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Flight Activity, Oviposition Pit Distribution, and Emergence Densities of Monochamus titillator and M. carolinensis in the Ozark-St. Francis National Forest in Arkansas

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Flight Activity, Oviposition Pit Distribution, and Emergence Densities of *Monochamus titillator*
and *M. carolinensis* in the Ozark-St. Francis National Forest in Arkansas

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Abstract

Monochamus (Coleoptera: Cerambycidae) are a genus of longhorn beetles commonly known as pine sawyers. They have a worldwide distribution coincident with pines (*Pinaceae*) and are vectors of the pinewood nematode (*Bursaphelenchus xylophilus*). In the United States, there are eight known *Monochamus* species and they have a sympatric distribution with at least one other *Monochamus* species throughout their range. *Monochamus* are known to attack stressed, dead, and dying conifers especially pines.

In the Ozark- St. Francis National Forest in Arkansas, there are two species of pine sawyers and they share this sympatric distribution observed throughout the United States, *M. titillator* (southern pine sawyer) and *M. carolinensis* (Carolina sawyer). Both species seem to occupy the same ecological niche – similar seasonal flight patterns, life history, and host material.

The objectives of this study were to determine diurnal or nocturnal height flight patterns of *M. titillator* and *M. carolinensis*, compare heights at which *Monochamus* fly, and examine within tree distribution of oviposition pits and emergence densities of both species using suspended shortleaf pine (*Pinus echinata*) bolts.

From these objectives we were able to determine *Monochamus* were active at night (6 PM – 6 AM), and were more often captured at the base of canopy than at breast height. There were no differences between species by diurnal or nocturnal height flight patterns. During colonization of suspended pine bolts there was a higher density of oviposition pits in the base of canopy than at breast height. There were no differences in the density of emerging *Monochamus* by height at

which bolts were suspended. The majority of *Monochamus* emergence from suspended bolts was *M. titillator*.

These studies confirmed diurnal and height flight patterns, within tree distribution, and emergence densities of *Monochamus* species in the Ozark St. Francis National Forest in Arkansas. However, my studies failed to elicit ecological differences between *M. titillator* and *M. carolinensis*. Understanding the biology and ecology of *Monochamus* species may allow refinement of trapping methods, as well as better understanding of how *Monochamus* species interact among each other.

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Chapter 1 - Literature review

Monochamus

Monochamus Dejean (Coleoptera: Cerambycidae) longhorn beetles are commonly known as sawyers. The common name originates from the loud sounds produced as mandibles of *Monochamus* larvae chew sapwood (Baker 1972). There are 112 species of *Monochamus* worldwide with the majority located in the tropics (Roguet 2015).

North American *Monochamus*

In North America there are eight species of *Monochamus*: *M. carolinensis* (Olivier), *M. clamator* (LeConte), *M. marmorator* Kirby, *M. mutator* LeConte, *M. notatus* (Drury), *M. obtusus* Casey, *M. scutellatus* (Say), and *M. titillator* (F.) (Bezark 2016). North American *Monochamus* primarily are pine (*Pinus* spp.) feeders but some species colonize other hosts such as *Picea, Abies*, and *Pseudotsuga* (Craighead 1923; Lingafelter 2007). North American *Monochamus* are generally secondary insect pests, attacking and colonizing recently dead and stressed conifers (Webb 1909; Baker 1972). There are, however, a few reports of *Monochamus* as colonizers of distressed but still healthy trees. Dodds and Stephen (2000) observed fresh resin flowing from *Monochamus* oviposition pits on trees that were recently mass attacked by southern pine beetle (*Dendroctonus frontalis* Zimmermann). Gandhi and colleagues (2007) reported *Monochamus* species as primary colonizers in a severely wind-disturbed area in Superior National Forest in Minnesota. Ethington (2015) tested whether southeastern *Monochamus* attracted by host volatiles, kairomones, and pheromones to healthy shortleaf pines could successful colonize them. Oviposition by *Monochamus* was confirmed but most of the eggs were killed by resinosis, and

only 0.5 % of eggs laid hatched to larvae and created galleries. There was no successful development of *Monochamus* from egg stage to adult.

Southeastern *Monochamus*

In the southeastern United States, there are two common *Monochamus* species, the southern pine sawyer *M. titillator*, and the Carolina sawyer *M. carolinensis*. *Monochamus titillator* has a light reddish brown integument with mottled patches of white, yellow, and dark brown pubescence and ranges in size from 17-31 mm. In contrast, *M. carolinensis* has a darker reddish brown integument with less distinct mottled patches and is slightly smaller, 15-23 mm (Linsley and Chemsak 1984; Lingafelter 2007). Both species are very similar in appearance, and were considered the same species until 1941, when they were taxonomically differentiated by the shape of the elytral apex (Dillon and Dillon 1941). The elytral apex of *M. titillator* is produced into a strong, but sometimes blunt spine, whereas the apex of *M. carolinensis* elytra forms a weak projection (Lingafelter 2007). In 1985, structural differences were described in the male genitalia of *M. titillator* and *M. carolinensis* (Pershing and Linit 1985). The apex of the median orifice in *M. titillator* produce a bluntly rounded apex, whereas a pointed apex in *M. carolinensis*.

Monochamus titillator is distributed across the eastern United States to Texas and North Dakota, while *M. carolinensis* occurs throughout the eastern United States to Texas and Minnesota (Linsley and Chemsak 1984). Both species appear to be sympatric over most of their ranges (Linsley and Chemsak 1984; Pimentel et al. 2014).

Life history

The life histories of *M. titillator* and *M. carolinensis* in the Piedmont of North Carolina were described by Alya and Hain (1985). Both species appear to have the same seasonal flight pattern. *Monochamus titillator* is active from March through October and *M. carolinensis* from April through September (Lingafelter 2007). Alya and Hain (1985) report that adult *Monochamus* flight activity increased in June with a peak in mid-June, then a second smaller peak in early September. The second peak suggests emergence of a partial generation. There are one and a half to two generations per year of *Monochamus* in the southeast (Webb 1909; Pershing and Linit 1986a) and development time can vary. Some eggs that are oviposited in spring, develop to adults and emerge in fall of the same year, about a four month period, whereas 85% of the population has been reported to overwinter as larvae and emerged late in the following spring (Webb 1909; Alya and Hain 1985).

Freshly emerged *Monochamus* adults undergo obligatory maturation feeding, during which adults feed on tender bark of small shoots and branches for approximately one to two weeks (Craighead 1923; Walsh and Linit 1985; Pershing and Linit 1986b). After they become sexually mature, *Monochamus* search for suitable hosts. Pines are the primary hosts for *M. titillator*, with *Abies*, and *Picea* reported as alternative hosts. *Monochamus carolinensis* has a narrower host range, confined to the genus *Pinus* (Lingafelter 2007).

In cerambycid beetles, chemical communication is very important in host location and recognition of mates (Hanks 1999; Ginzel and Hanks 2005; Bezark 2016). *Monochamus* species are attracted to host material by pine volatiles and bark beetle kairomones (Billings and Cameron 1984; Allison et al. 2001; Groot and Nott 2004; Miller et al. 2011). *Monochamus* use short range pheromones and cuticular hydrocarbons to find mates on host material (Hanks 1999).

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Sexually mature female *Monochamus* use their mandibles to chew oviposition pits through the outer bark of hosts. Variability in oviposition pit size, and depth occurs based on bark thickness. In thicker bark, *Monochamus* chew a conical pit, but in thin bark, create a slit-like pit (Walsh and Linit 1985; Edwards and Linit 1991). Female *Monochamus* oviposit between the phloem and first layer of outer bark. One to about nine eggs, depending on the species, are then deposited (Webb 1909; Craighead 1923; Alya and Hain 1985). *Monochamus titillator* oviposit about three to nine eggs per pit radially around the oviposition pit, whereas *M. carolinensis* normally oviposit one (but as many as three) eggs adjacent to one another (Alya and Hain 1985; Walsh and Linit 1985). Not all oviposition pits contain eggs. Walsh and Linit (1985) reported only 86% of oviposition pits contained eggs or newly hatched larvae. Rastok (2015) also reported that on caged shortleaf pines 40% of *M. titillator* oviposition pits contained no eggs. This could be due to lack of free moisture in the bark (Yamasaki et al. 1989), or lack of chemicals such as D-catechin (Islam et al. 1997), for inducing oviposition response.

Monochamus eggs hatch in five to nine days (Webb 1909; Alya and Hain 1985). *Monochamus carolinensis* have three to six larval instars (Pershing and Linit 1989). The number of instars for *M. titillator* is unknown. Larvae feed in the phloem for one to two months, packing their frass between the phloem and outer bark, causing the bark to loosen. Eighteen to 32 days after hatching, larvae begin to mine into the sapwood to create a U-shaped gallery in the xylem in which to pupate. Larvae continue to forage in phloem, cambium, and outer sapwood during this period. *Monochamus* larvae are unable to digest cellulose on their own, and must acquire fungal enzymes through fungus-infected food (Kukor and Martin 1986). Emerging adult *Monochamus* extend the U-shaped chamber towards the bark surface of the host, and with their mandibles

create perfectly round exit holes (Webb 1909; Craighead 1923; Baker 1972; Alya and Hain 1985).

Competition

Both species of *Monochamus* in the southeast appear to occupy the same ecological niche (Webb 1909; Alya and Hain 1985; Walsh and Linit 1985). Host pines are suitable for *Monochamus* colonization only for a relatively short period of time. Felled pines, after seven days, were suitable for *Monochamus* colonization with peak colonization occurring after two weeks (Alya and Hain 1985).

During larval development in host trees, *Monochamus* compete with conspecific larvae, other *Monochamus* species, and members of the southern pine beetle guild for phloem. *Monochamus titillator* has been reported as a competitor of the southern pine beetle (Coulson et al. 1976; Coulson et al. 1980). Feeding larvae rapidly deplete pine phloem once the tree is colonized. With a short host suitability timeline and an ephemeral resource, available pine phloem is limited. For both species of *Monochamus* to coexist on that same ephemeral pine phloem resource, they must exhibit some form of separation from one another to lessen competition.

Intraspecific competition for a limited resource can lead to systems such as social hierarchies, or territoriality that reduce aggression between group members (Matthews and Matthews 2009). Intraspecific competition has been observed among *Monochamus* for mates (Hughes and Hughes 1982), oviposition sites (Webb 1909; Hughes and Hughes 1982), and phloem (Victorsson and Wikars 1996; Dodds et al. 2001). In all of these studies, the larger *Monochamus* were able to hold onto and guard mates, secure preferred oviposition sites, and within the phloem, kill and consume smaller larvae.

Through interspecific competition, extreme conflicts are expected (Matthews and Matthews 2009). These conflicts reflect the competitive exclusion principle: two species in the same location, which utilize the same resource, at the same time, cannot coexist (Gause et al. 1934). The loser will be driven to extinction or forced to modify its feeding behavior (DeBach 1966; Matthews and Matthews 2009; Price et al. 2011). However, in nature there are varying degrees of coexistence or displacement that depend on multiple factors (DeBach 1966; Denno et al. 1995). Interspecific competition between *Monochamus* and members of the southern pine bark beetle guild has been studied (Coulson et al. 1976; Coulson et al. 1980; Miller 1985; Dodds et al. 2001; Schoeller et al. 2012; Ethington 2015). In all studies *Monochamus* was the larger species and produced a negative effect on survival of the smaller competitors. Interspecific competition of *Monochamus* for oviposition sites was reported in *M. notatus* and *M. scutellatus* on white pine (*Pinus strobus* L.) logs (Hughes and Hughes 1987). *Monochamus notatus* was able to supplant *M. scutellatus* resource holders without fighting, by assessing the competitor with antennal contact and sometimes lashing of the antennae (Hughes and Hughes 1987). Since *M. notatus* has a low rate of oviposition compared to *M. scutellatus*, stealing another's newly created oviposition pit could increase ovipositions by *M. notatus* (Hughes and Hughes 1987).

Competition may be viewed as symmetric or asymmetric; however, asymmetric competition is more common in phytophagous insects (Kaplan and Denno 2007). In asymmetric competition, one species utilizes a disproportionally larger share of resources and has more of a negative effect on the other competitor. Asymmetric competition has been reported among *M. titillator* and *Dendroctonus frontalis* in loblolly pines (*P. taeda* L.) (Coulson et al. 1976; Coulson et al. 1980). *Monochamus titillator* is larger than *D. frontalis* and is reported to consume 20% of the available pine phloem. When both species occur in the same host, *M. titillator* significantly

reduces survival of *D. frontalis* through interspecific competition and facultative intraguild predation (Dodds et al. 2001).

Competition among *Monochamus* species for pine phloem can lead to coexistence through resource partitioning or niche divergence. Resource partitioning is the differentiation in the shared resource by temporal or spatial differentiation (Pfennig and Pfennig 2012). Niche divergence arises from competition or other factors that reduce the overlap of ecological niches of two species (Price et al. 2011). The degree of resource partitioning or niche divergence necessary to ensure coexistence in nature is still unknown (Price et al. 2011).

Pinewood nematode

The greatest worldwide economic impact of *Monochamus* is undoubtedly because of its ability to vector pinewood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) (Nematoda: Aphelenchoididae), a non-obligate plant parasitic nematode that is the cause of pine wilt disease (PWD) (Mamiya 1983; Akbulut and Stamps 2012). At least six of the eight North American *Monochamus* vector pinewood nematode: *M. carolinensis*, *M. marmorator*, *M. mutator*, *M. obtusus*, *M. scutellatus*, and *M. titillator* (Wingfield and Blanchette 1983; Linit 1988).

Bursaphelenchus xylophilus is indigenous to North America and has been reported from 20 species of pines and seven other conifers (Robbins 1982). Pines native to North America appear to be resistant to death from PWD (Robbins 1982; Linit and Tamura 1987). Pines not native to North America have shown high susceptibility to death from PWD (Robbins 1982; Mamiya 1983). In 1981, pine forests infested by *B. xylophilus* reached 2.6 million ha (25% of Japan's pine forest area), and PWD caused death of nearly 10 million trees (Mamiya 1983).

Pine wilt disease is caused by the blocking of xylem channels in susceptible trees by large numbers of *B. xylophilus*, cutting off water transport throughout the tree (Mamiya 1983). Symptoms of PWD, initially represented in the crown, occur in four distinct stages of pine needles fading in color, or in branch flagging. The four stages are pine needles fading from green to: A: light grayish green; B: yellowish green; C: yellowish brown; D: light chocolate to rusty brown. During hot weather, all four stages were observed over a four-month period. Up to a year after first appearance of PWD, adult and propagative *B. xylophilus* were recovered from host material (Malek and Appleby 1984).

Bursaphelenchus xylophilus dauer juveniles are transmitted to new hosts primarily by *Monochamus* vectors (Mamiya and Enda 1972; Akbulut and Stamps 2012). Transmission to new hosts is initiated when lipid reserves inside *Monochamus* are low, and *B. xylophilus* are strongly attracted to pine host volatiles (Stamps and Linit 1998). During primary transmission, *Monochamus* create open wounds in host material through maturation feeding, upon which *B. xylophilus* enters pine host. Secondary transmission occurs when *B. xylophilus* enters hosts through *Monochamus* oviposition pits (Linit 1988).

Inside host trees *B. xylophilus* are present in the sapwood surrounding *Monochamus* larval galleries. *Bursaphelenchus xylophilus* feed on the cells lining resin canals, and on fungi that colonize recently dead, or dying pines, and flourish on fungi around insect pupal chambers (Futai 2013). As the subcortical environment of the host degrades, there is a switch in nematode development from the reproductive to dispersal phase. In the dispersal phase, *B. xylophilus* aggregate around *Monochamus* pupal chambers. When *Monochamus* pupae eclose and remain in pupal chambers as callow adults, dauer juveniles of *B. xylophilus* enter the vectors' bodies through the spiracles and proceed to the trachea (Kondo 1986; Necibi and Linit 1998; Futai

2013). Prior to *Monochamus* emergence the highest numbers of *B. xylophilus* are found in the trachea and under elytra (Mamiya and Enda 1972). Humphry and Linit (1989) determined that high density of *B. xylophilus* had a negative effect on *M. carolinensis* flight duration.

Research objectives

The primary goal of this study is to examine selected competitive interactions between *M. titillator* and *M. carolinensis* in shortleaf pine (*Pinus echinata*) in the Ozark- St. Francis National Forest in Arkansas. My first objective is to determine hourly, and seasonal flight activity of both species by active trapping of adults arriving at shortleaf pines. My second objective is to determine, by active trapping, whether the two species exhibit spatial differences on standing shortleaf pines. My last objective is to examine interspecific competition for oviposition sites by examining within tree distribution of oviposition pits, and emergence densities of both species. I chose to focus on these objectives because they are factors that should respond to competition, and show possible differences between the two species. I hypothesized that due to competition over a highly desired but ephemeral resource, pine phloem, the two sympatric species of *Monochamus* will exhibit differences in flight activity, colonization patterns, or emergence densities.

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Chapter 2 - Flight activity of *Monochamus titillator* and *M. carolinensis*

Introduction

The genus *Monochamus* Dejean (Coleoptera, Cerambycidae) is comprised of 112 species of worldwide distribution with eight recognized species in North America (Roguet 2015; Bezark 2016). North American *Monochamus* are typically secondary tree pests colonizing weakened and recently dead trees primarily in the genus *Pinus* although *Abies, Picea,* and *Pseudotsuga* are also attacked by some species (Webb 1909; Craighead 1923; Baker 1972; Furniss and Carolin 1977; Linsley and Chemsak 1984).

It is common to find two species of *Monochamus* co-occurring in North America where the genus is present (Pimentel et al. 2014). For instance, the southern pine sawyer (*M. titillator* (F.)), and the Carolina sawyer (*M. carolinensis* (Olivier)) co-occur in the Ozark – St. Francis National Forest of Arkansas. *Monochamus titillator* and *M. carolinensis* are sympatric over most of their range (Linsley and Chemsak 1984). They have similar host preferences and larvae feed on phloem of weakened and dead pines (Webb 1909; Craighead 1923; Furniss and Carolin 1977; Linsley and Chemsak 1984; Lingafelter 2007). They are reported to have up to two generations per year with overlapping brood (Webb 1909; Pershing and Linit 1986; Dodds and Stephen 2000). They also have the same seasonal flight pattern from April through October (Linsley and Chemsak 1984; Alya and Hain 1985).

Felled pines were determined to be susceptible hosts after seven days, with peak colonization occurring after two weeks (Alya and Hain 1985), suggesting that pine phloem, the common resource for *Monochamus* is available for a relatively short period of time. Pine phloem is rapidly depleted by feeding larvae and fungal colonization. For both species of *Monochamus* to

co-exist on this limited resource, they likely exhibit some form of separation from one another in regards to resource partitioning (Pfenning and Pfenning 2012) or niche divergence (Price et al. 2011). This may occur through competitive displacement (DeBach 1966; Denno et al. 1995; Price et al. 2011) and asymmetric competition (Price et al. 2011).

Understanding how *Monochamus* species interact and compete for resources is valuable in understanding the biology and ecology of *Monochamus*. This study was designed to examine specific life history traits and possible interactions of *M. titillator* and *M. carolinensis* in the Ozark – St. Francis National Forest. The first objective was to determine hourly, and seasonal flight activity of both species by active trapping of adults arriving at shortleaf pine (*Pinus echinata* Mill.). The second objective was to determine by active trapping, whether the two species exhibit height differences in arrival on standing shortleaf pines.

Materials and methods

Research site

The research site was an approximately 32 ha pine stand within the Wedington Management area of the Ozark-St. Francis National Forest, located 32 km west of Fayetteville (Washington Co.), Arkansas. Transects were created to allow random sampling at fixed points over time in the same habitat. Traps were initially suspended from pine trees along a 290 m and a 335 m transect, running roughly north-south and parallel to each other. The two parallel transects ranged in distance from 63-133 m from each other and were separated by a 7-16 m clearing used as a logging skid trail during thinning operations in September-October 2014. The 335 m transect was shortened to 277 m after 20 July 2015 by eliminating the northernmost sample tree when the experimental design was altered as described below. Overall composition of the stand was

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mostly shortleaf pine with small amounts of *Quercus alba* L., *Q. stellata* Wang., *Q. rubra* L., *Q. velutina* Lam., *Prunus nigra* Ait., *Juniperus virginiana* L., and *Carya tomentosa* Nutt. The average pine basal area was 27.2 m^2/ha and average basal area of non-pine species was 3.4 m^2/ha.

Tree preparation

Selected trees were similar in diameter at breast height, total height, and overall health. Tree health was characterized as green canopy and no visible signs of stress or damage such as missing bark or presence of resin exudate on the bole. Breast height diameter ranged from 29 – 37 cm and trees were located 48 – 92 m apart. Dead limbs were removed up to the base of live crown to reduce variation among trees and to allow access for climbing equipment and the raising and lowering of traps. To attach hardware trap supports, trees were climbed using either a climbing deer stand (Warren & Sweat Manufacturing Company, Grand Island, FL, USA) or Wibe[®] sectional climbing ladders (Hultafors Group, Sweden). Trap supports consisted of angle brackets constructed from 2.54 cm x 0.3175 cm (1 in x 1/8 in) angle iron. Braided nylon 0.48 cm (3/16 in) diameter rope was used to raise and lower the trap by utilizing a 0.48 cm (3/16 in) threaded removable chain-link attached to the distal end of the bracket as a simple pulley. Hardware was mounted on the northeast aspect of the tree bole, but offset slightly so that traps would not interfere with one another while being raised or lowered. Two 5.08 cm x 0.79 cm (2 in x 5/16 in) lag bolts secured the brackets to the supporting trees (Figure 1).

Monochamus trapping

Active trapping for *M. titillator* and *M. carolinensis* adults was initiated 25 May 2015. Initially eighteen black panel traps (APTIV[TM]) were suspended on 12 shortleaf pines using one of four treatment designs (A: one trap at breast height; B: one trap at mid-bole; C: one trap at base of

canopy; and D: one trap each at breast height, mid-bole, and base of canopy) (Table 1). Traps at breast height were 1.5 m from the ground, mid-bole 5.3 m, and base of canopy ranged from 10.7 – 12.6 m. Individual trees were located 48 – 92 m apart. After 20 July 2015, traps were redistributed to nine trees, with one trap located at breast height and one at base of canopy on each tree, for a total of 18 traps (Table 1). Traps were redistributed to insure a more powerful statistical design to distinguish differences in *Monochamus* species captured at each trap tree. Breast height and base of canopy traps were placed at the same heights described above. Traps were baited with commercially available *Monochamus* attractants and eluted at the following rates: (Monochamol 750 μg/day at 20°C), host volatiles (α-pinene 150 mg/day at 20°C, and ethanol 20 mg/day at 20°C), and *Ips* bark beetle pheromones (ipsenol 800 μg/day at 20°C) (Synergy Semiochemicals, Burnaby, BC, Canada). Trap collection cups contained propylene glycol (Splash Products Incorporated, St. Paul, MN, USA). Lures were replaced every four to six weeks depending on temperature.

Trap contents were collected under two distinct regimes: (weekly) with traps collected at seven to nine day intervals; or (intensive) with traps collected at time intervals during three 24-hour periods throughout a week. Intensive time intervals consisted of trap collection starting at 6:00 AM and subsequently at 9:00 AM, 12:00 PM, 3:00 PM, 6:00 PM, 9:00 PM, 12:00 AM, and 6:00 AM (Table 2). Time of trap exposure for the initial sample at 6:00 AM ranged from 12 hours, to one week, since last trap collection. It therefore was not included in the results of intensive sampling. Intensive sampling was conducted on a Monday, Wednesday, and Friday schedule of the same week. Initial intensive sampling efforts revealed that *Monochamus* flight primarily occurred from sunset to sunrise. After 20 July 2015, intensive trapping was altered to most efficiently correspond with *Monochamus* activity. Intensive samples were collected at three-hour

time intervals starting at 6:00 PM with subsequent collections at 9:00 PM, 12:00 AM, 3:00 AM, and 6:00 AM (Table 2). Initial trap catches at 6:00 PM were not included in the results of overnight sampling because the trap exposure ranged from one week to 12 hours since last collection. Trap contents were collected and placed into 1.2 L plain Whirl-Pak bags (Whirl-Pak®, Nasco, USA) with labels containing date, time, and trap identification. Whirl-Pak bags were returned to the laboratory and stored at 3.8°C until specimen identification. *Monochamus* were identified to species by the curvature at the apex of the elytra and color and pattern of pubescence on integument as described in the dichotomous key by Lingafelter (2007). There were varying degrees of elytra curvature in the *Monochamus* captured with individuals having one elytra resembling *M. titillator* and the other elytra characteristic of *M. carolinensis*. In those instances the color and pattern of pubescence on the integument resembled *M. titillator* and were marked as *M. titillator*. After identification, specimens were stored at -12°C in clear .06 L Whirl-Pak bags filled with 70% ethanol.

Statistical analyses

All of the data were analyzed in SAS® version 9.4. The intensive traps – 24 hour and 12 hour data sets were analyzed with a Proc GLIMMIX analyzing the counts of *Monochamus* as a Poisson distribution with each tree as a random effect to examine differences among *Monochamus* captured by time period and trap height. The weekly data were analyzed with a Proc GLIMMIX analyzing the counts of *Monochamus* as a Poisson distribution with tree as a random effect to determine differences among *Monochamus* captured by date throughout the season. The weekly data were also analyzed with a Proc GLIMMIX analyzing the counts of *Monochamus* captured as a Poisson distribution with tree and date as a random effect to examine differences among *Monochamus* captured by trap height throughout the season. The intensive

traps – 24 hour and 12 hour data, and weekly data sets were analyzed with a Proc GLIMMIX analyzing the proportion of *Monochamus* species and sex separately as a binomial distribution with each tree as a random effect to examine differences among *Monochamus* species and sex respectively. When appropriate Turkey's HSD was used for mean separation. For all figures proportions of *Monochamus* captured were calculated from the statistical means. The proportions of *Monochamus* captured in the tables were calculated from the raw data.

Results

TRAPPING

The most common insects caught in this study were *M. titillator*, and *M. carolinensis*. Other insect associates found in all traps were *Acanthocinus* sp. (Cerambycidae), *Thanasiumus dubius* (Cleridae), multiple species of Buprestidae, and numerous bark and ambrosia beetles.

Intensive traps – 24 hours

A total of 610 *Monochamus* were captured over six sampling dates. Peak *Monochamus* flight activity occurred in the three time intervals from 6 PM – 6 AM and encompassed 97% of all *Monochamus* captured (Figure 2). Within the 6 PM – 6 AM time period, *Monochamus* flight activity significantly increased from 6 PM until 12 AM then decreased for the rest of the time periods ($F = 66.07$, $p = 0.0001$) (Figure 2). Trap height had an effect on proportion of *Monochamus* captured with more *Monochamus* captured at breast height and base of canopy than at the mid-bole ($F = 18.9$, $p = 0.0092$) (Figure 3). There was no significant difference in the proportion of *M. carolinensis* to *M. titillator* captured based on trap height with the average proportion of 28% *M. carolinensis* to 72% *M. titillator* ($F = 0.98$, $p = 0.36$) (Table 3). There was also no significant difference in proportion of female to male *Monochamus* captured by trap height with the average proportion of 57% female to 43% male ($F = 1.25$, $p = 0.31$) (Table 3).

Breast height: In the 24-hour study a total of 217 *M. titillator* and 73 *M. carolinensis* were captured at breast height accounting for 46% of total trap catches (Table 3, Figure 3). At breast height *M. titillator* accounted for 75% of the total trap catches where as 25% for *M. carolinensis* (Table 3). Of the total *Monochamus* captured at breast height 57% were male and 43% female (Table 3).

Mid-bole: At mid-bole there were 93 *M. titillator* and 45 *M. carolinensis* captured which accounts for 17% of total trap catches (Figure 3). Of which 67% were *M. titillator* and 33% *M. carolinensis* (Table 3). For *Monochamus* captured at mid-bole 62% were female and 38% were male (Table 3).

Base of canopy: There were a total of 129 *M. titillator* and 53 *M. carolinensis* captured at the base of canopy of which accounted for 37% of total trap catches (Figure 3). For *Monochamus* captured at the base of canopy 71% were *M. titillator* and 29% *M. carolinensis*. Of which 53% were female and 47% male.

Intensive traps – 12 hours

A total of 713 *Monochamus* were captured over nine sampling dates (Table 3). *Monochamus* flight activity increased significantly from 6 PM to 12 AM then decreased significantly for the rest of the time periods ($F = 76.56$, $p = 0.0001$) (Figure 4). There were significantly more *Monochamus* captured at traps in the base of canopy than at breast height ($F = 11.55$, $p = 0.0094$) (Figure 5). There were no significant differences in the proportion of *M. carolinensis* to *M. titillator* captured by trap height ($F = 1.26$, $p = 0.27$). There were also no significant differences in proportion of female to male *Monochamus* captured due to trap height ($F = 1.98$, $p = 0.17$).

Breast height: In the 12-hour study a total of 214 *M. titillator* and 97 *M. carolinensis* were captured at breast height accounting for 44% of total trap catches (Figure 5, Table 4). Of the total trap catches at breast height for the 12 hour study 70% were *M. titillator* and 30% *M. carolinensis* (Table 4), of which 55% were female and 45% male.

Base of canopy: At the base of canopy a total of 292 *M. titillator* and 110 *M. carolinensis* were captured, accounting for 56% of total trap catches (Figure 5). From the total trap catches at the base of canopy for the 12-hour study 73% were *M. titillator* and 27% were *M. carolinensis* (Table 4). Of which 49% were female and 51% male (Table 4).

<u>Weekly traps</u>

A total of 5731 *Monochamus* were captured over 15 sampling dates. Trap contents consisted of 4445 *M. titillator* and 1286 *M. carolinensis*. The number of captured *Monochamus* throughout the season varied week to week but proportionally higher numbers were captured from late July through late September (F = 141.75, p = 0.0001) (Figure 6). September accounted for the highest proportion of *Monochamus* captured throughout the study, accounting for 46% of total catches (Figure 6). Ninety two percent of all *Monochamus* captured in this study were collected by the end of September (Figure 6). Throughout the season significantly more *Monochamus* were captured in traps at the base of canopy than at breast height from late July through August and then from mid to late September (F = 9.81, p = 0.0001) (Figure 7). From early to mid-September then October through November, there were no significant differences between proportions of *Monochamus* captured by trap height (Figure 7). The proportion of *M. carolinensis* to *M. titillator* remained relatively the same throughout the season with more *M. titillator* than *M. carolinensis* except on 19 September 2015 and 2 November 2015, where the proportion of the

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two species captured were almost equal (F = 14.28, p = 0.0001) (Figure 8). There were no differences in the proportion of female to male *Monochamus* captured each date throughout the season (F = 1.50, p = 0.11).

Breast height: The weekly study recovered a total of 1862 *M. titillator* and 637 *M. carolinensis* at breast height accounting for 44% of total trap catches (Table 4). Of the total trap catches at breast height for the weekly study, 75% were *M. titillator* and 25% *M. carolinensis*, of which 55% were female and 45% male.

Base of canopy: There were a total of 2583 *M. titillator* and 649 *M. carolinensis* recovered at the base of canopy accounting for 56% of total trap catches (Table 4). From the total trap catches of *Monochamus* at the base of canopy for the weekly study 80% were *M. titillator* and 20% *M. carolinensis*, of which 50% were female and 50% male.

Discussion & conclusions

Overall, more *M. titillator* (4445) were captured than *M. carolinensis* (1286). This is opposite of what was reported in the piedmont of Carolina (Alya and Hain 1985) or the northeastern United States (Dodds 2014). Our research locations in Arkansas are about 1600 km west of these studies and host tree species are different.

During intensive sampling, we found that both *Monochamus* species are most active at night with primary activity from 9 PM – 12 AM (Figures 2, 4). There were no significant differences between proportion of *Monochamus* captured by traps at breast height and base of canopy during the intensive 24 hour period (Figure 3). However, there were proportionally more *Monochamus* captured in the base of canopy than at breast height in the 12-hour sampling (Figure 5). This

suggests that in the beginning of the season, May through June, *Monochamus* do not appear to favor breast height or base of canopy. But later in the season, July through September, *Monochamus* tend to favor positions higher in the bole of trees. The base of canopy may be more valued for *Monochamus* activity due to more access to mates or closer approximation to new twigs and shoots in the canopy upon which adults feed.

Both 24 hour and 12 hour intensive sampling revealed no differences between the proportions of *M. carolinensis* to *M. titillator* captured by trap height. However, overall, *M. titillator* was captured in higher numbers (Table 4). During the weekly sampling the proportion of *M. carolinensis* to *M. titillator* was relatively similar throughout the season except on two weeks, 19 September 2015 and 2 November 2015, when the species were almost captured at the same proportion (Figure 8). Both species of *Monochamus* were active at about the same proportion each date as well as by trap height.

There were no differences in the proportion of female to male *Monochamus* captured by trap height for the 24 hour and 12 hour intensive sampling. There were also no differences in proportion of female to male *Monochamus* captured by date throughout the weekly sampling. This shows that both sexes of *Monochamus* were active at the same proportions by date as well as by trap height.

The seasonal activity of *Monochamus* varied week to week but proportionally more (92%) were recovered between July through September compared to October through November (Figure 6). There were large peaks in *Monochamus* activity in July, and early September, which may be indicative of partial generation emergence (Figure 6). Alya and Hain (1985) also reported

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evidence for partial generation emergence of *Monochamus* in September. Activity of *Monochamus* then declined from mid-September through November.

Monochamus titillator and *M. carolinensis* were captured in the same proportion by time periods, and by trap height on the same resource, suggesting *Monochamus* are not partitioning resources by time or place of arrival on host material. Coexistence of both species could be due to asymmetric competition with other phloem inhabiting competitors. In Arkansas, *Monochamus* are competing with conspecific larvae, other *Monochamus* species, and three species of *Ips* bark beetles for host material. *Ips* species attack and colonize the same host material through chemically mediated behaviors and have slightly different arrival patterns from each other (Nebeker 2011). These factors have helped *Ips* fully utilize their shared resource, by temporal and spatial resource partitioning. *Monochamus* are attracted through the release of host volatiles, and kairomonal response to bark beetle pheromones to the same host material as the *Ips* speceis (Billings and Cameron 1984; Allison et al. 2001; Miller et al. 2011).

Monochamus are drastically larger than *Ips* species and are also known to suppress competition for phloem through intraguild predation (Coulson et al. 1980; Dodds and Stephen 2000; Graber 2000; Stephen 2011; Schoeller et al. 2012). *Monochamus* larvae express behaviors to avoid intraspecific predation, or to secure resources such as maintaining and building walls in their galleries, and by sound production (Graber 2000). These walls are made with frass and may help in isolating sounds and keeping competitors out of their galleries. Sound production by *Monochamus* larval feeding may aide in communication between larvae. These behaviors could help in resource partitioning among *Monochamus* species. The advantage of *Monochamus* being

larger, and preying upon competitors, could outweigh the negative interspecific competition that could result in injury or death among *Monochamus* species.

Results from this study suggest that the best times for monitoring *Monochamus* adult activities in Arkansas are at night from 9 PM to 12 AM. Ideal trap placement to monitor for *Monochamus* species in Arkansas is in the base of the canopy. Trap placement is similar to what was recommended by Dodds (2014) in the northeastern United States.

Tables and figures

Table 1: Study design placement of black panel traps on shortleaf pines.

	BH	MB	C	BH, MB, and C	Total
25 May 2015					
Traps	3	3	3	9	18
Trees	3	3	3	3	12
25 July 2015					
Traps	9	0	9	0	18
Trees	9	0	9	0	9

BH = breast height, MB = mid-bole, C = base of canopy

Table 2: Sample dates of checked traps

Date 2015	Intensive	Weekly		Date 2015	Intensive	Weekly
25-May	+			13-Aug	+	
27-May	+			15-Aug	+	
29-May	+			24-Aug		+
8-Jun		+		1-Sep		+
15-Jun		+		8-Sep		+
22-Jun	+	+		14-Sep		+
24-Jun	+			15-Sep	+	
26-Jun	+			17-Sep	+	
6-Jul		+		19-Sep	+	
13-Jul		+		28-Sep		+
20-Jul		+		5-Oct		+
27-Jul	+	+		12-Oct		+
29-Jul	+			19-Oct		+
1-Aug	+			26-Oct		+
10-Aug		+		2-Nov		+
11-Aug	+					

25 May – 26 Jun: Intensive sampling – contents first collected 6:00 AM and subsequently at 9:00 AM, 12:00 PM, 3:00 PM, 6:00 PM, 9:00 PM, 12:00 AM, and 6:00 AM.

27 July – 19 Sep: Intensive sampling - trap contents first collected at 6 PM and subsequently at 9:00 PM, 12:00 AM, 3:00 AM, and 6:00 AM.

Table 3: Total number and proportion of *Monochamus* captured by treatment per trap height for intensive 24 hour sampling.

Treatment	Trap	Number	Prop
Species			
M.t.	Bh	217	0.75
M.c.		73	0.25
M.t.	Mb	93	0.67
M.c.		45	0.33
M.t.	C	129	0.71
M.c.		53	0.29
Sex			
♀	Bh	165	0.57
♂		125	0.43
♀	Mb	86	0.62
♂		52	0.38
♀	C	97	0.53
♂		85	0.47

Treatment: *M.t. = Monochamus titillator*. *M.c.= M. carolinensis*. Sex: ♀ = female. ♂ = male. Trap = trap height: Bh = breast height, Mb = mid-bole, C = base of canopy. Number = total number of *Monochamus* captured per trap. Prop = proportion of total *Monochamus* by treatment.

Table 4: Total number and proportion of *Monochamus* captured by trap height per treatment for intensive – 12 hour, 24 hour, and weekly sampling.

Treatment	Sample	Trap	Number	Prop
Species				
M.t.	12 hr	Bh	214	0.70
M.c.			97	0.30
M.t.	24 hr	Bh	217	0.75
M.c.			73	0.25
M.t.	Weekly	Bh	1862	0.75
M.c.			637	0.25
M.t.	12 hr	C	292	0.73
M.c.			110	0.27
M.t.	24 hr	C	129	0.71
M.c.			53	0.29
M.t.	Weekly	C	2583	0.80
M.c.			649	0.20
Sex				
♀	12 hr	Bh	170	0.55
♂			141	0.45
♀	24 hr	Bh	165	0.57
♂			125	0.43
♀	Weekly	Bh	1410	0.56
♂			1089	0.44
♀	12 hr	C	197	0.49
♂			205	0.51
♀	24 hr	C	97	0.53
♂			85	0.47
♀	Weekly	C	1615	0.50
♂			1617	0.50

Treatment: *M.t. = Monochamus titillator*. *M.c.= M. carolinensis*. Sex: ♀ = female. ♂ = male. Trap: Bh = breast height. Mb = mid-bole. C = base of canopy. Sample: 12 hr = 12 hour intensive sampling. 24 hr = 24 hour intensive sampling. Weekly = weekly sampling. Trap = trap height: Bh = breast height, C = base of canopy. Number = total number of *Monochamus* captured per trap. Prop = proportion of total *Monochamus* by treatment.

Figure 1: Black panel trap suspended from a tree by an angle bracket

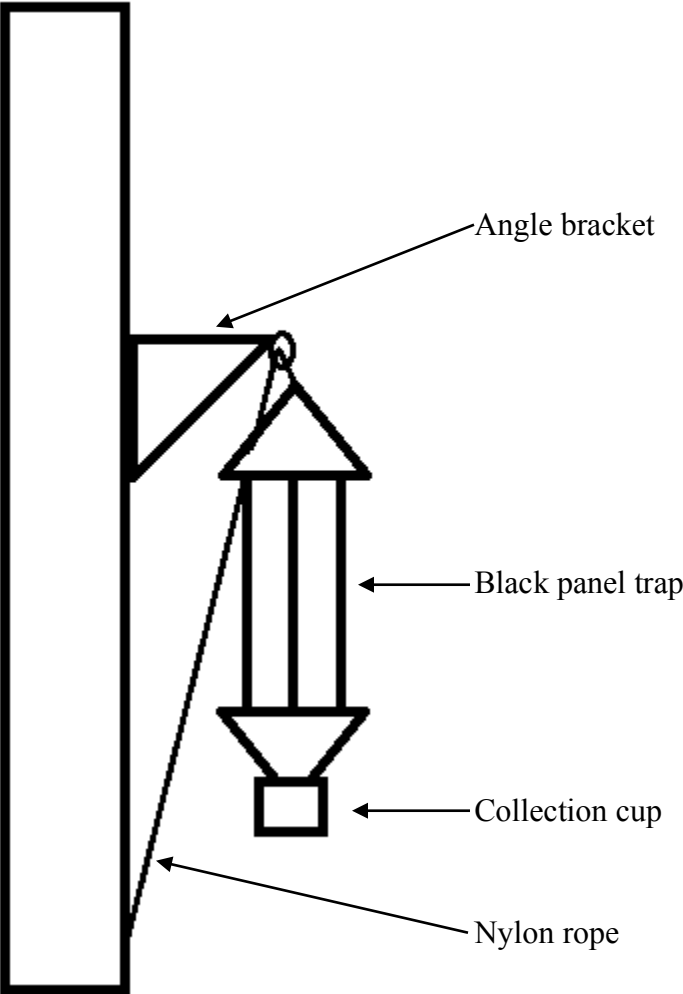Angle bracket

Black panel trap

Collection cup

Nylon rope

Figure 2: Proportion of flight activity of both *Monochamus* species combined over 24-hour intensive sampling periods. A total of 610 *Monochamus* were captured over six sampling dates from 5/25/15 through 6/26/15. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparisons at α = 0.05.
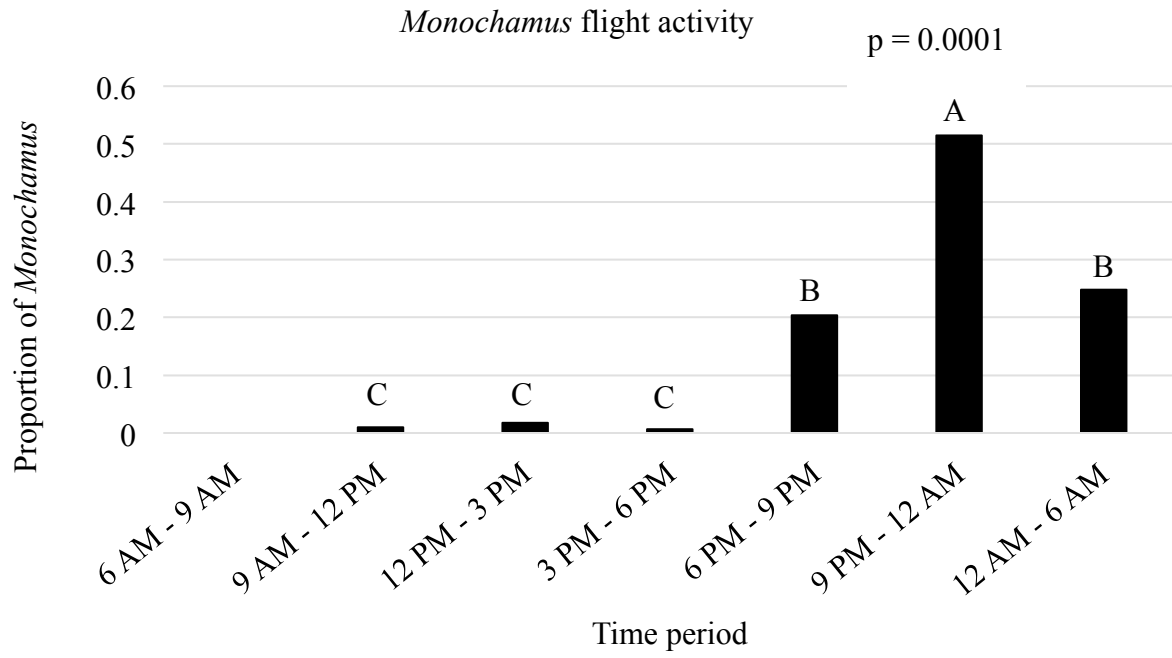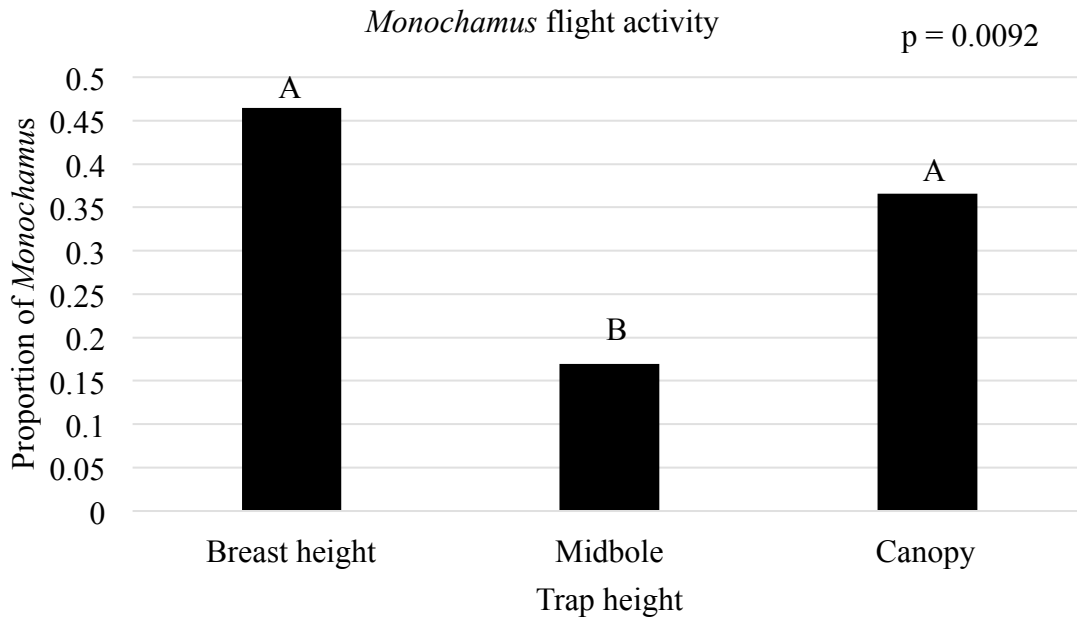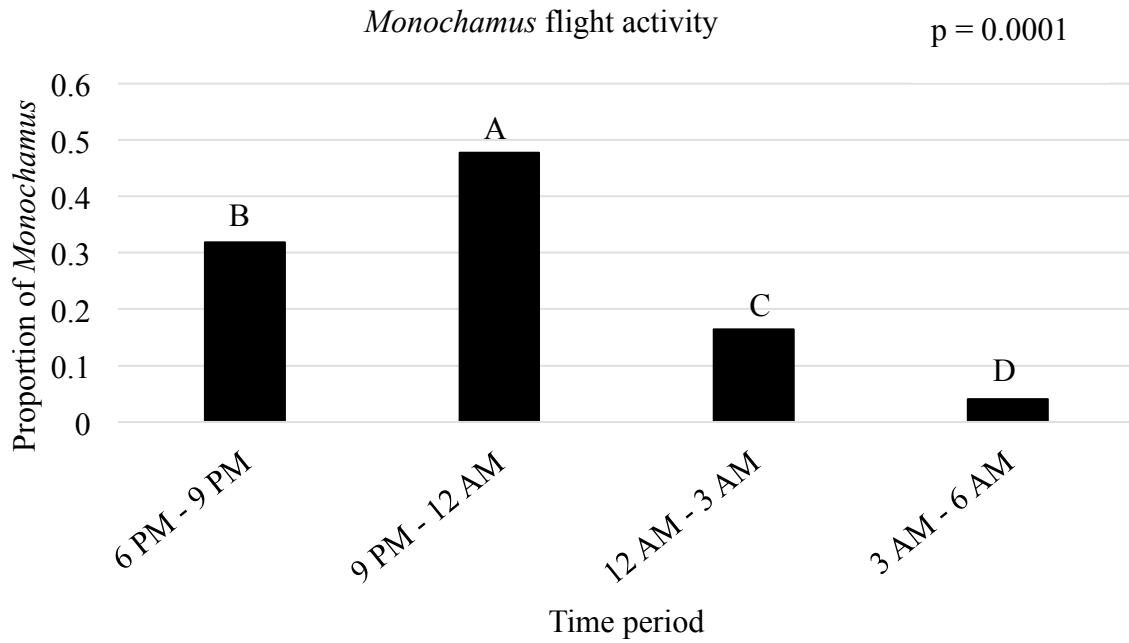
Figure 3: Proportion of flight activity of *Monochamus* species by trap height over 24-hour intensive sampling periods. A total of 610 *Monochamus* were captured over six sampling dates from 5/25/15 through 6/26/15. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparisons at $\alpha = 0.05$.

Figure 4: Proportion of flight activity of *Monochamus* species combined over 12-hour intensive sampling periods. A total of 713 *Monochamus* were captured over nine sampling dates from 7/27/15 through 9/18/15. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison at $\alpha = 0.05$.

Figure 5: Proportion of flight activity of *Monochamus* species by trap height over 12-hour intensive sampling periods. A total of 713 *Monochamus* were captured over nine sampling dates from 7/27/15 through 9/18/15. Different letters above columns denote means that are significantly different according to Tukey's HSD means comparison at α = 0.05.
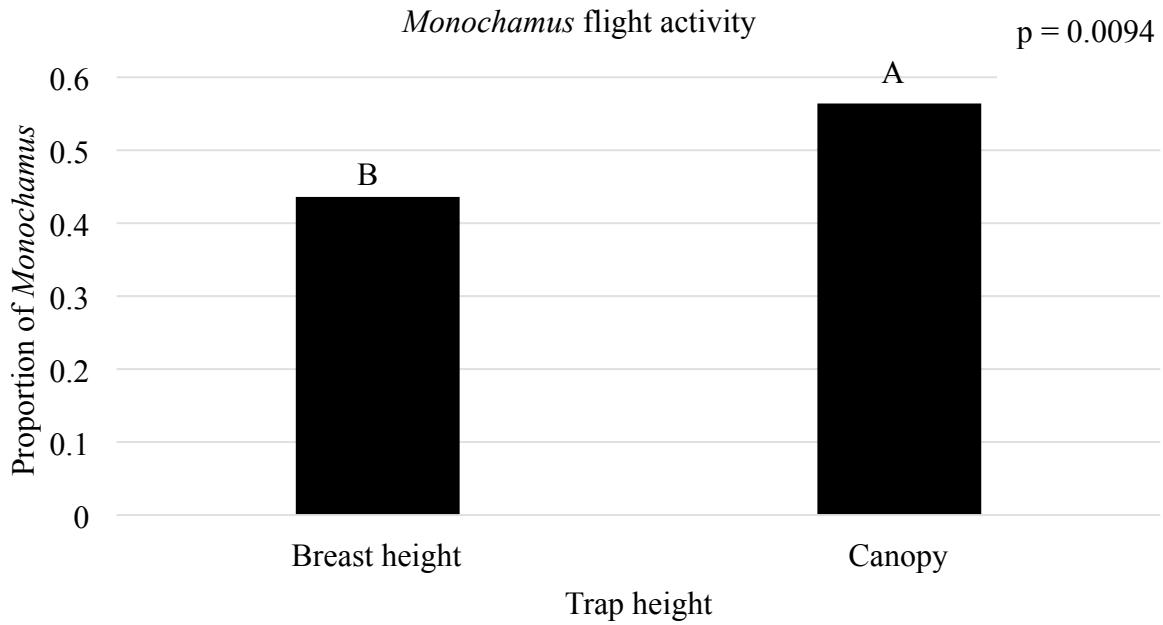
Figure 6: Proportion of seasonal flight activity of *Monochamus* species combined. A total of 5731 *Monochamus* were captured over fifteen sampling dates from 7/27/15 through 11/02/15. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison at $\alpha = 0.05$.
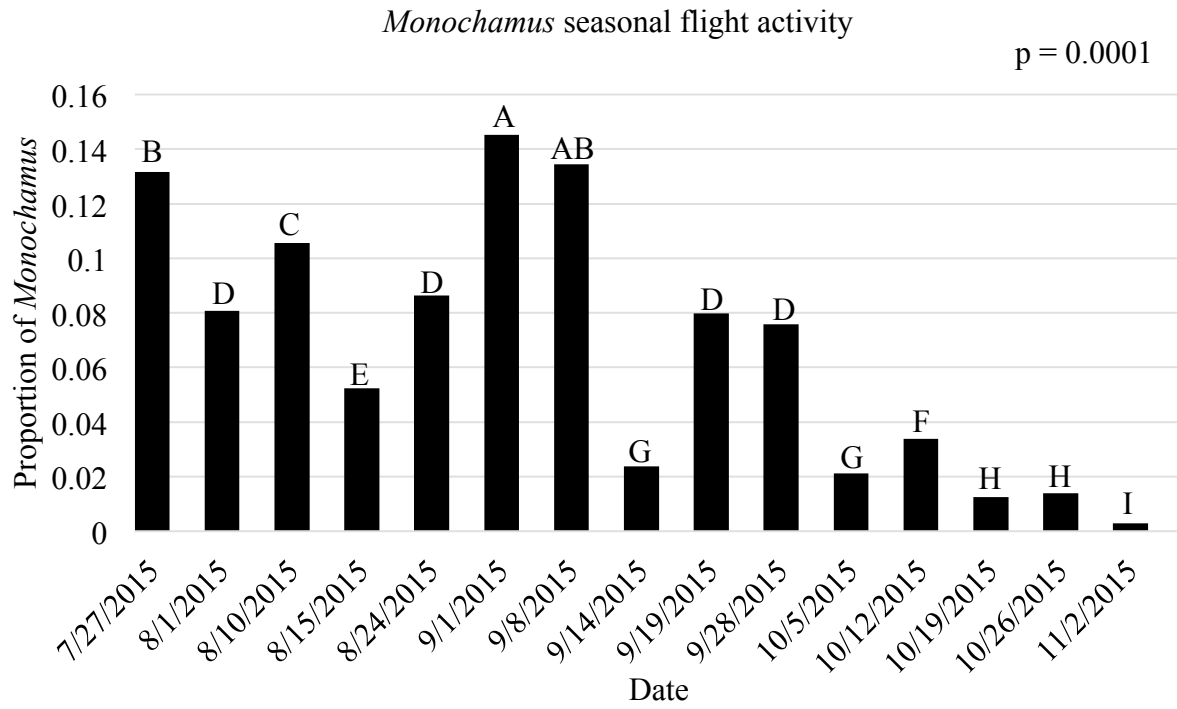
Figure 7: Proportion of *Monochamus* species combined by trap height. A total of 5731 *Monochamus* were captured over fifteen sampling dates from 7/27/15 through 11/02/15. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison at α = 0.05.

Monochamus seasonal flight activity

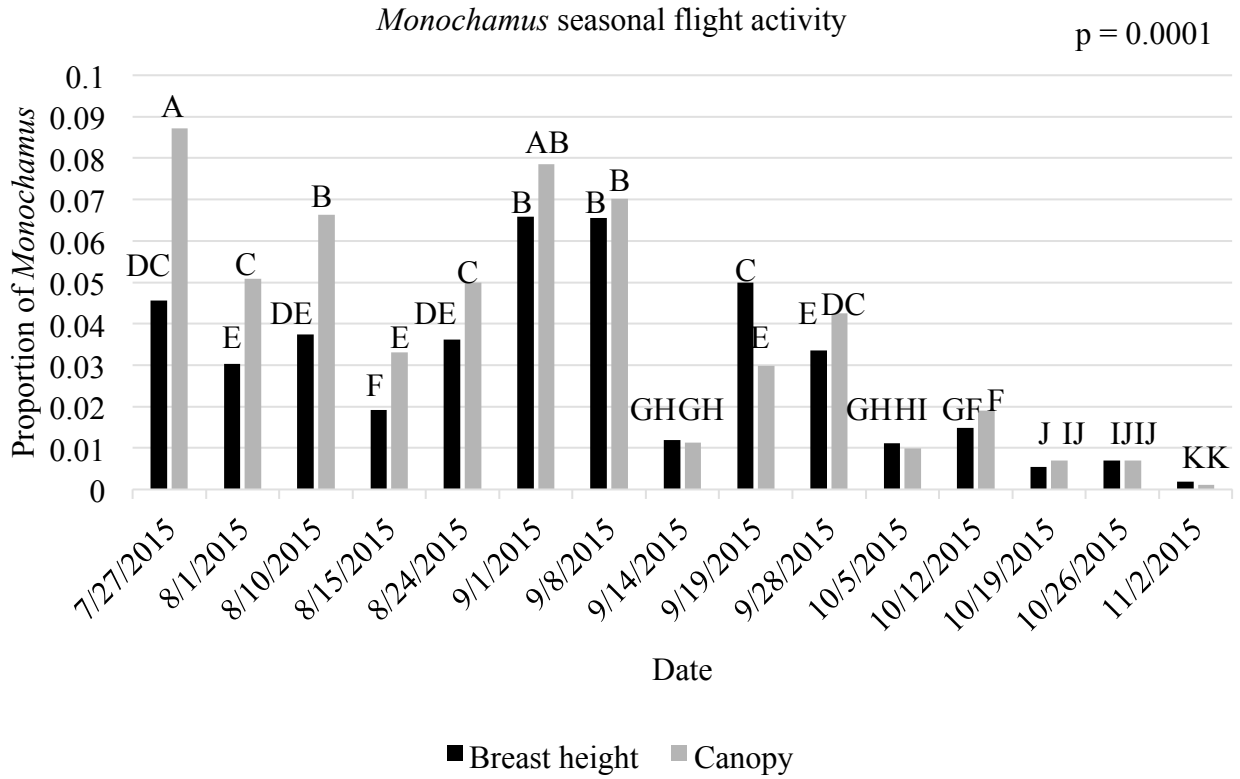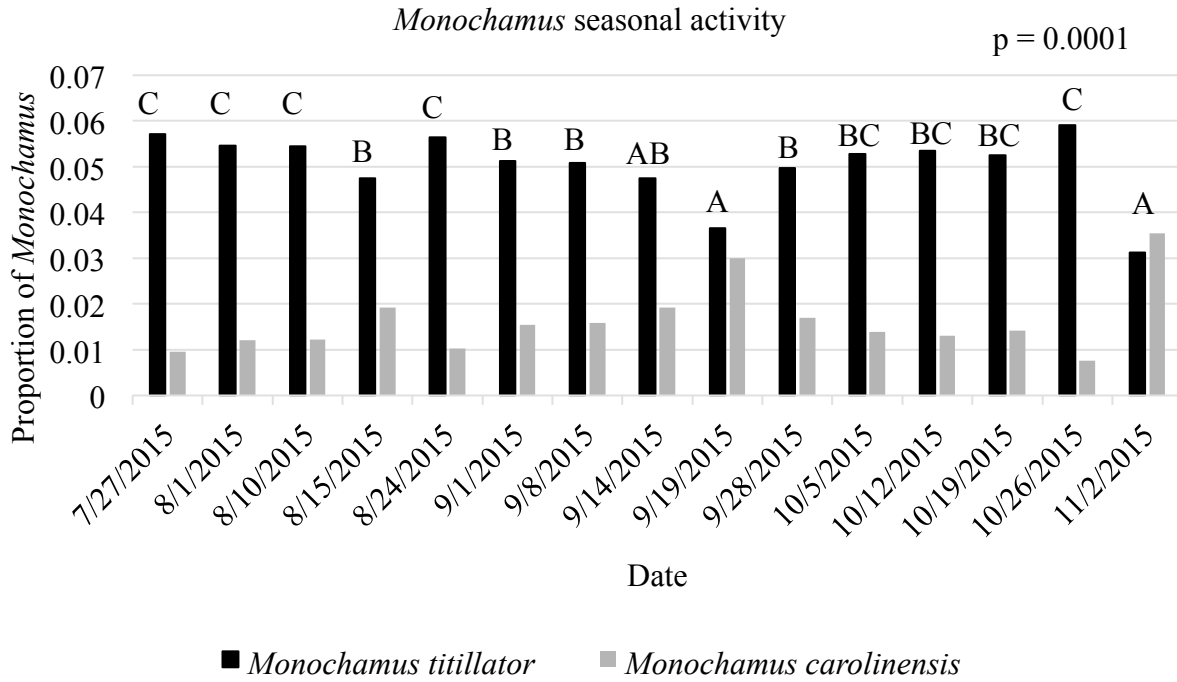p = 0.0001

■ Breast height ■ Canopy

Figure 8: Proportion of *Monochamus* seasonal flight activity by species. A total of 5731 *Monochamus* were captured over fifteen sampling dates from 7/27/15 through 11/02/15. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison at α = 0.05.

Monochamus seasonal activity

p = 0.0001

■ *Monochamus titillator*　■ *Monochamus carolinensis*

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Chapter 3 - Oviposition pit distribution and emergence densities of *Monochamus titillator* and *M. carolinensis*

Introduction

The southern pine sawyer (Coleoptera: Cerambycidae: *Monochamus titillator* (F.)) and the Carolina sawyer (*M. carolinensis* (Olivier)) are longhorned wood boring beetle species, both native to the eastern and southern United States (Webb 1909; Linsley and Chemsak 1984). Both species of *Monochamus* seem to be sympatric and occupy the same ecological niche (Webb 1909; Alya and Hain 1985; Walsh and Linit 1985).

Monochamus locate hosts through male-produced aggregation pheromones Monochamol, plus pine volatiles, and bark beetle kairomones (Billings and Cameron 1984; Allison and Borden 2001; Groot and Nott 2004; Miller et al. 2011; Ryall et al. 2014). Short range pheromones and cuticular hydrocarbons enable *Monochamus* to find mates on host material (Hanks 1999). To create oviposition pits, sexually mature female *Monochamus* use their mandibles to chew through the outer bark of the host. Number and pattern of eggs oviposited by *Monochamus* depends on the species. *Monochamus titillator* oviposits up to about nine eggs per pit in a radial pattern (Webb 1909; Craighead 1923; Alya and Hain 1985; Rastok 2015). *Monochamus carolinensis* oviposits up to three eggs per pit adjacent to one another (Alya and Hain 1985; Walsh and Linit 1985). Eggs hatch five to nine days after oviposition (Webb 1909; Alya and Hain 1985).

During colonization of host material by *Monochamus*, intraspecific and interspecific competition for mates and oviposition sites has been observed (Webb 1909; Hughes and Hughes 1982; Hughes and Hughes 1987). Inside the host phloem *Monochamus* larvae are competing with conspecifics, other *Monochamus* species, and other phloem feeders such as pine bark beetles, for

the limited supply of pine phloem (Coulson et al. 1976; Coulson et al. 1980; Miller 1985; Victorsson and Wikars 1996; Dodds et al. 2001; Schoeller et al. 2012; Ethington 2015).

To better understand how two sympatric species of *Monochamus* are able to coexist on the same limited resource, I investigated how *Monochamus* species interact, compete, and utilize resources. This study was designed to examine colonization and emergence of *Monochamus titillator* and *M. carolinensis* in the Ozark-St. Francis National Forest in Arkansas. The first objective was to determine distribution and density of oviposition pits created by both *Monochamus* species on pine bolts suspended at three heights on standing pine trees. The second objective was to determine emergence densities and emergence pattern of the two *Monochamus* species from the experimental bolts.

Materials and Methods

Research site

The research site was a 32 ha pine stand located in the Wedington Management area of the Ozark-St. Francis National Forest, located 32 km west of Fayetteville (Washington Co.), Arkansas. The stand had been thinned in September-October 2014. The stand was primarily *P. echinata* with a small amount of *Quercus alba* L., *Q. stellata* Wang., *Q. rubra* L., *Q. velutina* Lam., *Prunus nigra* Ait., *Juniperus virginiana* L., and *Carya tometosa* Nutt. Average pine basal area was 21.6 m^2/ha and average basal area of non-pine species was 4.1 m^2/ha. The average pine diameter at breast height was 27.4 cm.

Tree preparation

A 300 m, roughly north to south transect, was created on which five standing shortleaf pines (*Pinus echinata* Mill.) were selected. Supporting shortleaf pine trees upon which bolts were

installed were climbed using either a climbing deer stand (Warren & Sweat Manufacturing Company, Grand Island, FL, USA) or Wibe® sectional climbing ladders (Hultafors Group, Sweden). Dead limbs were removed up to base of canopy to reduce structural variation among trees and allow clear access to climbing equipment. Choker cables, 0.64 cm (1/4 in) steel aircraft cables (three lengths (2.45 m, 2 m, and 1.66 m), fixed into a continuous loop with an aluminum ferrule swagged eye on one end, were fastened on the supporting tree bole at breast height (2 m), mid-bole (~5.65 m), and base of canopy (~9.2 m) respectively.

Bolt preparation

Five additional shortleaf pines, within 50 m of supporting trees, were felled in both June and August. From each of the felled trees, two, 1m long pine bolts were cut from breast height, mid-bole, and base of canopy. Pine bolts were labeled with tree identification, position in relation to tree bole height, date, and side of tree on which bolt was to be suspended (east or west). One meter long, 0.64 cm dia (¼ in) aircraft steel cable with swagged eyes on both ends were attached on the sides, at the top end of each bolt using 5.08 cm x 0.95 cm (2 in x 3/8 in) galvanized lag bolts through short lengths of 0.64 cm (¼ in) proof coil chains (Figure 1). All holes into the pine bolt were pre-drilled with a 0.31 cm (5/16 in) drill bit.

***Monochamus* oviposition and emergence**

On 10 June, 2015, six shortleaf pine bolts were suspended on each of five standing shortleaf pines, each located 68 m – 85 m apart, to assess oviposition and emergence densities of *Monochamus* species. This was repeated on 30 August 2015. Two choker cables were placed at breast height (1.5 m), mid-bole (5.25 m – 6 m), and base of canopy (8 m – 10 m) on all trees. The pine bolts were then suspended at corresponding heights to the cables, one on the east and other on the west side, on the five standing trees (Figure 2). Each standing tree received a set of

pine bolts from the same felled tree. Pine bolts were suspended for one week to allow reduction in pine bolt resin defenses. After one week, supported pines were climbed and the numbers of accumulated oviposition pits on each suspended pine bolt were counted. Commercially available lures, comprised of *Monochamus* attractants, eluting at the following rates (Monochamol 750 µg/day at 20°C), host volatiles (α-pinene 150 mg/day at 20°C, and ethanol 20 mg/day at 20°C), and *Ips* bark beetle pheromones (ipsenol 800 µg/day at 20°C) (Synergy Semiochemicals, Burnaby, BC, Canada) were attached to the supporting tree bole between each pair of suspended pine bolts at breast height, mid-bole, and base of canopy.

Pine bolts were suspended for two weeks. Pine bolts were then collected, brought to the laboratory, and stored at 23°C in either individual temporary trash cans with a mesh screen (Figure 3), or Lumite mesh bags (Figure 4) until a wooden emergence cage was built (Figure 5). Average diameter, average bark thickness, and number of *Monochamus* oviposition pits were recorded. Emergence cages were checked weekly for emerging *Monochamus* beginning 60 days after pine bolts were collected from the field until 3 February 2016. On the 11 January 2016 bolts in wooden emergence cages were relocated to an unheated greenhouse subject to winter temperatures (max 22.7°C, min -4°C) until the 2nd of March 2016. Bolts were then placed at constant 23°C and checked weekly for emerging *Monochamus* until May, when the pine bolts were destructively sampled.

Insect rearing

Rearing cages were constructed of 1.9 cm (¾ in) plywood with 0.19 cm (3/16th in) plywood sliding doors. Cages are 2.4 m x 40 cm x 10.16 cm (8 ft x 15 ¾ in x 4 in) sectioned either 40.64

cm (16 in) for large bolts or 30.48 cm (12 in) for small bolts. Once plywood-rearing cages were built, bolts were then stored inside them and held until May 2016.

Bolt dissections

After *Monochamus* emergence declined in May 2016, life stage development information was collected from each bolt. Bark was removed with a drawshave, and bolts were then cut into one to one and a half cm. wide planks using a band saw. The number of *Monochamus* larvae, pupae, and adults was recorded.

Statistical analyses

Data were analyzed in JMP®, version 12. SAS Institute Inc., Cary, NC, 1989-2007. Unless otherwise stated, all density estimates are expressed in numbers per m^2 of bark surface area. Data were expressed as counts then log-transformed to decrease variability. To create graphs by density of *Monochamus* per m^2 data were transformed with the exponential function. Data were analyzed using fit mixed model with tree, and tree by bolt, suspension as random effects and bolt suspension, date of bolt suspension, and bolt suspension by date suspension as fixed effects. When appropriate Tukey's HSD was used for mean separations with significant level of 0.05.

Results

Oviposition

The total number of *Monochamus* oviposition pits for all 60 pine bolts was 4,246 (Table 1). The total density of *Monochamus* oviposition pits was highest at base of canopy and lowest at breast height ($F = 25.69$, $p = 0.0003$) (Figure 6). The base of canopy accounted for 51% of all *Monochamus* oviposition pits, with 33% being found at mid-bole, and 16% at breast height. The total *Monochamus* oviposition pit density was higher in June than August ($F = 8.10$, $p = 0.014$)

(Figure 7). There was no difference between *Monochamus* oviposition pit density in bolt suspension by date suspended (F = 0.34, p = 0.72).

Emergence

Overall: Total *Monochamus* emergence holes in the bark of the pine bolts suspended in both June and August was 531 (Table 1). A total of 425 *Monochamus* emerged from both (June and August) sets of pine bolts. Of which 40% emerged from pine bolts at breast height, 35.5% at mid-bole, and 24.5% at base of canopy (Table 2). Emergence densities of *Monochamus* by species were different with *M. titillator* (97%) accounting for the majority with very few *M. carolinensis* (3%). There were also no differences between male (45%) and female (55%) *Monochamus* that emerged from the pine bolts (F = 0.265 p = 0.61) (Table 2). Due to the low emergence counts of *M. carolinensis* it was not possible to determine differences among species by height at which pine bolts were suspended in this study. There was no difference in *Monochamus* emergence density by pine bolt suspension (F = 1.07, p = 0.39). The density of *Monochamus* emergence was higher in the pine bolts suspended in June as opposed to August (F = 9.975, p = 0.0082) (Figure 8). There was no difference between density of *Monochamus* emergence by pine bolt suspension by date suspended (F = 0.068, p = 0.93).

Bolt dissections

All life stages of *Monochamus* were dissected from pine bolts, with live *Monochamus* larvae as the most abundant life form (Table 3). Adult *Monochamus* were also found inside surface cocoons in the outer bark (Figure 9, Table 3). Other insects were found in bolts of which 506 were larvae, 77 pupae, and 60 adults (Table 3). The other insects were comprised of *Xylotrechus* sp. (Cerambycidae), *Acanthocinus* sp. (Cerambycidae), and *Thanasiumus dubius* (Cleridae).

All of the life stages of *Monochamus* dissected from pine bolts were combined with all emerged

Monochamus to determine the total population density of *Monochamus* inside the pine bolts. The

total population density of *Monochamus* increased significantly from breast height to the base of

canopy (F = 14.06, p = 0.0024) (Figure 10). The total population density of *Monochamus* was

higher in the bolts suspended in June as opposed to August (F = 12.93, p = 0.0037) (Figure 11).

In June, the total population density of *Monochamus* was highest in the mid-bole and base of

canopy (F = 3.96, p = 0.0479) (Figure 12). In August the total population density of

Monochamus increased significantly from breast height, to mid-bole, to base of canopy (F =

3.96, p = 0.0479) (Figure 12).

Development time

Development times from oviposition to emergence of adults were recorded for 425 *Monochamus*

in the suspended pine bolts. Development time was only calculated from emerged adult

Monochamus (both species combined, owing to low emergence of *M. carolinensis*) (Table 2).

Development times of emerged *Monochamus* were significantly different by pine bolt

suspension, with the least amount of days required at the base of the canopy (147 days) and

longer development at breast height (168 days) (F = 21.30, p = 0.0255). Development times of

emerged *Monochamus* were significantly different based on date of pine bolt suspended, with the

June bolts having the shortest development time at 112 days and 236 days for the pine bolts

suspended in August (F = 736.93, p = 0.0001). Development times of emerged *Monochamus*

were significantly different by date suspended and height at which pine bolts were suspended (F

= 6.22, p = 0.0062). The pine bolts suspended in June had the shorter development time, with the

least amount of time required in the base of canopy and no difference in the pine bolts suspended

between mid-bole and breast height (Figure 13). The pine bolts suspended in August had the

longest amount of time required to complete *Monochamus* development, with bolts suspended at mid-bole requiring the most days, followed by the pine bolts suspended at breast height than canopy (Figure 13).

Discussion & Conclusions

Monochamus species develop inside a subcortical environment, making it difficult to observe specific life history traits. Without destructive sampling, it is challenging to observe life history traits and interactions that occur. Determining differences between the two species of *Monochamus* larvae is nearly impossible owing to a lack of diagnostic characteristics or genetic markers. Host quality also plays a role in insect development, therefore pine bolt size and source trees were standardized.

Monochamus compete for suitable resources by scramble competition (Price et al. 2011). This competition may occur through intraspecific and interspecific competition for mates, for oviposition sites during colonization of host material (Webb 1909; Hughes and Hughes 1982; Hughes and Hughes 1987), and for phloem which larvae must consume to complete development. During colonization of suspended pine bolts by *Monochamus,* there was a higher density of oviposition pits in the base of canopy than at breast height (Figure 6). The availability of pine phloem decreases from breast height to the base of canopy, due to the diameter of a tree decreasing from the base to the canopy. With less available resources in the base of canopy compared to breast height, and higher density of oviposition sites, suggests scramble competition was observed during colonization of suspended pine bolts by *Monochamus* species.

More evidence to suggest scramble competition was observed is from comparing the emergence densities of *Monochamus* in the suspended pine bolts. There were no differences in emergence

density of *Monochamus* between the base of canopy and breast height, even though there were a higher density of oviposition pits in the base of canopy. During larval development of *Monochamus* inside the pine bolts, *Monochamus* face intraspecific and interspecific scramble competition (Webb 1909; Hughes and Hughes 1982; Hughes and Hughes 1987). With fewer available resources present in the base of canopy, and higher density of *Monochamus* oviposition pits, the chance of *Monochamus* larvae encountering each other increases. Thus, competitive interactions among larval *Monochamus* in pine bolts at the base of canopy are greater than compared to breast height. This higher level of competition among larval *Monochamus* in the base of canopy may be why the overall emergence density of *Monochamus* was not different than at breast height.

Emergence densities of *Monochamus* by species were different, with 97% *M. titillator* emergence and only 3% emergence of *M. carolinensis*. There were no differences in sex ratio of emerging *Monochamus* (Table 2). *Monochamus titillator* accounted for the majority of emergence, which may be due a variety of reasons such as to slight differences in their biology, competition; abiotic and biotic factors, etc. *Monochamus* species differ in the number of eggs each species can oviposit. *Monochamus titillator* oviposit eggs (3-9) per oviposition pit compared to *M. carolinensis* (1-3) (Webb 1909; Alya and Hain 1985; Walsh and Linit 1985; Rastok 2015), which in this study may explain why *M. titillator* accounted for the majority of *Monochamus* emergence.

The results of this research demonstrate how *Monochamus* species colonize and utilize available resources. Even though there is greater competition among *Monochamus* larvae observed in the base of canopy, there are no differences in emergence densities compared to bolts at breast height. This competition may explain how *Monochamus* species can co-exist.

In this study *Monochamus titillator* appears to be the stronger competitor. However, *M. carolinensis* are still able to compete and emerge on the same resource. Since both species of *Monochamus* appear to occupy the same ecological niche, and are sympatric, further studies will need to be conducted to further understand how *M. titillator* and *M. carolinensis* utilize and compete for the same resources. Further studies could focus on differences in the number of eggs oviposited, and larval competition, separate and in the presence of the other species.

Tables and figures

Table 1: Total numbers of *Monochamus* oviposition pits and emergence holes on 60 suspended shortleaf pine bolts (30 pine bolts in June, and 30 in August).

Date	Bolt Suspension	Cardinal	Oviposition pits	Prop Ovi pits	Emergence holes	Prop Emerg
06/10/2015	Breast height	East	233	0.09	79	0.22
	Breast height	West	224	0.08	64	0.18
	Mid-bole	East	565	0.29	55	0.16
	Mid-bole	West	326	0.20	62	0.12
	Canopy	East	780	0.21	59	0.15
	Canopy	West	534	0.12	42	0.17
08/04/2015	Breast height	East	114	0.07	34	0.24
	Breast height	West	120	0.08	28	0.19
	Mid-bole	East	253	0.26	21	0.14
	Mid-bole	West	264	0.26	22	0.14
	Canopy	East	419	0.16	21	0.14
	Canopy	West	414	0.17	20	0.15

Prop Ovi pits = proportion of *Monochamus* oviposition pits. Prop Emerg = proportion of *Monochamus* emergence holes.

Table 2: Total numbers of *Monochamus* that emerged from 60 suspended shortleaf pine bolts (30 pine bolts in June, and 30 in August). A total of 418 *Monochamus titillator* and eight *M. carolinensis* emerged from 60 suspended shortleaf pine bolts.

Month	Suspension	Species	Sex	N	Prop month	Prop total
June	Breast height	*M. titillator*	Male	39	0.15	0.09
			Female	63	0.24	0.15
		M. carolinensis	Male	1	0.00	0.00
			Female	1	0.00	0.00
June	Mid-bole	*M. titillator*	Male	45	0.17	0.11
			Female	52	0.20	0.12
		M. carolinensis	Male	0	0.00	0.00
			Female	0	0.00	0.00
June	Canopy	*M. titillator*	Male	34	0.13	0.08
			Female	30	0.11	0.07
		M. carolinensis	Male	1	0.00	0.00
			Female	0	0.00	0.00
August	Breast height	*M. titillator*	Male	37	0.23	0.09
			Female	26	0.16	0.06
		M. carolinensis	Male	1	0.01	0.00
			Female	2	0.01	0.01
August	Mid-bole	*M. titillator*	Male	28	0.18	0.07
			Female	25	0.16	0.06
		M. carolinensis	Male	0	0.00	0.00
			Female	1	0.01	0.00
August	Canopy	*M. titillator*	Male	19	0.12	0.05
			Female	19	0.12	0.05
		M. carolinensis	Male	0	0.00	0.00
			Female	1	0.01	0.00

N = total number. Prop month = proportion of individual *Monochamus* that emerged by month (June and August separate). Prop total = proportion of individual *Monochamus* that emerged by the total emergence count.

Table 3: Development stage of *Monochamus* recovered in laboratory dissection of suspended pine bolts.

	Date suspended					
	06/10/2015			08/04/2015		
	Suspension			Suspension		
	BH	**MB**	**CP**	**BH**	**MB**	**CP**
Live *Monochamus* adult	2	1	3	3	12	10
Live *Monochamus* pupae	9	4	2	6	13	13
Live *Monochamus* larvae	14	29	29	7	12	43
Dead *Monochamus* adult	11	3	3	4	2	0
Dead *Monochamus* pupae	0	0	0	0	0	0
Dead *Monochamus* larvae	2	3	5	0	1	1
Other adult	11	5	1	27	16	0
Other pupae	18	18	1	28	11	1
Other larvae	122	72	51	125	78	58

Suspension: BH = pine bolts suspended at breast height; MB = pine bolts suspended at mid-bole; CP = pine bolts suspended at base of canopy. Other – adult, pupae, larvae = insects that are not *Monochamus* comprised of *Xylotrechus* sp. (Cerambycidae), *Acanthocinus* sp. (Cerambycidae), and *Thanasiumus dubius* (Cleridae).

Figure 1

Hardware attached to the top portion of a shortleaf pine bolt. Used for suspending pine bolt in another tree.

Figure 2
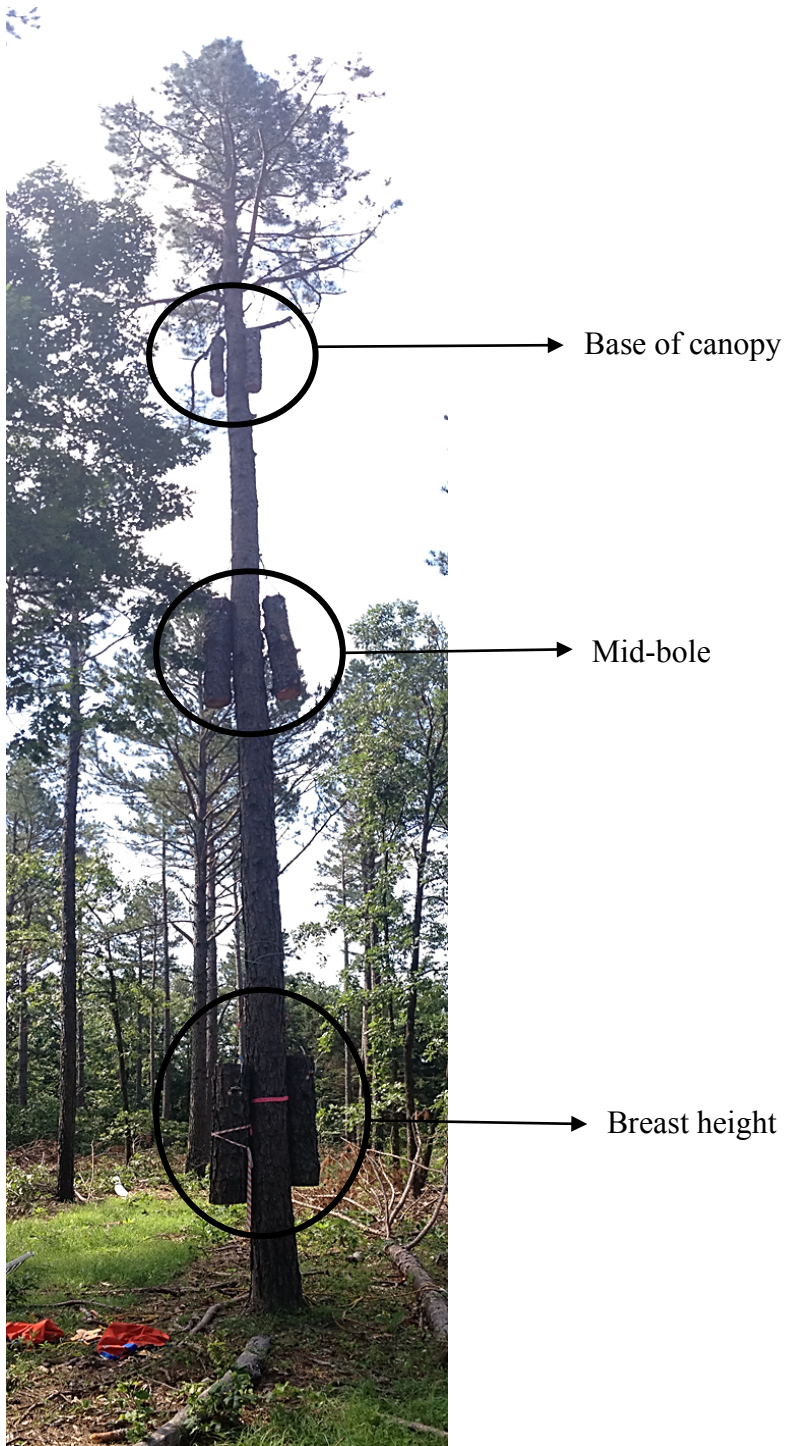
Individual shortleaf pine bolts suspended at breast height, mid-bole, and base of canopy on the east and west side of a shortleaf pine.

Figure 3

Modified trash can with mesh lid containing experimental shortleaf pine bolt. Used to quarantine and easily check for emerging *Monochamus* spp.

Figure 4

Individual experimental shortleaf pine bolts wrapped in Lumite mesh and screwed to a flat plywood base for stability. Used to quarantine and easily check for emerging insects.

Figure 5

Wooden emergence container with *Monochamus* infested shortleaf pine bolt. Emergence container used to quarantine and easily check for emerging insects.

Figure 6: Density of *Monochamus* oviposition pits per pine bolt suspension. A total of 60 pine bolts were used. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison, $\alpha = 0.05$.
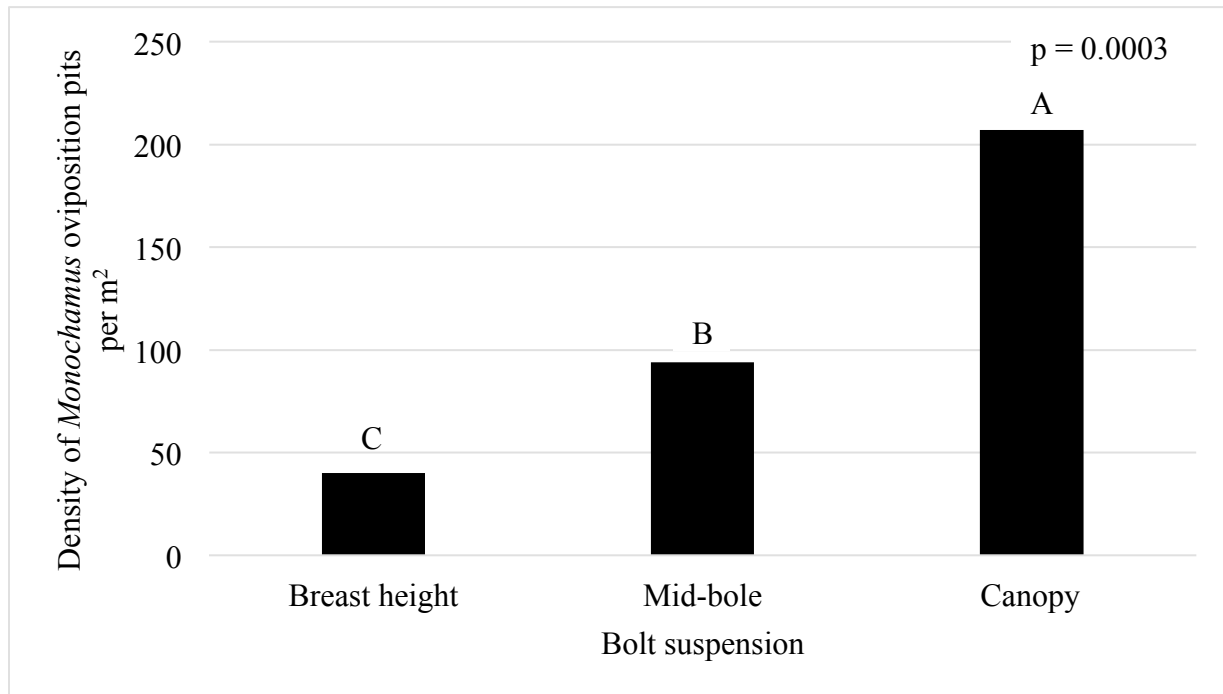
Figure 7: Density of *Monochamus* oviposition pits per pine bolt suspension date. A total of 60 pine bolts were used. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison, α = 0.05.
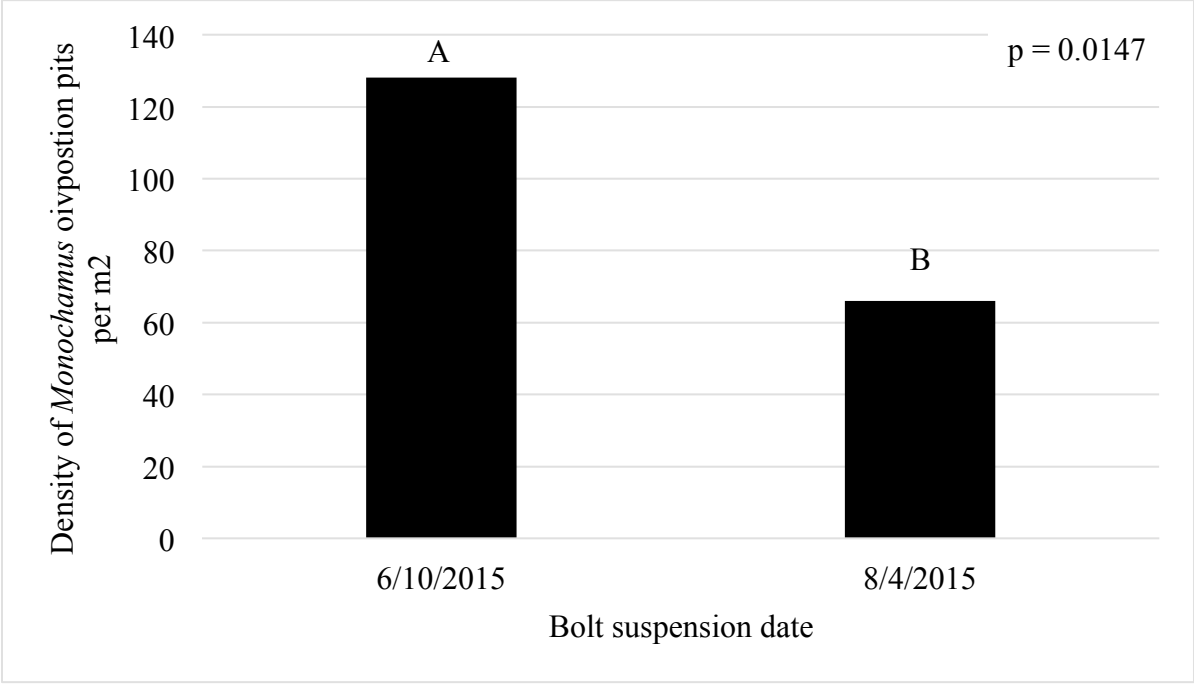
Figure 8: Emergence density of *Monochamus* by suspension date. A total of 60 pine bolts were used. Different letters above columns denote means that are significantly different according to Tukey's HSD mean comparison, α = 0.05.
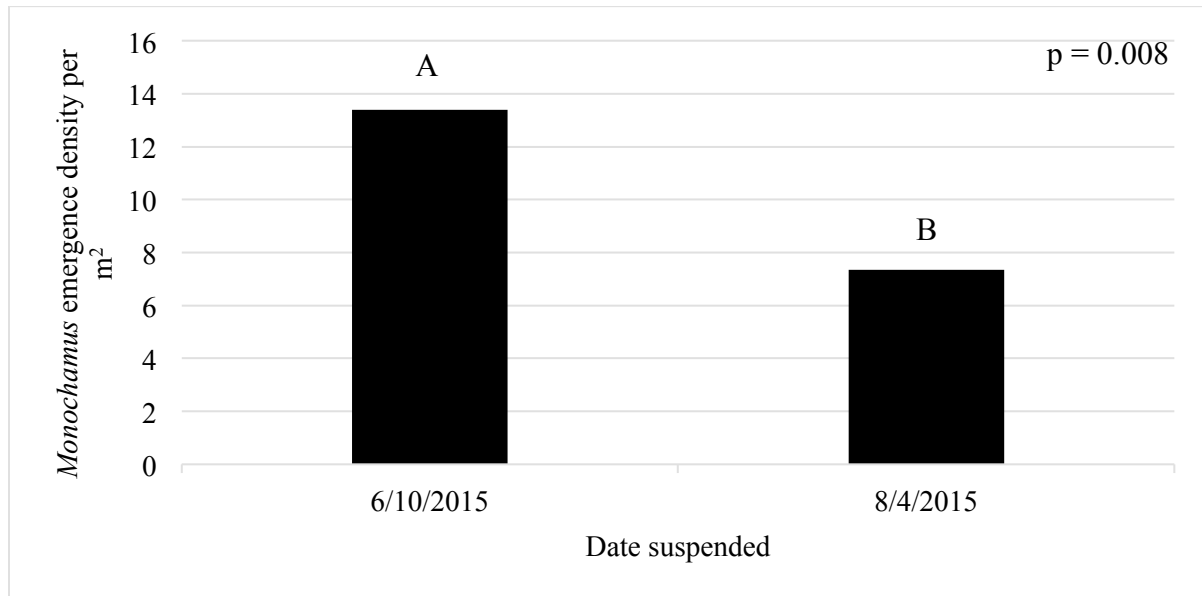
Figure 9: Adult *Monochamus* that were found inside surface cocoon in outer bark of suspended shortleaf pine bolts.
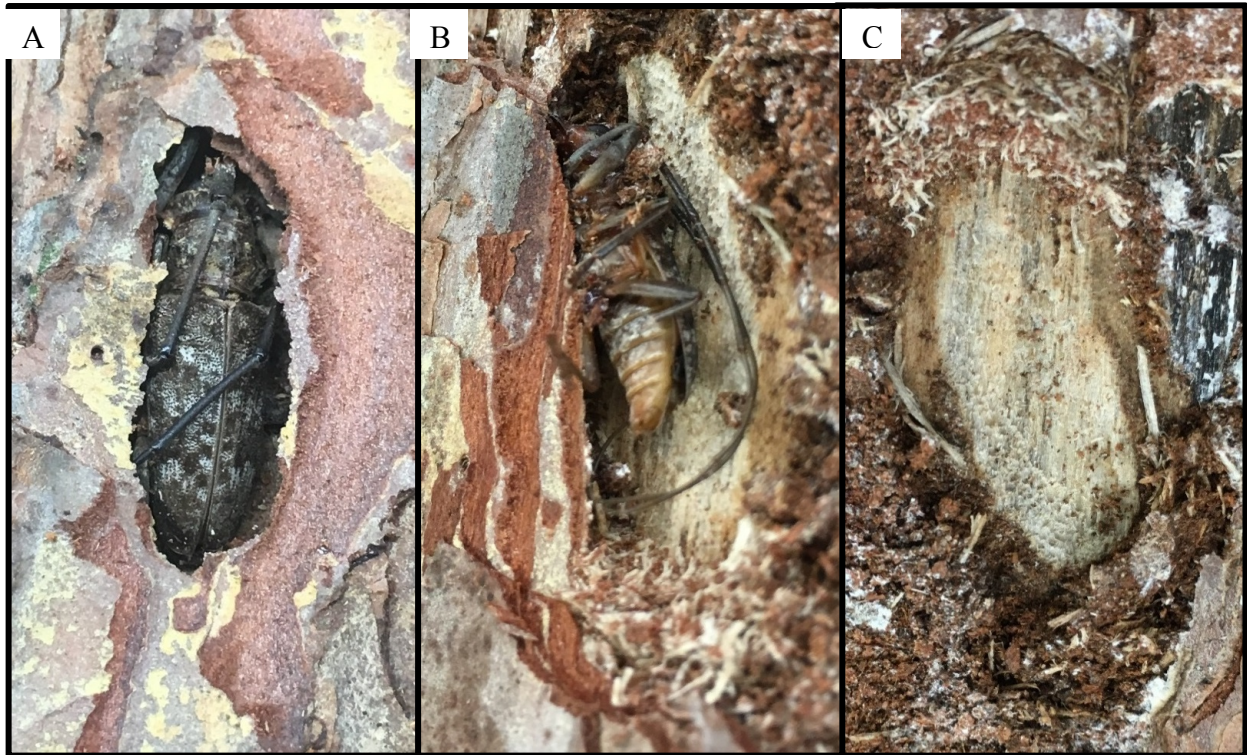
Notes: A, B: *Monochamus* adult inside surface cocoon in outer bark. C: *Monochamus* surface cocoon with no scar visible in xylem.

Figure 10: Total population density of *Monochamus* per m^2 by pine bolt suspension. A total of 60 shortleaf pine bolts were suspended.
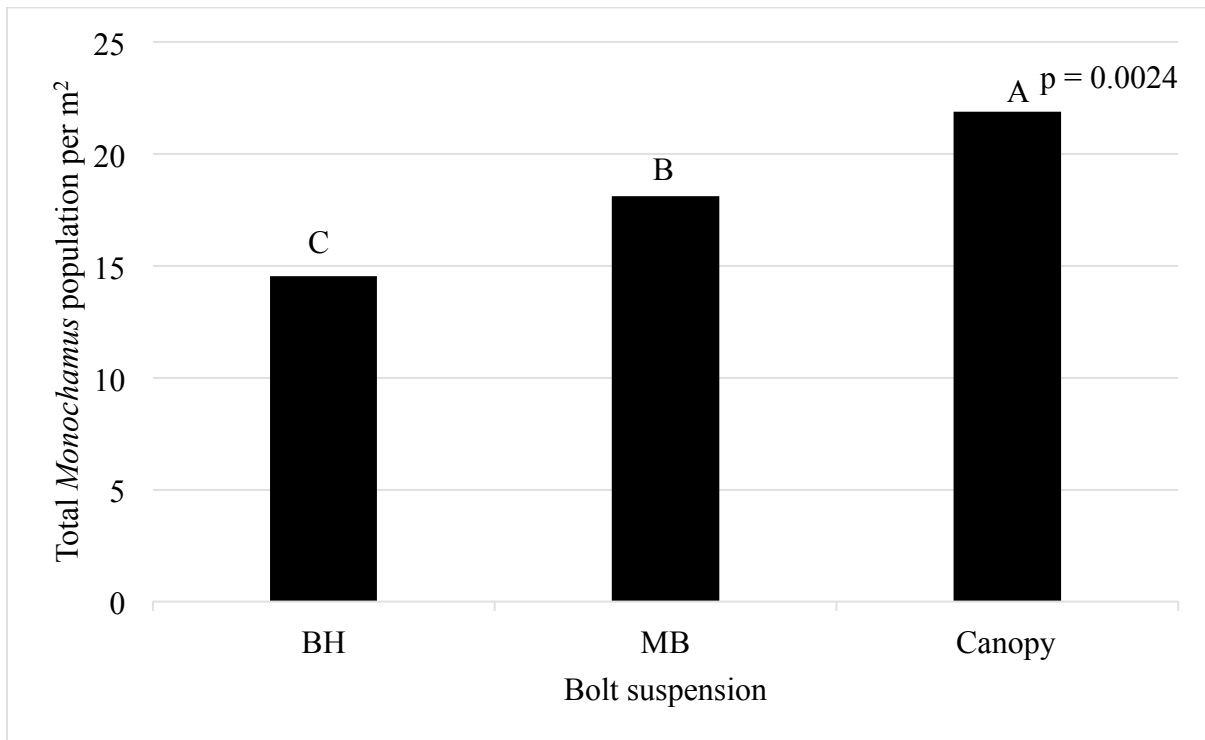
Figure 11: Total *Monochamus* population density per m^2 by date of pine bolt suspension. A total of 60 shortleaf pine bolts were suspended.
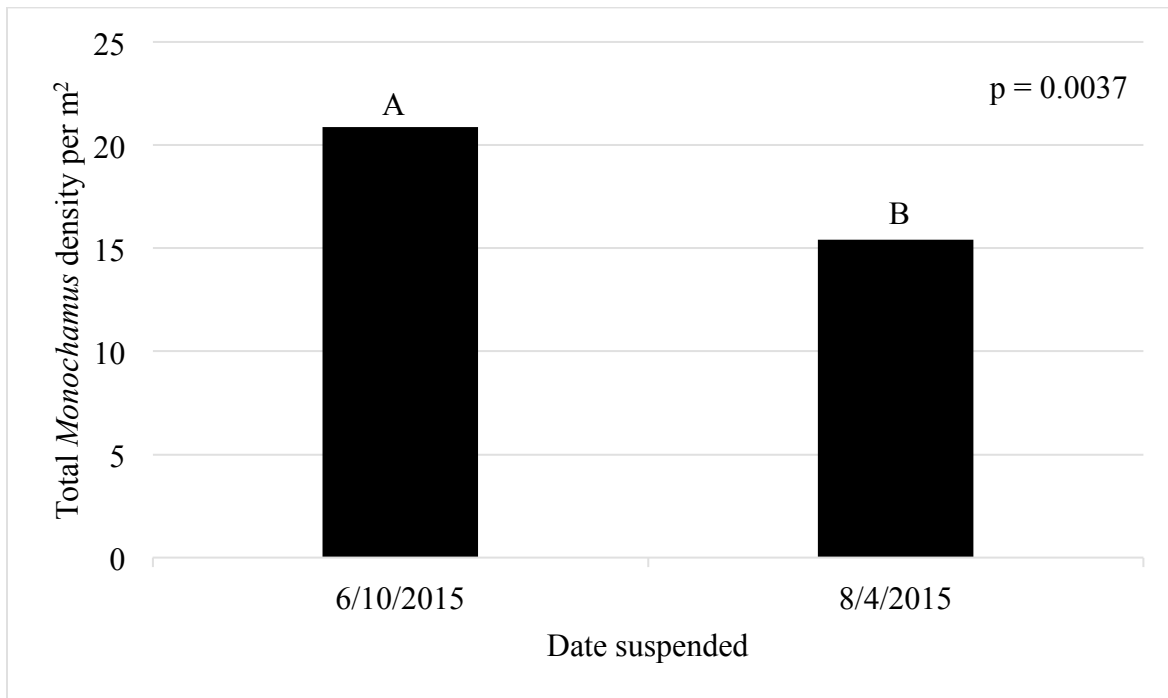
Figure 12: Total *Monochamus* population density per m^2 by pine bolt suspension and date of suspension. A total of 60 shortleaf pine bolts were suspended.
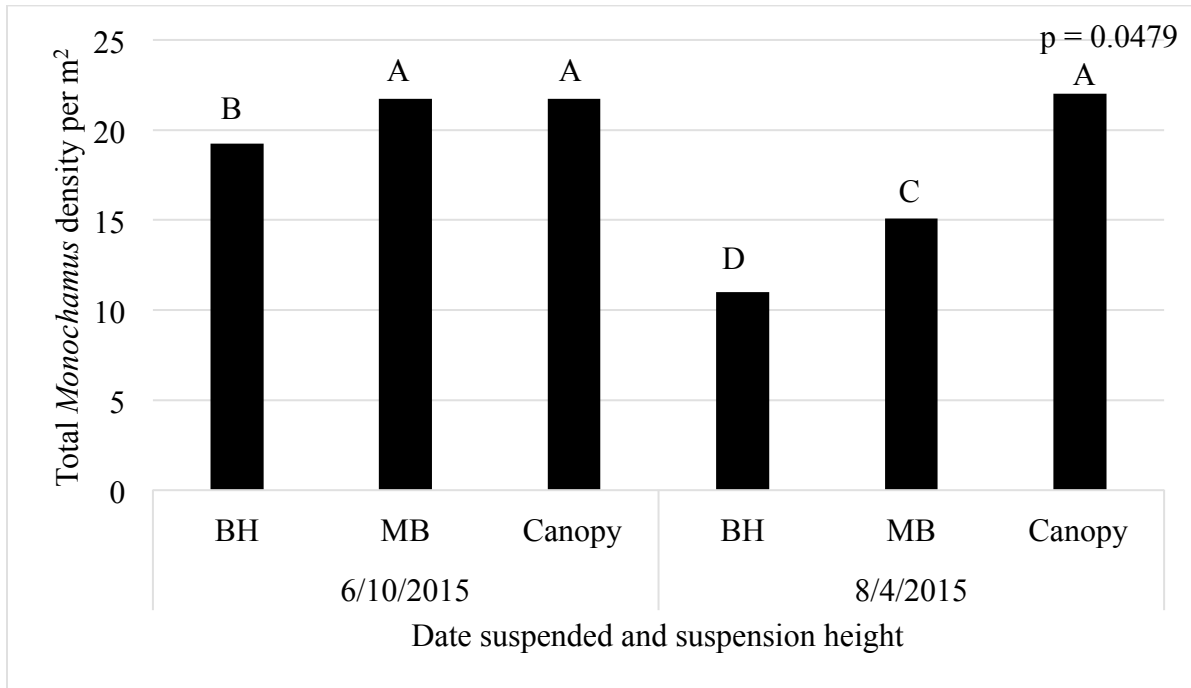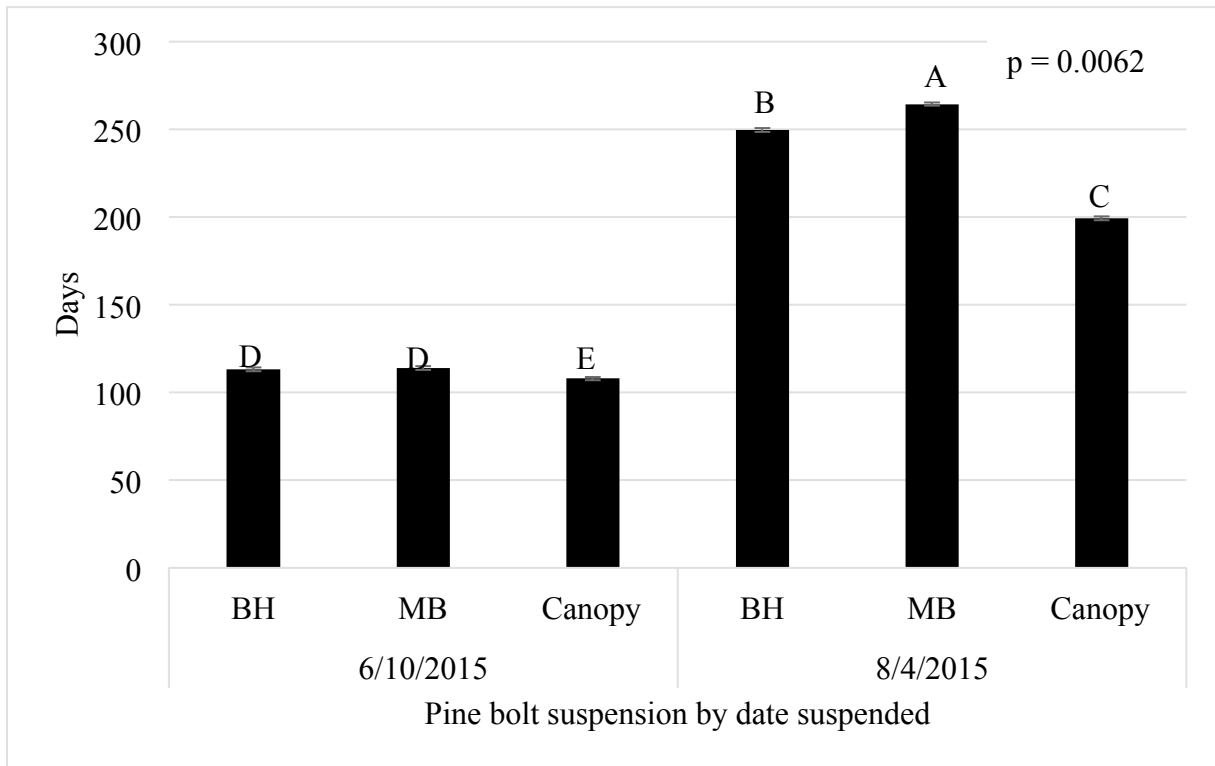
Figure 13: Development time of emerged *Monochamus* from pine bolts suspended in June and August. Thirty pine bolts were suspended in both June and August. Of the total 60 pine bolts suspended 425 *Monochamus* emerged.

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Conclusion

These studies were designed to better demonstrate ecological differences between the two species of pine sawyers found in the Ozark St. Francis National Forest in Arkansas, *Monochamus titillator* (southern pine sawyer) and *M. carolinensis* (Carolina sawyer). Both species seem to occupy the same ecological niche – similar seasonal flight patterns, life history, and host material.

Although these studies failed to establish ecological differences between species, they did help in understanding the ecology of *Monochamus* by elucidating diurnal or nocturnal height flight patterns, heights at which *Monochamus* fly, and within tree distribution of oviposition pits and emergence densities using suspended shortleaf pine (*Pinus echinata*) bolts.

These studies determined that both species of *Monochamus* were active at night, and most often captured at the base of canopy. There was a higher density of oviposition pits in the base of canopy during colonization of suspended pine bolts, and no differences in density of emerging *Monochamus* by height at which bolts were suspended.

These results help in understanding the biology and ecology of these two *Monochamus* species, which may allow us to refine trapping methods, as well as beginning to understand of how *Monochamus* species interact among each other, and with their associated pine phloem feeding guild.

Further studies will need to be conducted to better understand how North American *Monochamus* can have a sympatric relationship with a sibling species while apparently occupying the same ecological niche.