimens collected within the experimental plots. Initial resin flow ranged from 0 to 4.75 cm with a mean of 1.68 cm in trees of the first treatment period and 1.39 cm in trees of the second treatment period. At the end of the field season, November 10th, resin flow ranged from 0 to 3.65 cm with a mean of 1.68 cm for first treatment period and 1.09 for second treatment period trees [Figure 3]. Average resin flow change was -0.20 cm. We found no significant correlation between resin flow change and the number of pits in the tree ($df = 47$, $F\text{-value} = 0.408$, $p\text{-value} = 0.526$).

Periodic tree inspection

The treated trees throughout the experiment showed no visible symptoms of branch flagging due to *Monochamus* attack. Resin was seen flowing from wounds caused by ladder prongs and oviposition pits. Some trees also experienced varying numbers of holes from sapsuckers.

A total of 574 pits was observed throughout the experimental periods. The range of oviposition pits in attacked trees for the first treatment period was 0 to 73, with a range of 0 to 65 for the second period. The highest total number of pits was seen on a tree treated with Ipsenol only. The number of oviposition pits varied by lure treatment. Our statistical analysis concluded that there were significant differences among treatments in number of oviposition pits ($df = 39$,

F-value = 32.94, p-value < 0.05) [Figure 4]. Treatments containing Ipsenol, both Ipsenol and Monochamol + ethanol + Ipsenol (MEI), had a significantly higher number of pits than those without Ipsenol or a lure treatment.

The spatial distribution of oviposition pits was also determined. Pits appeared to be highly clustered around the lure bag and measurements confirmed that pit placement was in a non-random pattern. A significantly greater number of pits was found within 0.5 m above or below the lure bag (df = 5, Kruskal-Wallis chi-squared = 77.007, p-value < 0.05) than in other areas of the bole where pits were present. Furthermore 90 percent of total pits were found on the same 180° of the bole as the lure, while only 10 percent of pits were found on the opposite side [Figure 5].

Bolt dissection

From the 18 trees destructively sampled 562 pits were identified and dissected. A total of 993 eggs was found within the pits, but only 62 percent of the oviposition pits contained any eggs. A high number of unused pits is often seen in *Monochamus* research (Webb, 1909; Walsh & Linit, 1985; Rastok, 2015). The number of eggs within pits ranged from 1 to 11 with an average of 2.85 eggs for those pits that contained eggs. From the pits dissected, only 20 larvae were found and of these, only 5 had produced any galleries. While dissecting the pits we also found that 95.6 percent of the total dissected pit areas contained a large amount of resin within them, indicative of resinosis.

2015 trapping study

A total of 955 adult *Monochamus* beetles were caught during the 7 weeks of trapping. Of 770 *M. titillator* captured 423 were male and 347 were female, for a male to female ratio of 1.22. Of 163 *M. carolinensis* captured 63 were male and 100 were female, for a male to female ratio of

0.63. There was no significant difference between sexes captured with each attractant ($\chi^2 = 3.7883$, $df = 3$, $p\text{-value} = 0.2853$) [Figure 6]. There was no significant difference in species captured by each attractant ($\chi^2 = 7.6531$, $df = 3$, $p\text{-value} = 0.05375$) [Figure 7]. A significant difference was noted in total beetles caught by lure combination with MEI and MIA capturing the most and ME the least (Kruskal-Wallis $\chi^2 = 53.117$, $df = 3$, $p\text{-value} < 0.05$) [Figure 8].

Discussion & Conclusions

The current research objective was to determine if southeastern *Monochamus* could, without coincident or simultaneous bark beetle attack, successfully colonize healthy shortleaf pines. The impetus behind this research was the report by Gandhi (2005) that *Monochamus* could be the primary cause of tree death in jack pine. With populations of similar *Monochamus* species present in both Minnesota and Arkansas study areas, and shared characteristics between jack pine and shortleaf pine stands, *Monochamus* may be able to colonize healthy shortleaf pines.

Southeastern *Monochamus* oviposited into healthy shortleaf pine, but eggs placed into healthy trees were almost always killed by resinosis. Only 2.5 percent of the eggs developed into larvae, and only 0.5 percent of eggs led to larvae which created galleries. No development of larvae to adults occurred. Large amounts of resin was observed in 95.6 percent of dissected pits, and resin was observed pooling in some of the oviposition pits on treated trees (pers. observation). Dodds and Stephen (2000) found that the egg stage of *M. titillator* is particularly vulnerable to mortality attributed to resinosis. Mortality due to resinosis also affects other subcortical insects (Langor & Raske, 1988). Hanks (1999) describes *Monochamus* species as colonizing only weakened or stressed hosts, but not vigorous or fully dead trees. This seems to place *Monochamus* in the precarious position of requiring trees sufficiently compromised that host defenses will not overcome their eggs, yet not so decayed that nutritional value in the sub-

cortical material has already been consumed or become too decomposed. In weakened or stressed trees constitutive and induced resin defenses may be compromised and unable to prevent *Monochamus* larval development. These results differ from those of Gandhi (2005), but this may be due to characteristics that differ between the hosts, such as resin flow, or at the time of her study the presence of larger populations of *Monochamus* in Minnesota. Gandhi (2005) reported that a significantly larger populations of *Monochamus* were present in wind disturbed plots than in fire-treated or control plots. Oviposition into healthy pines occurred in the wind-disturbed plots and abundant maturation feeding occurred on young pines surrounding these plots. In theory a large enough population of *Monochamus* could act as primary colonizers and overwhelm host defenses simply by mass attacks in a short period of time. Mass attack was not observed in this study, although *Monochamus* were not overly abundant during the experimental periods.

Host finding by *Monochamus* utilizes sensory perception of chemical attractants to discover susceptible host trees. Greater oviposition occurred on trees treated with bark beetle pheromones than with *Monochamus* pheromones in this study. Strong attraction to the bark beetle pheromone Ipsenol has been seen in other experiments (Billings, 1985; Allison et al., 2001; Miller & Asaro, 2005). *Ips* bark beetles, which produce Ipsenol, attack hosts in a similar weakened or stressed state to *Monochamus* hosts. The ability of *Monochamus* to detect a kairomone (e.g. Ipsenol) promotes encounters with susceptible hosts and increased oviposition in hosts at the proper weakened or stressed state for larval development. The attraction to Ipsenol may even be an ancestral state as some *Monochamus* are still attracted to Ipsenol when Ipsenol-producing bark beetles are absent in their native range (Pajares et al., 2004). The *Monochamus* may have been poorly attracted to the sex pheromone Monochamol due to its short range of

attraction. Sex pheromones in other cerambycids have been found to operate only at a distance of 5 cm or less (Wang et al., 1991; Lacey et al., 2004). Alternatively the concentration of Monochamol is much higher than would be present in nature and this repels conspecifics rather than attracting them. Kairomones appear to have a much stronger long-range influence on attraction of *Monochamus* while Monochamol may be a short-range attractant that operates once conspecifics are on the same host tree.

This trapping study supported the notion that host volatiles and kairomones serve as primary, long-range attractants which were highly attractive and synergistic while the sex pheromone Monochamol was the least attractive by itself. Ipsenol was more attractive to adult beetles than the combination of Monochamol + ethanol. Synergistic lure combinations were shown to be the most attractive with combinations of host volatiles, kairomones, and male-produced pheromones capturing more than kairomones or host volatiles on their own.

The placement of oviposition pits and eggs in host trees should be a trait highly regulated by evolution in order to minimize intraspecific competition. Eggs placed a sufficient distance from conspecific eggs would allow for development without strong competition for resources or high likelihood of cannibalism. The spatial location of oviposition pits in this study was non-random with a majority of pits concentrated within 0.5m from the lure. In addition, 90 percent of pits were found on the same side of the bole as the lure. This behavior would seem to lead to increased intraspecific competition, but this is not the only example of cerambycids exhibiting this behavior in the presence of bark beetle kairomones. Pits were highly concentrated around lures containing Ipsenol, which, as has already been mentioned, is highly attractive to *Monochamus* species. The cerambycid *Acanthosinus aedilus* will place its eggs in a concentrated spatial pattern near, or even into, the entrance holes of the bark beetle *Tomicus*

piniperda (Schroeder, 1997). An additional cerambycid, *Acanthosinus nodosus*, has been found to lay more than 99 percent of its eggs in entrance holes and ventilation tubes of southern pine beetle (Dodds et al., 2002). Perhaps this behavior increases the possibility of gaining nutrients from facultative predation of bark beetles, which has been shown to occur with southeastern *Monochamus* species (Dodds et al., 2001; Schoeller et al., 2012). The benefits of extra nutrition gained by predation may outweigh the costs of intraspecific competition where strong kairomonal sources are present.

The study objective was to determine if southeastern *Monochamus* can successfully develop in healthy shortleaf pines. The results demonstrate that, although oviposition may occur in healthy pines, resin defenses lead to the death of almost all eggs and successful development to adults does not occur.

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Table 1: Lure treatments applied to trees during treatment periods in 2014. The numbers represent trees treated with each lure during each treatment period. The 1st full treatment period extended from May 23 - August 1, the "test" period July 3 - August 1, and the 2nd full treatment period August 1 - September 26. A total of 52 trees was treated with attractants. Four trees remained untreated as controls throughout all treatment periods.

	Monochamol + ethanol (ME)	Monochamol + ethanol + Ipsenol (MEI)	Ipsenol	Cis-3- pinene-2-ol (CIS-OL)	Control	Total
1st Full Treatment Period	12	--	--	--	4	16
"Test" Treatment Period	--	4	4	--	--	8
2nd Full Treatment Period	8	8	8	8	--	32
Total	20	12	12	8	4	56

Figure 1: Oviposition pits of *Monochamus* species. Three pits are shown that demonstrate the variability in pit structure due to thickness of bark. Deeper pits are created in thick bark while thin bark may only necessitate a small slit. Photo by Ryan Rastok.



Figure 2: Modified cross-vane panel trap used in 2015 trapping study. (a) Shows the cross-vane panel trap attached to an opening in the lid of (b) a 121-liter garbage can. Holes are cut in the sides of the can and covered with mesh to allow for ventilation. The total trap height was 175 cm. The trap was hung by a rope between two non-host trees to place the can bottom approximately 0.5m off the ground. Trap designed by Larry Galligan, diagram used with permission from Rastok (2015).

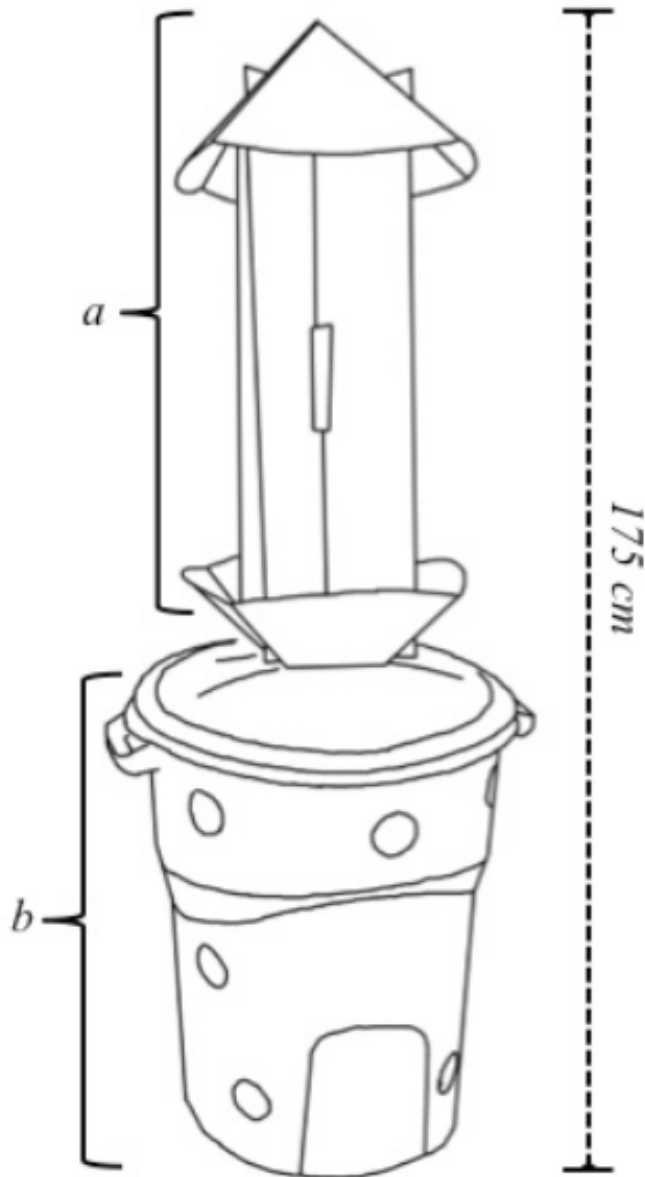


Figure 3: Initial and season end resin measurements for 56 trees in four plots during 2014. Experimental season lasted between May 23 and September 26. Thick bar shows median while box shows interquartile range and outer lines show range. Season end resin flow was not significantly different from initial resin flow ($t = 0.96821$, $df = 93.988$, $p\text{-value} = 0.3354$).

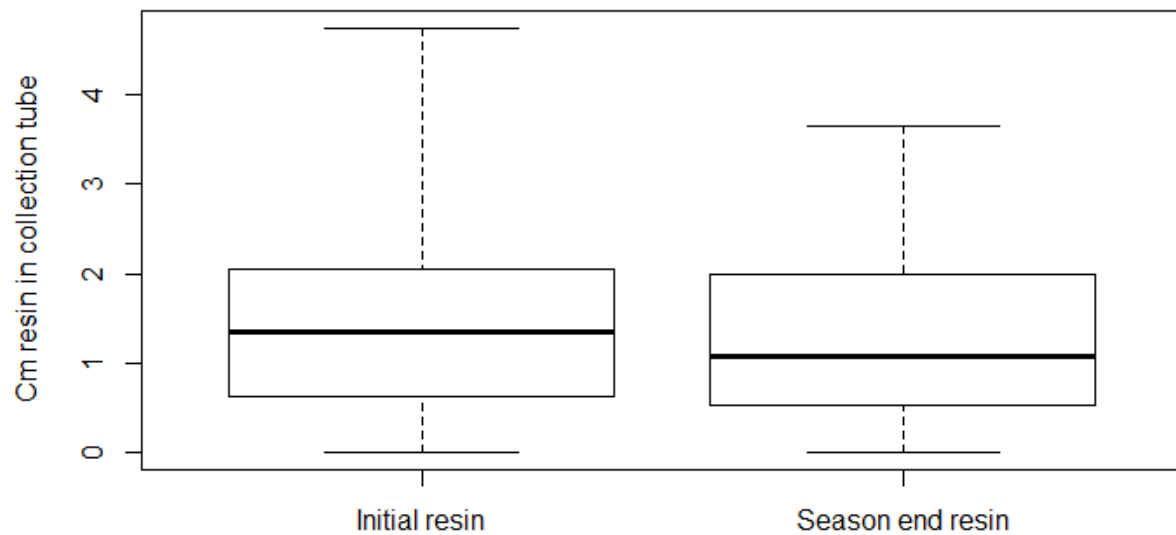


Figure 4: Mean number of oviposition pits by lure combination. The graph represents oviposition pits found on 56 trees in four plots during May 23 – September 26, 2014. Treatment combinations were Ipsenol, Monochamol + ethanol + Ipsenol (MEI), Monochamol + ethanol (ME), and cis-3-pinene-2-ol (CIS-OL). Significant differences in mean pit numbers are designated by different letters. Treatments containing Ipsenol were shown to be more attractive than those lures without (F-value = 32.94, df = 39, p-value < 0.05).

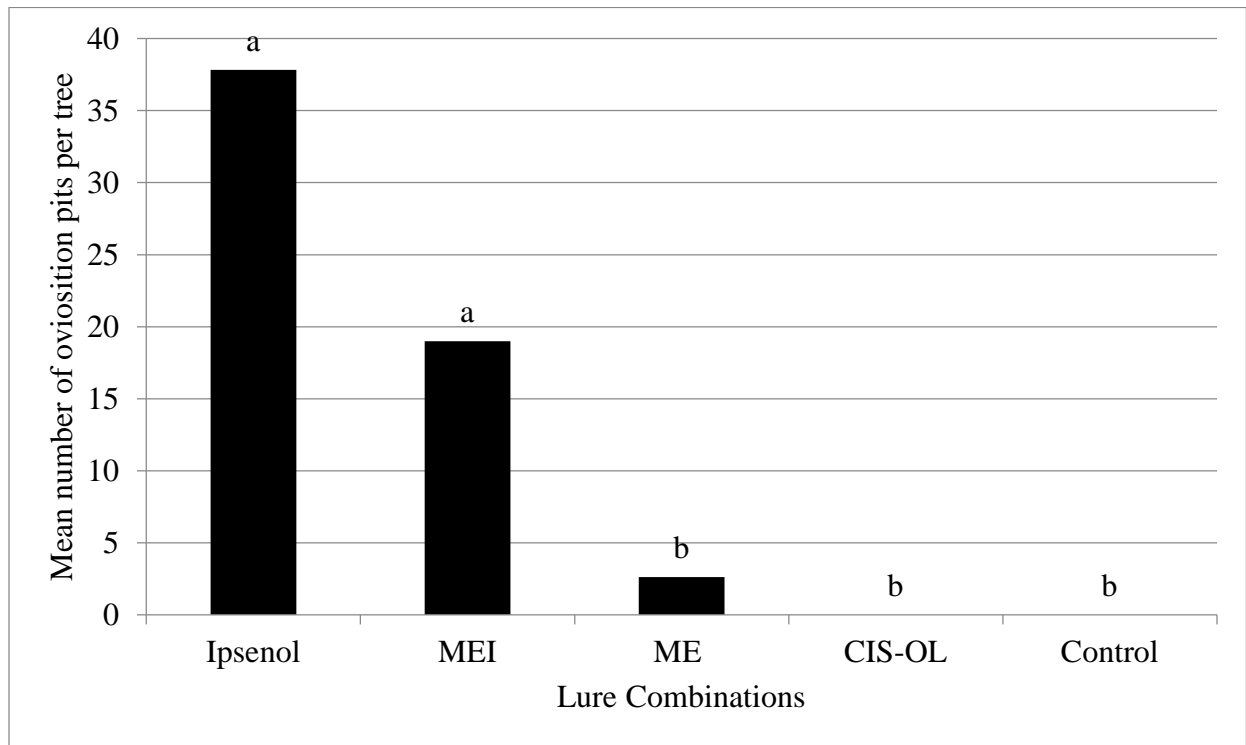


Figure 5: Number of oviposition pits found on tree boles by 0.5m section above and below lure bags. Pits were observed on 52 total treated trees in four plots during May 23 – September 26, 2014. “Front” refers to the same face of the bole where the lure bag was located, whereas “back” refers to the opposite side. A significant difference in oviposition pits by location is designated by different letters over columns. We found significantly more pits in the 0.5m above and below the lure bag than in other bole sections containing pits (Kruskal-Wallis $\chi^2 = 77.007$, $df = 5$, p -value < 0.05). Of total pits 90% were found on the front while 10% were on the back.

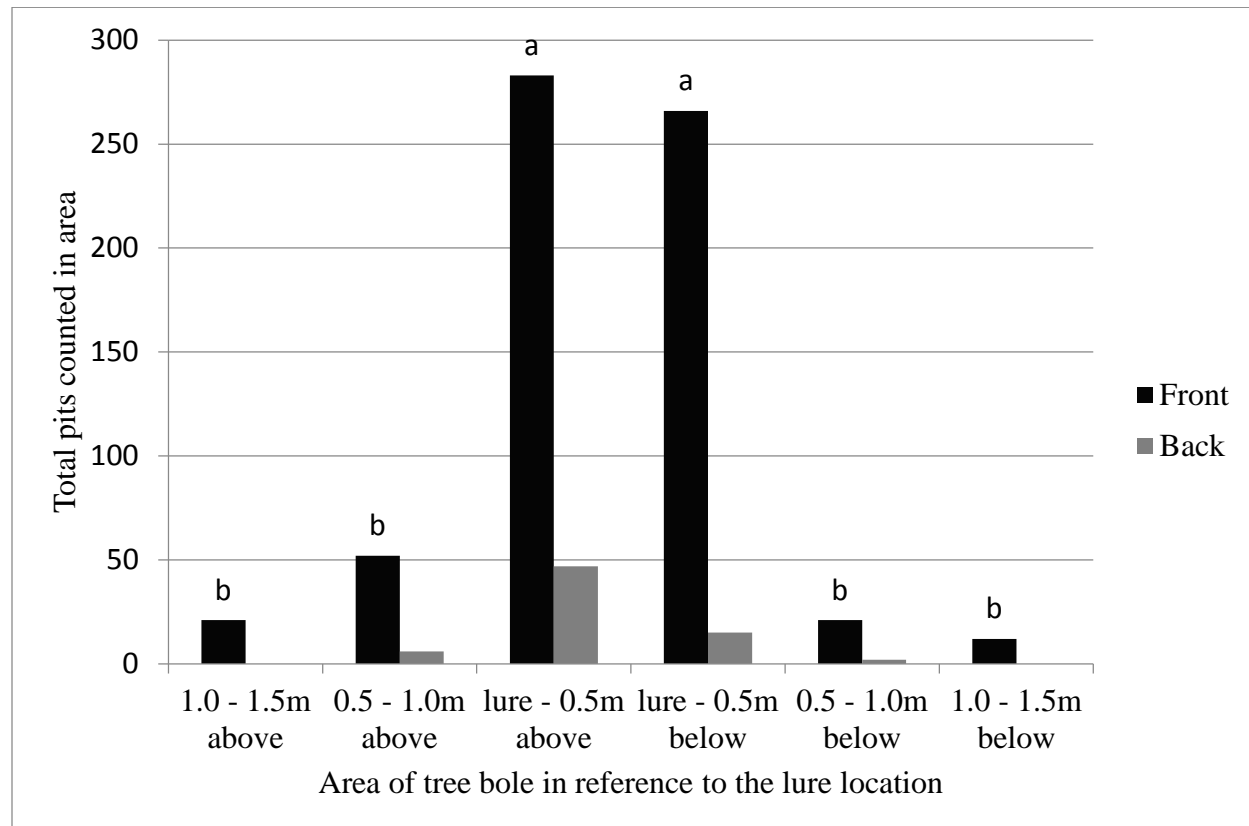


Figure 6: Proportions of live *Monochamus* of each sex captured by lure combinations. Beetles were captured in a modified cross-vane panel trap, as previously described, May 26 – July 21, 2015. Beetles were caught in traps baited with Ipsenol, Monochamol + ethanol (ME), Monochamol + ethanol + Ipsenol (MEI), and Monochamol + Ipsenol + UHR alpha-pinene (MIA). There were no significant differences in the proportion of beetles of each sex caught by each lure combination ($\chi^2 = 3.7883$, $df = 3$, $p\text{-value} = 0.2853$), i.e. no one lure combination caught significantly more males than females or vice-versa.

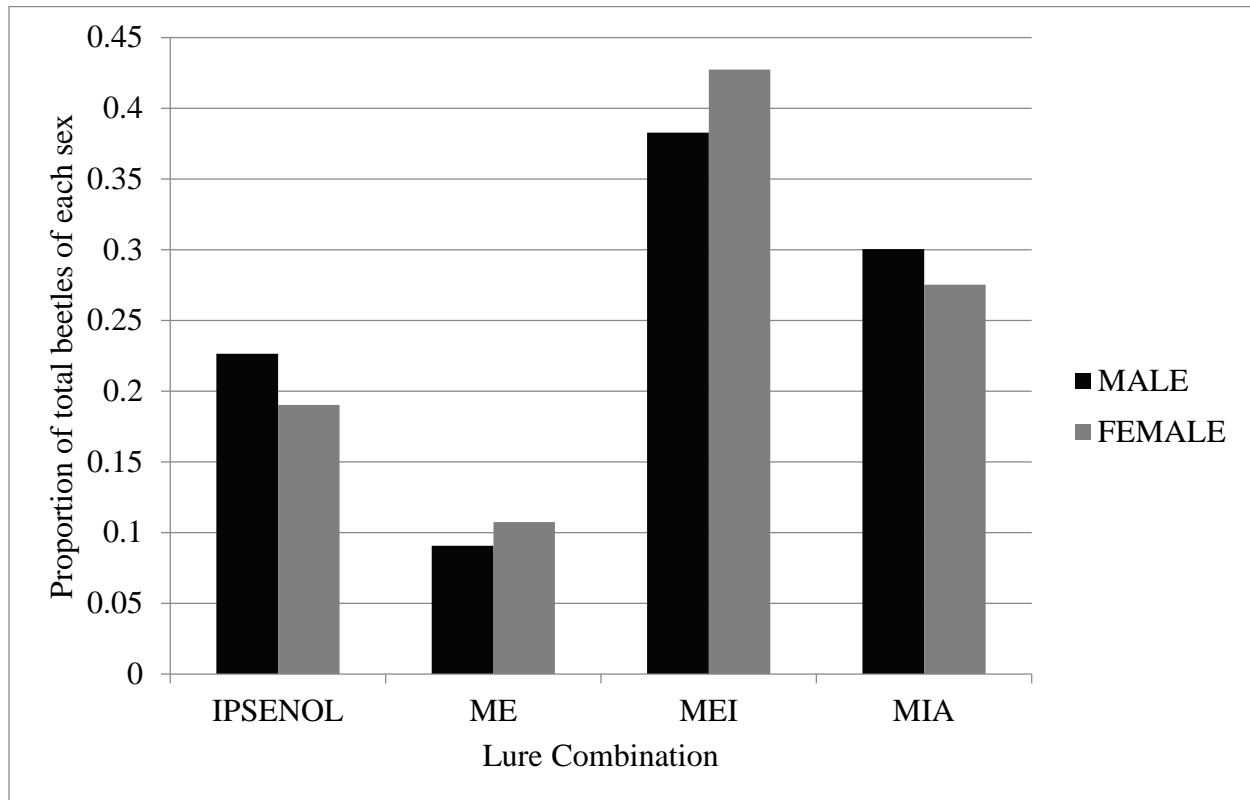


Figure 7: Proportions of live *M. titillator* (MT) and *M. carolinensis* (MC) captured by lure combinations. Beetles were captured in a modified cross-vane panel trap, as previously described, May 26 – July 21, 2015. Beetles were caught in traps baited with Ipsenol, Monochamol + ethanol (ME), Monochamol + ethanol + Ipsenol (MEI), and Monochamol + Ipsenol + UHR alpha-pinene (MIA). There were no significant differences in species of beetles caught by each lure attractant ($\chi^2 = 7.6531$, $df = 3$, $p\text{-value} = 0.05375$), i.e. no one lure combination caught significantly more MT than MC or vice-versa.

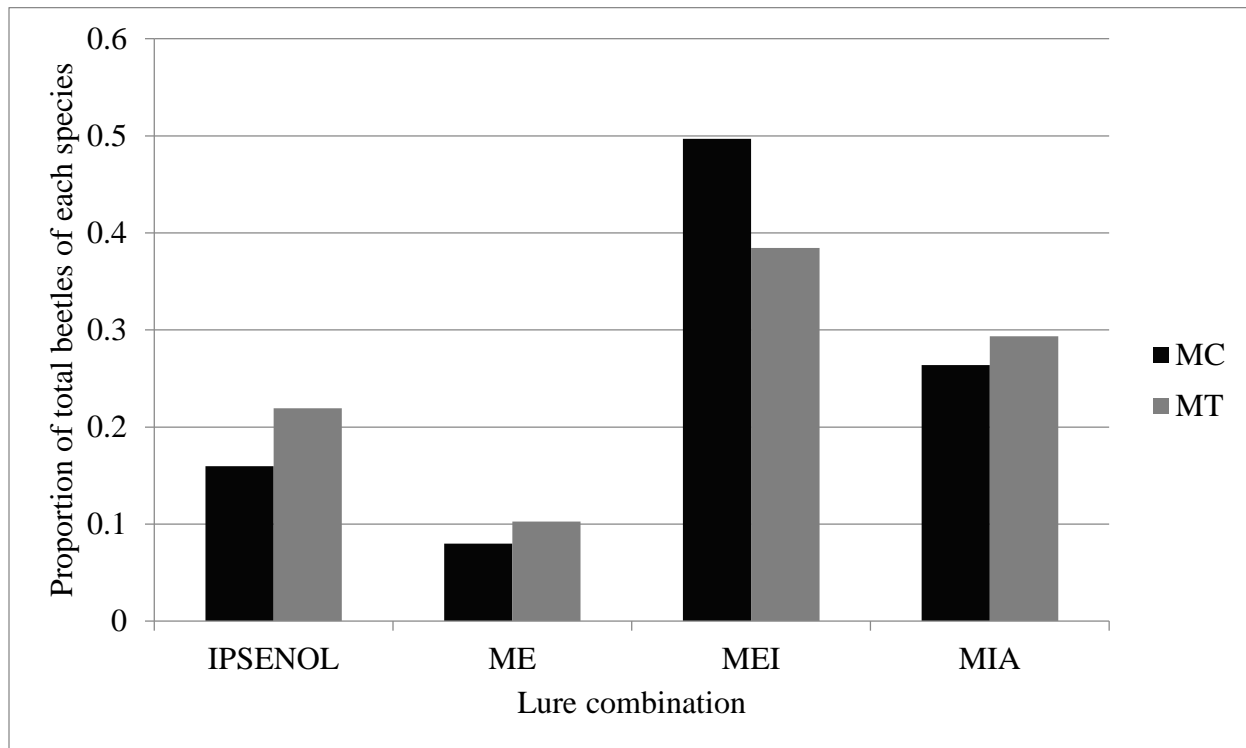
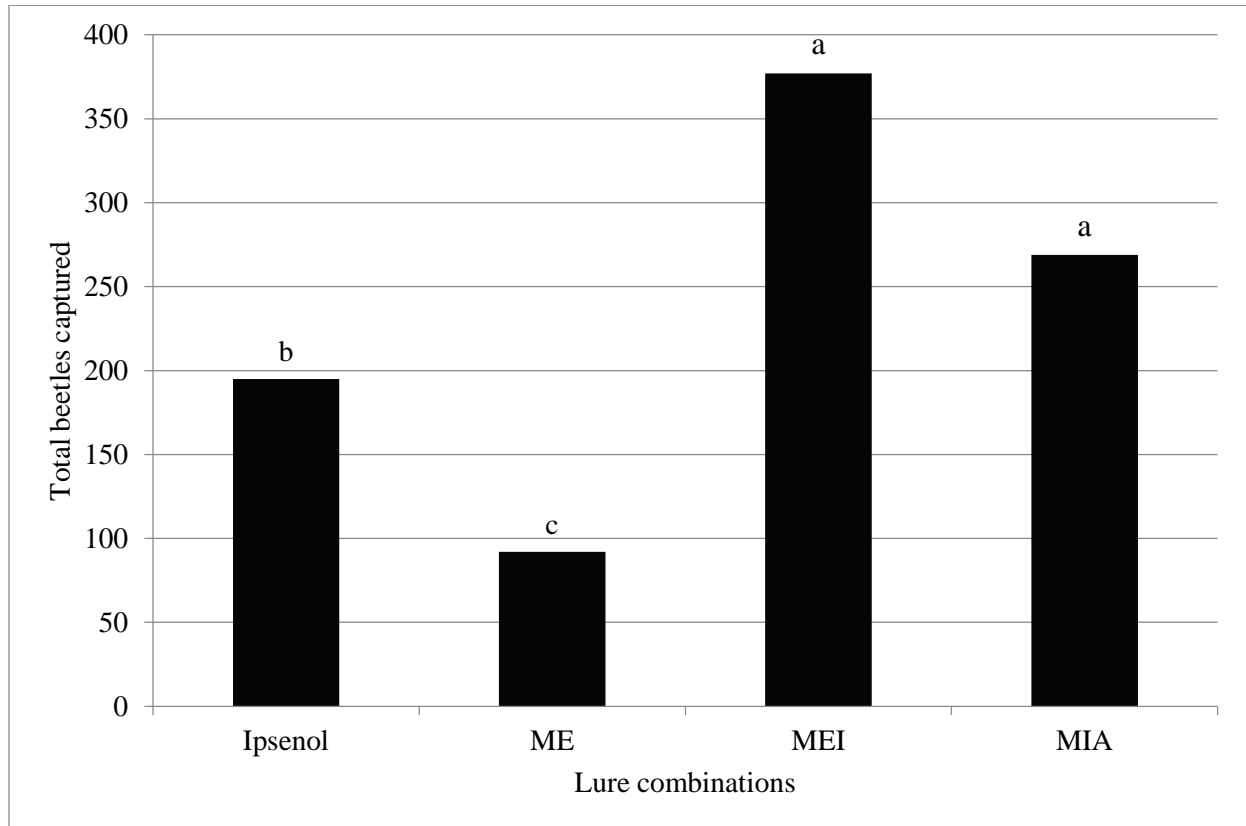


Figure 8: The total number of beetles captured by each lure combination May 26 – July 21, 2015. Beetles were caught in traps baited with Ipsenol, Monochamol + ethanol (ME), Monochamol + ethanol + Ipsenol (MEI), and Monochamol + Ipsenol + UHR alpha-pinene (MIA). Beetles were collected from two traps for each treatment combination each week. Columns with different letters are significantly different from each other. MEI and MIA were found to capture significantly more beetles than the other lure combinations (Kruskal-Wallis $\chi^2 = 53.117$, $df = 3$, $p\text{-value} < 0.05$).



Introduction

Beetles in the genus *Monochamus* Dejean are moderate to large wood-boring insects in the longhorn family Cerambycidae. The majority of *Monochamus* species are endemic to tropical ecosystems, but a number of species are present in the temperate Northern Hemisphere (Roguet, 2015). Temperate species develop primarily in pines (*Pinus*), but will also utilize fir (*Abies*) and spruce (*Picea*) as hosts for larval development (Linsley & Chemsak, 1984). While aspects of their life history and economic damage have been explored, interactions with many associated insects remain unclear.

Sexually mature *Monochamus* are attracted to hosts at long distances by a variety of host volatiles and associated insect kairomones (Fatzinger, 1985; Allison et al., 2001; Miller et al., 2013). Upon arriving at hosts, attraction of females to congeneric males is mediated by short-range, male-produced pheromones (Allison et al., 2012; Fierke et al., 2012; Macias-Samano et al., 2012). As congenics are encountered, males palpate the elytra to detect cuticular hydrocarbons and determine if mating will occur (Ibeas et al., 2009). Following copulation females search on hosts for suitable sites to excavate oviposition pits (Alya & Hain, 1985; Edwards & Linit, 1991). One to nine eggs are deposited below the bark in a circular pattern around the oviposition pit (Webb, 1909). After eggs hatch the larvae develop by consuming inner bark, cambium, and phloem (Rose, 1957; Alya & Hain, 1985). Later in development larvae create long galleries deep into the sapwood, although they return to feed on nitrogen-rich cambium in the host sub-cortical zone (Webb, 1909). Pupal chambers are formed, in larval galleries, where the larvae will pupate and eclose as adults, chewing circular emergence holes in the bark (Webb, 1909; Alya & Hain, 1985). Newly emerged adults must feed on needles, twigs,

or small branches of host trees for seven to twelve days in order to become sexually mature (Linsley & Chemsak, 1984; Walsh & Linit, 1985). After sexual maturation adults are attracted to suitable host trees and the cycle repeats.

Bark beetles in the genus *Ips* De Geer are common associates of *Monochamus* in the Northern Hemisphere. The hosts of *Ips* are primarily pines, but spruces are also colonized. These beetles normally colonize weakened or stressed trees, but in outbreak populations, or in situations where hosts are weakened, living trees may be attacked and killed as well (Garraway, 1986; Nebeker, 2011). Colonization of susceptible trees is initiated by males creating entrance galleries and nuptial chambers below the bark (Anderson, 1948; All & Anderson, 1972). Aggregation of both sexes results from secondary attraction to male-produced pheromones (Anderson, 1948; Vite & Renwick, 1971). Specificity of these pheromones appears to decrease competition for resources and complements niche partitioning among *Ips* and associated bark beetle species (Werner, 1972; Paine et al., 1981). After females join males in nuptial galleries mating occurs and galleries are created leading away from the nuptial chamber. Eggs are deposited in niches in the sides of the galleries. Larval development is supported primarily by consumption of sub-cortical phloem and cambium, although symbiotic fungi are also utilized for nutrition in several species (Wood & Stark, 1968). After pupation and eclosion, adult beetles chew small round emergence holes and fly to infest other trees.

A number of *Monochamus* and *Ips* species inhabit large pine and mixed pine-hardwood forests in the southeastern United States. There are four *Monochamus* species whose geographic distributions extend to this area, but the most common and abundant are *M. titillator* Fabricius and *M. carolinensis* Olivier (Linsley & Chemsak, 1984; Alya & Hain, 1985). The most common species of *Ips* in the southeast are *I. avulsus* Eichoff, *I. grandicollis* Eichoff, and *I. calligraphus*

German. These three *Ips* species, along with the bark beetles *Dendroctonus frontalis* Zimmermann and *D. terebrans* Olivier, comprise the southern pine beetle (SPB) guild (Nebeker, 2011). Larval development of both *Monochamus* and *Ips* in the southeast occurs in pines weakened or stressed by biotic or abiotic factors (Hanks, 1999; Nebeker, 2011). Many *Monochamus* species show strong attraction to *Ips* species aggregation pheromones, specifically including those most common in the southeast (Ibeas et al., 2007; Miller et al., 2011). Attraction of both genera to *Ips* aggregation pheromones commonly leads to shared hosts where *Ips* colonization occurs shortly before the arrival of colonizing *Monochamus* (Gardiner, 1957). Adult flight and colonization of hosts by southeastern *Ips* commences in early spring, but from late spring to early fall *Ips* and *Monochamus* colonize shared hosts (All & Anderson, 1972; Alya & Hain, 1985; Schoeller & Allison, 2013). The large number of shared characteristics make interactions between southeastern *Monochamus* and *Ips* species highly likely.

Interspecific interactions between *Monochamus* and *Ips* can include competition for shared resources or direct facultative predation (Dodds et al., 2001). *Monochamus titillator* compete with *D. frontalis* leading to diminished survival and emergence of the bark beetle (Coulson et al., 1976, 1980). Miller (1986a) found that the presence of *M. titillator* in logs contributed to more than 50 percent of total *I. calligraphus* brood loss. Large and mobile *Monochamus* larvae may also consume bark beetles when encountered during sub-cortical foraging. Several studies have demonstrated that southeastern *Monochamus* consume members of the SPB guild (Dodds et al., 2001; Schoeller et al., 2012).

Trapping studies using *Ips* aggregation pheromones in pine stands within the Ouachita National Forest in Arkansas revealed that, over several years, the most commonly collected *Ips* and *Monochamus* species are *I. grandicollis* and *M. titillator* (Barton, 2015; Galligan

unpublished data). Both *I. grandicollis* and *M. titillator* also have a similar, non-concentrated, staggered adult emergence, unlike other bark beetles and common associates (Berisford & Franklin, 1971; Alya & Hain, 1985). In addition, southeastern *Monochamus* species may be even more likely to interact with *I. grandicollis* due to strong attraction to Ipsenol, the aggregation pheromone produced by *I. grandicollis* (but not other southeastern species) (Vite & Renwick, 1971; Miller & Asaro, 2005). Because of these shared characteristics it is highly likely that sub-cortical interactions between *M. titillator* and *I. grandicollis* occur very frequently in the southeastern United States.

The current research objectives are to determine (1) if sub-cortical interactions between *M. titillator* and *I. grandicollis* affect survival and emergence of *I. grandicollis*, and (2) if so, how these effects differ in relation to the time between *I. grandicollis* colonization and *M. titillator* arrival on hosts.

Materials & Methods

To determine if sub-cortical interactions, or landing time differences, affected *I. grandicollis* survival *I. grandicollis* adults were introduced to shortleaf pine bolts and then *M. titillator* females introduced at different times after *I. grandicollis* colonization. Emergence of *I. grandicollis* was counted to determine the effects, if any, of sub-cortical interaction and difference of arrival time.

Experimental tree bolts

Experimental bolts were taken from shortleaf pine trees (*P. echinata* Mill.) found in mixed pine-hardwood stands in the Ozark National Forest in northwest Arkansas. Nine trees were felled and the boles cut into 75 cm long bolts. Of these nine, three trees were felled on 23 July, 2014, two trees on 29 August, 2014, two on 11 March, 2015, and two on 19 May, 2015.

Bolts were transported to the University of Arkansas Forest Entomology Lab and held in a large cooler at 1.1°C for storage. After three to five days bolts were removed from the cooler and cut ends were sealed with paraffin wax to prevent desiccation. Bolts were subsequently returned to the cooler and stored until used for the experiments.

Surface area of experimental bolts was determined using the equation $A = C \times L$, where C is the circumference, L the length of the bolt, and A is the area. The circumference was determined using the equation $C = \pi D$, where C = circumference and D = average diameter of the bolt. To determine average bolt diameter both ends of the bolt were measured using a tree diameter tape. The measurements were summed and divided by two to give the average bolt diameter.

Monochamus titillator

Adult *M. titillator* were collected from several sources throughout the experimental period. Shortleaf pine bolts with *Monochamus* oviposition pits were cut from ice damaged trees found in the Ouachita National Forest on July 12, 2014. These bolts were placed in 121-liter plastic garbage cans laid horizontally in a wooden frame [Figure 1]. Emerging *Monochamus* adults were collected every two to three days from the cans and identified to species and sex using Lingafelter (2007). Adult *M. titillator* were separated by sex and placed in separate cages with fresh loblolly pine (*P. taeda*) branches for at least ten days to allow for sexual maturation feeding. Fresh pine branches were placed in the cages every three to four days. After ten days both sexes were placed into the same cage with additional feeding material for at least five days to allow for mating. After the five-day mating period female beetles were considered ready for experimental use. Five female *M. titillator* were dissected after development and the number of mature eggs were compared to the egg load of five dissected field-collected females (described

below). Female egg load varied in both caged and field collected *M. titillator*, but average number of mature eggs was not significantly different between the groups ($F = 0.0044$, $df = 9$, $p\text{-value} = 0.948$).

During May to July, 2015, adult *Monochamus* were collected in live traps in mixed pine-hardwood plots within the Ozark National Forest, in Arkansas. These beetles were collected using a black cross-vane panel trap (APTIVTM Intercept) attached to the lid of a closed 121-liter plastic garbage can. A hole in the lid allowed the panel trap to funnel beetles into the can. The can also had mesh-covered holes to allow for ventilation [Figure 2]. Fresh pine branches were placed in the can for feeding and to provide refuge, as a means to avoid aggressive male interactions. The traps were baited with a combination of pine host volatiles (ethanol and alpha-pinene) and a beetle pheromone (Ipsenol) which attract adult *Monochamus*. Beetles were collected each week and all *Monochamus* were identified to species using Lingafelter (2007). All collected *M. titillator* were placed in a cage with fresh pine branches for at least 24 hours to ensure that females had mated. After 24 hours these beetles were considered ready for use in the experiments.

Ips grandicollis

Adult *Ips* beetles were collected from several sources throughout the experimental period. *Ips* were collected as they emerged in garbage cans from bolts collected from the Ouachita National Forest, as described above. Emerged beetles were collected every 2-3 days using a small aspirator. The beetles were identified to species using body length and number of spines present on the elytral declivity (Wilkinson & Foltz, 1982). Adult *I. grandicollis* were used for experiments the day they were collected only if they remained active. This was done to avoid beetles that had depleted their limited energy reserves.

Between May to July, 2015, adult *I. grandicollis* were also collected as they emerged from bolts and large branches of shortleaf pine gathered from a recently harvested stand in the Ozark-St. Francis National Forest in Arkansas. All beetles were collected, living or dead, and identified to species using the elytral declivity and number of spines. Again only actively moving adults were used, on the day of collection, for the experiments.

On each day that bolts were infested with adult *I. grandicollis* a sub-sample of 25 of the unused collected beetles were used to estimate the sex ratio of males to females for that day. Females were differentiated from males by dissecting heads from the remainder of the bodies and looking for stridulatory organs under a 160x stereomicroscope. Females are identified by the presence of a stridulatory organ on the posterior, dorsal portion of the head (Wilkinson, 1962).

Beetle rearing receptacles

Large 170-liter plastic tubs were used for initial introduction of beetles to pine bolts. The tubs had several mesh-covered holes cut out of each side, as well as the lid, to allow for gas exchange. The tubs were kept in a large, long shed with ambient temperature between 21°C and 29°C and relative humidity between 20 and 45 percent.

Bolts containing emerging beetles were held in 121-liter plastic garbage cans which facilitated beetle collection. The garbage cans were laid horizontally on a wooden rack in a large rearing shed [Figure 1]. To allow for ventilation several holes were cut and covered with fine mesh in the sides and lid of the can. To assist in continual air flow, which minimizes fungal growth on bolts, a tube was inserted into a small hole in one corner of the can. This tube was attached to a system of ducts allowing a fan to pass air through each can. The lid of the can had a hole with a 236.5-ml mason jar screwed on to allow ambient light to filter through and attract

beetles to the jar for easier collection. The garbage cans were kept in the same building and environmental conditions as the tubs.

Experimental setup

When adult beetles were available for introduction, one shortleaf pine bolt was placed horizontally on top of small wood slats in a plastic tub. If *Monochamus* were to be introduced to the bolt, several branches of fresh loblolly pine were included in the tub. Thirty adult *I. grandicollis* were carefully placed on top of the bolt. The *Ips* were allowed at least 30 m with the tub lid closed to colonize the bolt before any introductions of *Monochamus* occurred. After 30 m *Ips* beetles were no longer seen walking on the bark leading to the assumption that they were creating entrance galleries in the bark. If bolts were control replicates, used to determine a baseline of *I. grandicollis* emergence, no *M. titillator* females were introduced. If bolts were to be treated with *Monochamus* either two or five female *M. titillator* were placed in the tub. Female *M. titillator* were introduced to bolts on the same day, 3 days after, or 6 days after *I. grandicollis* infestation [Table 1]. Male *M. titillator* were omitted from the tubs to prevent damage to females by overeager mating or fighting (pers. observation).

The beetles had at least two weeks to colonize the bolts in the tubs. After at least two weeks the bolts were taken out of the tubs and placed into the horizontal garbage cans [Figure 1]. Emerging *I. grandicollis* and *M. titillator* were collected every two to three days. Adult *I. grandicollis* were collected for 120 days before removing bolts from the can. Bolts treated with *M. titillator* were moved to another emergence shed for further research.

Statistical analysis

All statistical analyses were done using the program R (R Core Team, 2015). Emergence of *I. grandicollis* was utilized as the response variable in all analyses. Where raw and

transformed numbers of *I. grandicollis* emergence failed the Shapiro-Wilk test of normality non-parametric analysis procedures were used to determine differences, or correlations, among the factors analyzed. All analyses were conducted at the 0.05 significance level.

The bolt surface area and number of *M. titillator* pits were analyzed to determine if bolt size was correlated with emergence of *I. grandicollis* adults. Raw numbers as well as log, square root, and arcsine transformation failed to normalize the response variable data. Due to lack of normality both factors were individually analyzed using the non-parametric Spearman's rank correlation coefficient.

The number of *M. titillator* introduced to logs, as well as the time between *I. grandicollis* and *M. titillator* introductions, were analyzed to determine if there were differences in *I. grandicollis* emergence between the different treatments. Non-parametric analysis of the individual factors as well as interactions were done using the Kruskal-Wallis test by ranks. Post-hoc testing of significant differences was accomplished using the `kruskalmc` function of the `pgirmess` package in R (Giraudoux, 2015).

Results

The dbh (diameter at breast height) of felled trees ranged from 17.0 to 19.2 cm. The mean dbh of all felled trees was 17.6 cm. The 75-cm bolts cut from the felled trees ranged from 9.75 to 20.5 cm in average diameter. The mean bolt diameter was 15.3 cm. Surface area of bolts ranged between .25 to .55 m², with a mean of .40 m².

A total of 2,460 adult *I. grandicollis* and 217 female *M. titillator* were used during the experiment. The male to female sex ratio of *I. grandicollis* adults collected in the introduction sub-samples ranged from 0.39 to 1.78. The mean sex ratio for all *I. grandicollis* used to infest experimental bolts was 0.96. A total of 8,885 *I. grandicollis* emerged from experimental bolts.

The number of emerged *I. grandicollis* ranged from 4 to 329 per bolt, with 107 beetles as the mean.

The number of oviposition pits per *Monochamus* treated bolt ranged from 15 to 192 pits, with a mean of 79 pits. Pit density in *Monochamus* treated bolts ranged from 39.3 to 599.2 pits per m². Average pit density was 39.3 pits per m².

No correlation was found between the surface area of bolts and *I. grandicollis* emergence ($S = 101061$, $p\text{-value} = 0.5861$, $\rho = -0.06063517$) [Figure 3]. The number of *M. titillator* oviposition pits exhibited a significant, but moderate, negative correlation with *I. grandicollis* emergence ($S = 58550$, $p\text{-value} = 9.804 \times 10^{-5}$, $\rho = -0.4744081$) [Figure 4]. The equation of the correlation trendline is $y = -0.5087x + 108.8$.

Statistical analysis revealed that bolts treated with *M. titillator* females led to significantly different *I. grandicollis* emergence ($\chi^2 = 34.2662$, $df = 2$, $p\text{-value} = 3.624 \times 10^{-8}$) [Figure 5]. Subsequent analysis determined that no significant difference in *I. grandicollis* emergence existed between treatments with two or five *M. titillator* females, but that both of these had significantly lower emergence than control bolts in which no *M. titillator* were introduced. Analysis found no significant differences in the time main effect ($\chi^2 = 2.3479$, $df = 2$, $p\text{-value} = 0.3091$) [Figure 6].

Discussion & Conclusions

Increased knowledge concerning interspecific interactions that affect population dynamics of economically important bark beetles may lead to better forest conservation and management practices. Interactions among predators and competing species can produce direct and indirect negative effects on bark beetle populations (Stephen, 2011).

Sub-cortical interactions between *Monochamus* and bark beetle species have been demonstrated in several species associations (Coulson et al., 1976; Miller, 1986b; Stephen, 2011). The current results support previous research showing decreased bark beetle emergence due to interaction with *Monochamus* species. Interactions between these two species in southeastern habitats depend on the presence and magnitude of each beetle population where hosts are available. As the results do not differentiate the types of interaction occurring (competition or facultative predation) further research may elucidate this information. The positive or negative effects of these interactions on *M. titillator* also remain undetermined at this time. In *M. carolinensis*, a sibling species of *M. titillator*, ingestion of bark beetle larvae led to larger larval and adult size (Dodds et al., 2001). The same is likely to occur in interactions between *M. titillator* and *I. grandicollis* as additional nutrition could be gained from bark beetle consumption.

The results of this study also demonstrate that sub-cortical interactions between *M. titillator* and *I. grandicollis* are not significantly different at different secondary colonization times of *M. titillator*, at least between 0 to 6 days. This result was unexpected due to the difference in development times between *M. titillator* and *I. grandicollis*. Development from egg to adult in *I. grandicollis* development can take 25 to 45 days (Morgan 1967), while *M. titillator* development takes 60 to 90 days with heavy phloem feeding only after 20 to 25 days after colonization (Webb, 1909; Morgan, 1967; Dodds & Stephen, 2000). Flamm et al. (1989) found that *Ips* bark beetles in the SPB guild escape interactions with *M. titillator* by developing quickly and leaving hosts before *Monochamus* become established. The results of this study do not support their findings, although these results only demonstrate the effects of up to 6 days in difference between colonization times. These results may also have been influenced by an

unusually high density of *M. titillator* oviposition pits, although when compared with pit densities from bolts exposed to field infestation, in the Ozark National Forest in Arkansas, pit density was similar (Bodart, unpublished data).

The results of this research support the occurrence of sub-cortical interactions between *M. titillator* and *I. grandicollis* leading to diminished *I. grandicollis* survival and emergence, although the difference in colonization time between the two species does not affect the interactions.

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Figure 1: Plastic 121-liter garbage cans laid horizontally in wooden frame to allow for beetle emergence. These cans were used first for emergence of adult *Monochamus* and *Ips* from field collected material. The blue tubs on the bottom row were used for experimental introduction of *I. grandicollis* and *M. titillator* adults. After two weeks experimental bolts were moved to the garbage cans where they were stored during subsequent emergence.



Figure 2: Diagram of trap used for collected live adult *Monochamus* during May to July, 2015. The top consists of a cross-vane panel trap (APTIVTM Intercept) connected to the top of a 121-liter plastic garbage can. Fresh pine material was placed inside to allow for feeding and to decrease male-produced damage to other beetles. Mesh-covered holes in the side of the can allowed for ventilation. Used by permission from Rastok (2015).

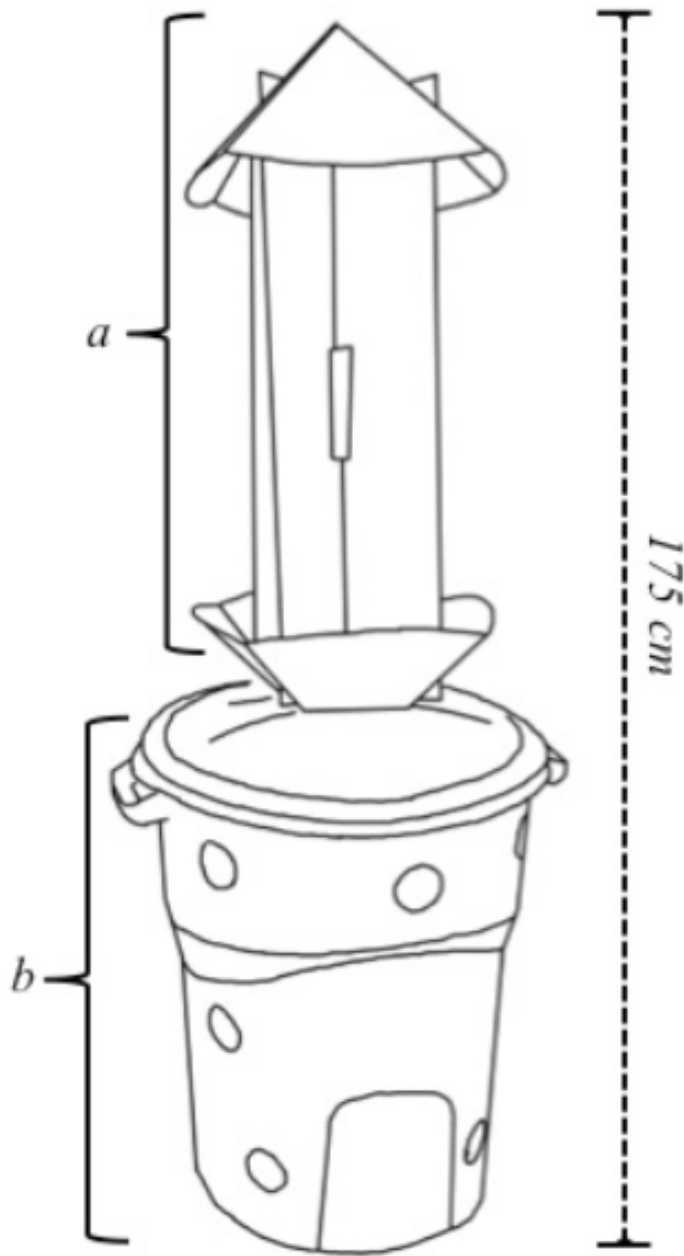


Table 1: Each experimental bolt in this 2 x 3 factorial experiment was first infested with 30 *I. grandicollis* adults. Control bolts received no further treatments. All other bolts were then treated with either 2 or 5 *M. titillator* females (“*M. titillator* introduced”). Treatment with *M. titillator* occurred on the same day, 3 days, and 6 days after *I. grandicollis* introduction (“Days after *I. grandicollis* introduction”). The number of replications for each treatment combination is shown below.

		<i>M. titillator</i> introduced			
Days after <i>I. grandicollis</i> introduction		Control	2 females	5 females	
		No. Bolts	No. Bolts	No. Bolts	Total No. Bolts
	0 days	7	10	10	27
	3 days	7	10	10	27
	6 days	7	11	11	29
	Total	21	31	31	83

Figure 3: Correlation between bolt surface area and *I. grandicollis* emergence. Each data point represents surface area and emergence data for one bolt. Each bolt was infested with 30 *I. grandicollis* and 0, 2, or 5 *M. titillator* females. Bolts were treated between August, 2014 and July, 2015. No significant correlation was found between bolt surface area and emergence of *I. grandicollis* ($S = 101061$, $p\text{-value} = 0.5861$, $\rho = -0.06063517$).

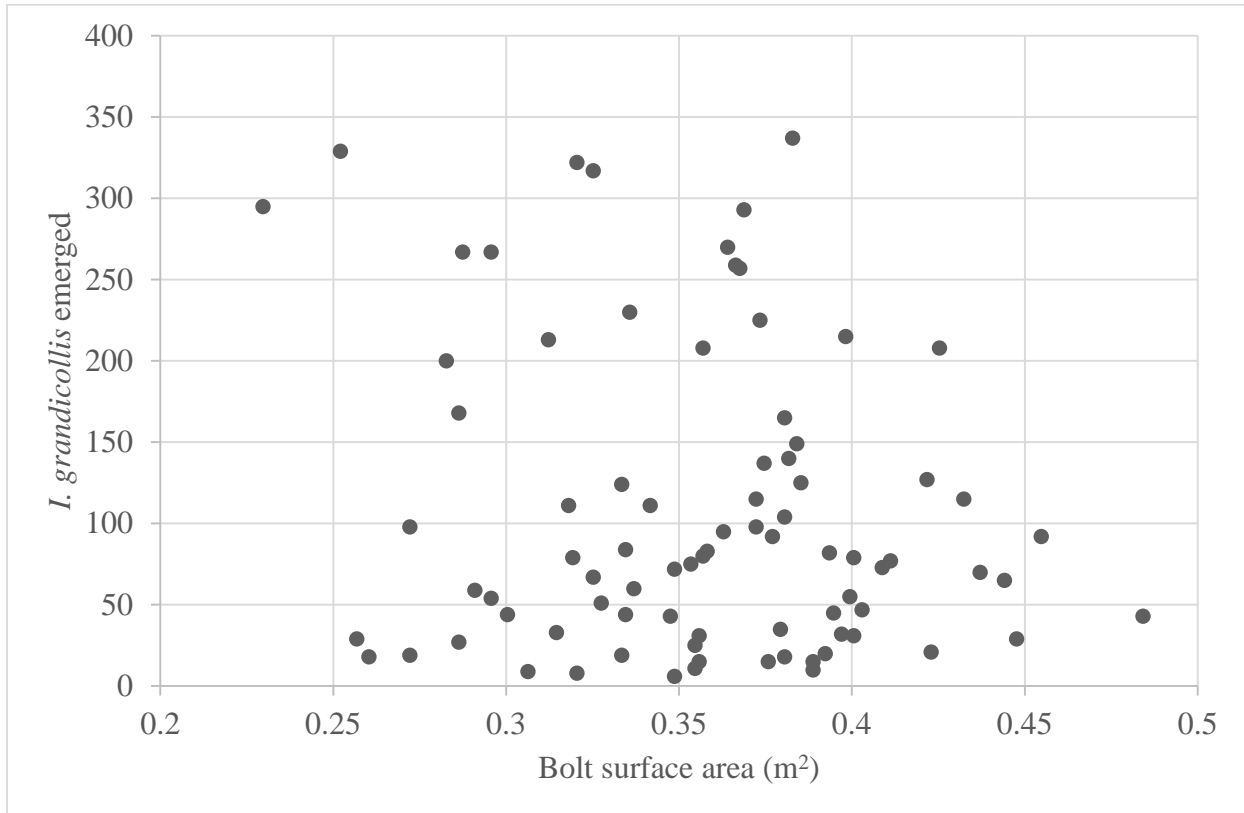


Figure 4: Correlation between *M. titillator* oviposition pits and *I. grandicollis* emergence. Each data point represents surface area and emergence data for one bolt. Each bolt was infested with 30 *I. grandicollis* and 0, 2, or 5 *M. titillator* females. Bolts were treated between August, 2014 and July, 2015. A moderate negative correlation was shown between the number of oviposition pits and *I. grandicollis* emergence ($S = 58550$, $p\text{-value} = 9.804 \times 10^{-5}$, $\rho = -0.4744081$). The equation of the trendline shown is $y = -0.5087x + 108.8$.

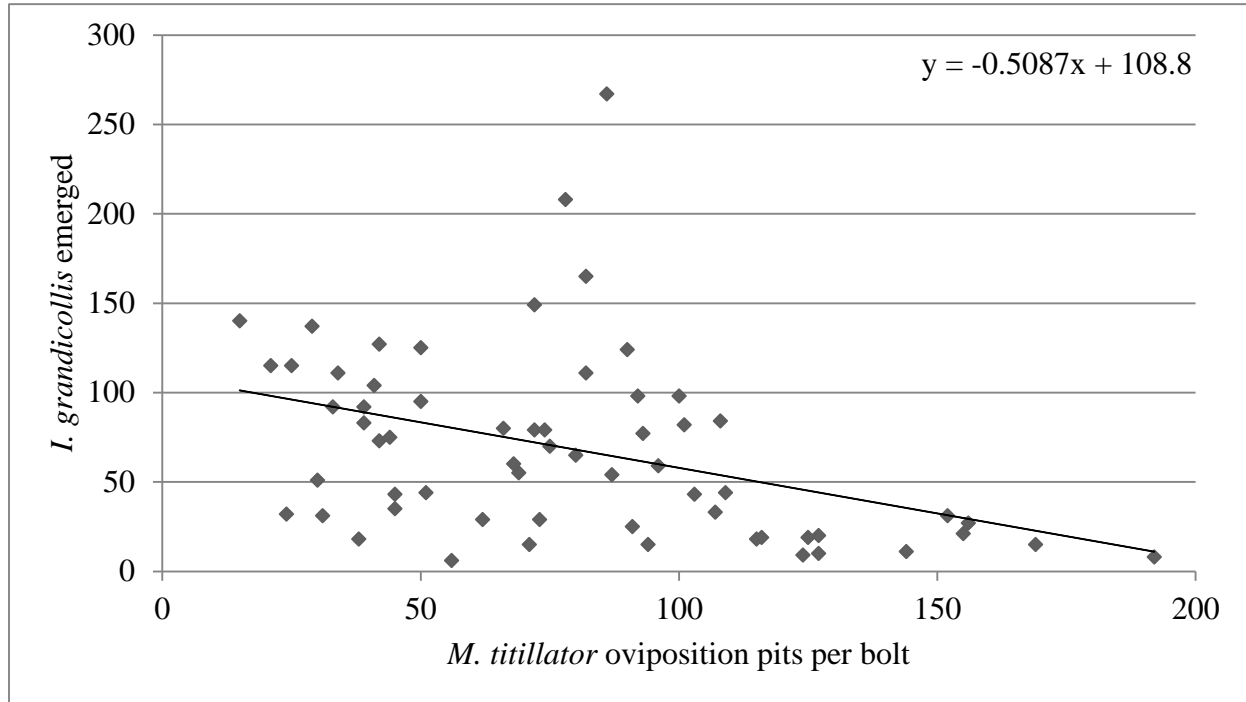


Figure 5: *I. grandicollis* emergence from bolts treated with 0, 2, or 5 *M. titillator* females. Columns represent mean *I. grandicollis* emergence for each *M. titillator* treatment. Bolts were treated between August, 2014 and July, 2015. Different letters show significant difference between treatments at 0.05 significance level ($\chi^2 = 34.2662$, $df = 2$, $p\text{-value} = 3.624 \times 10^{-8}$). Post hoc analysis found that control treatments were significantly different from both *Monochamus* treatments, but no significant difference existed between the two *Monochamus* treatments.

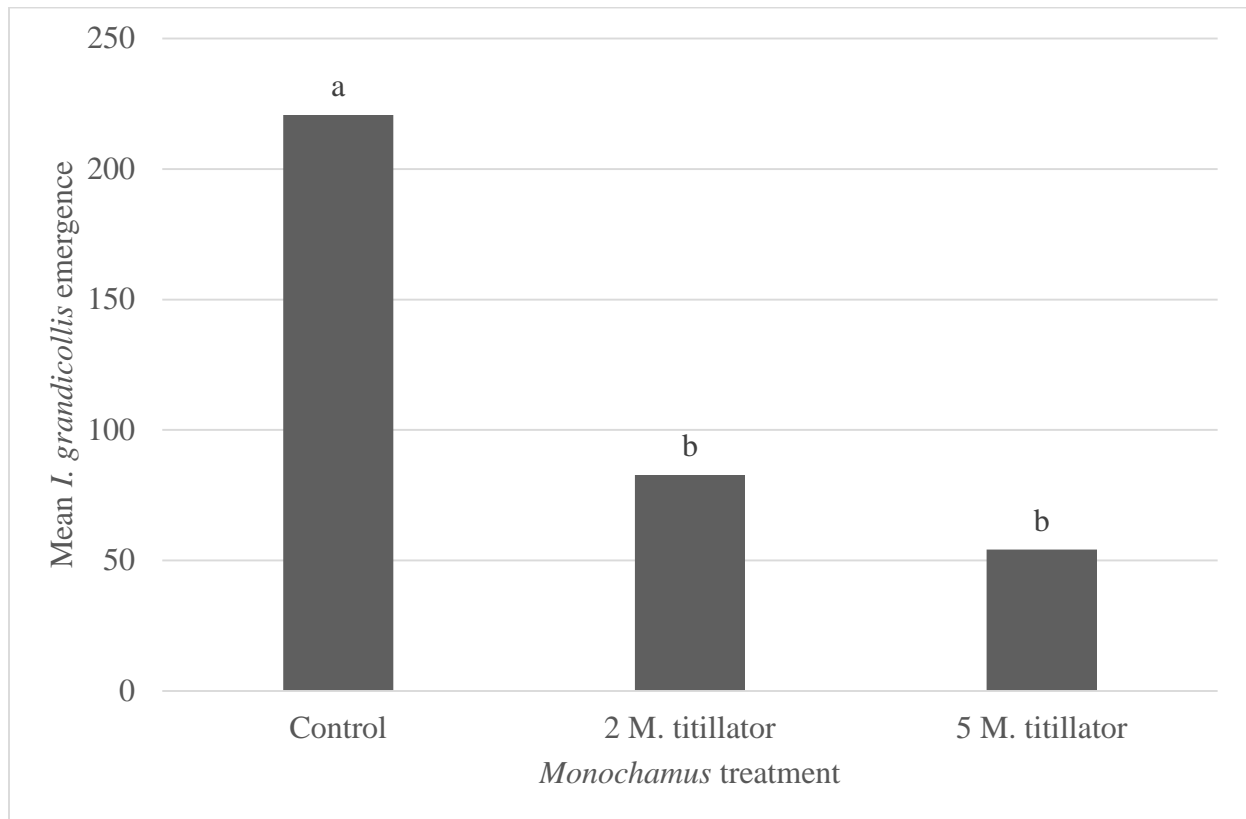
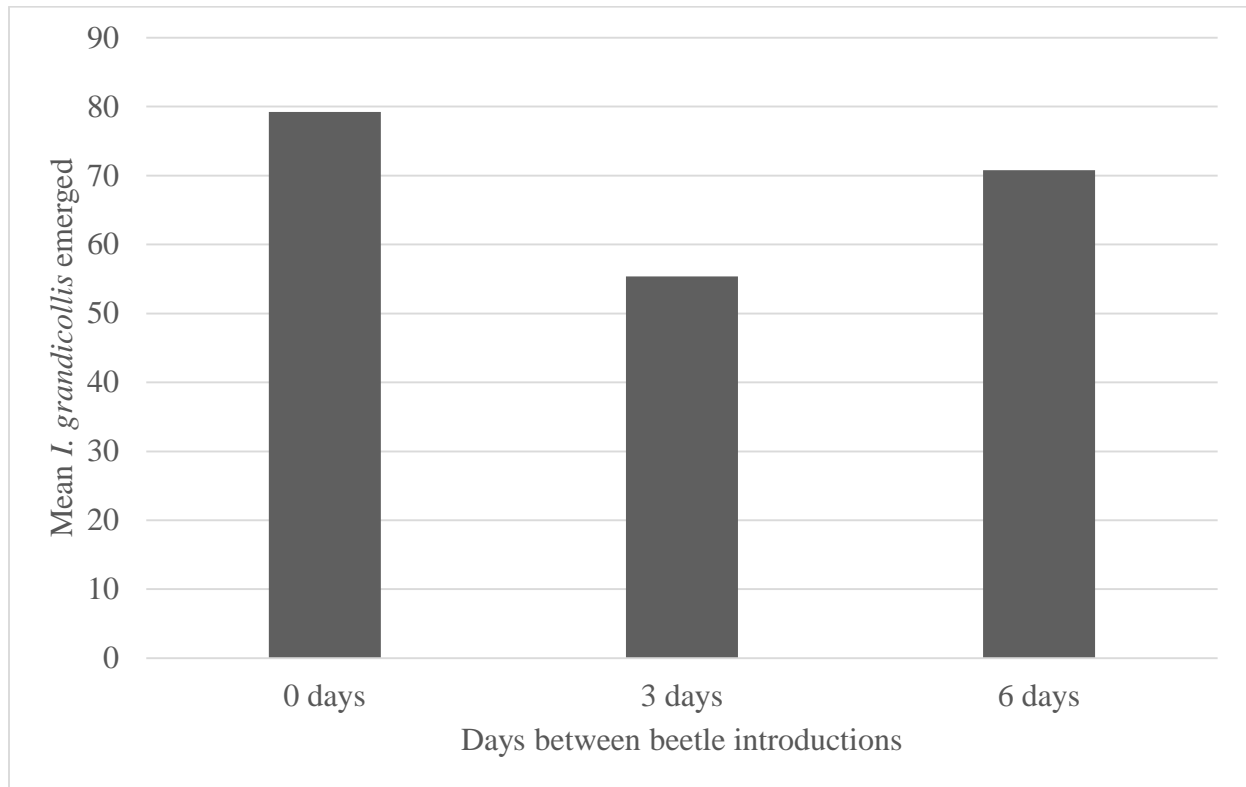


Figure 6: *I. grandicollis* emergence from bolts treated with *M. titillator* females at different introduction times. Columns represent mean *I. grandicollis* emergence for each day treatment (0, 3, or 6 days between *I. grandicollis* and *M. titillator* introduction). Bolts were treated between August, 2014 and July, 2015. No significant differences were found between day treatments at the 0.05 significance level ($\chi^2 = 2.3479$, $df = 2$, $p\text{-value} = 0.3091$).



Conclusion

Species of *Monochamus* interact with both host conifer trees and associated bark beetles. I tested several aspects of the interactions between southeastern *Monochamus* species and healthy hosts as well as an associated bark beetle species.

Historically *Monochamus* have been classified as saprophagic insects, but recent research has demonstrated the ability of *Monochamus* to be colonize hosts as primary insects. My results demonstrate that interactions between southeastern *Monochamus* and healthy shortleaf pine hosts can occur, but that oviposition in healthy trees does not lead to larval development. Larval development in healthy trees appears to be inhibited by resinosis. Strong attraction to the *Ips* beetle pheromone Ipsenol leads to hosts being shared by both *Monochamus* and *Ips* species, and may also contribute to facultative predation by *Monochamus* larvae.

Interactions between *Monochamus* and *Ips* beetles occur in shared host trees, but some of these interactions have not been fully explored, especially by designed experiments. My results demonstrate that sub-cortical interactions between *M. titillator* and *I. grandicollis* leads to diminished survival and emergence of *I. grandicollis*, although these interactions are not dependent on the difference of colonization time between the two species. Although colonization time differences did not lead to significantly different survival of *I. grandicollis* in my experiments this may change as the time difference increases beyond six days.

My results add to knowledge regarding the ecological interactions between *Monochamus* species and their hosts, as well as interactions with associated species. This additional knowledge expands our understanding of the ecological roles that *Monochamus* play within conifer forests of the Northern Hemisphere.