TRAPPING METHODS FOR LARGE WOODBORING INSECTS IN

SOUTHEASTERN U.S. FORESTS

by

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(Under the Direction of Kamal J.K Gandhi)

ABSTRACT

Large woodboring insects (Buprestidae, Cerambycidae, Elateridae, and Siricidae) are ecologically and/or economically important in the southeastern U.S. forests. Surveys are conducted annually in the region to monitor populations of native and exotic woodboring beetle species. My research objectives were as follows: 1) to assess the efficacy of various trapping techniques for surveying of native siricids (woodwasps) and their hymenopteran parasitoids in southeastern pine (*Pinus* spp.) stands; and 2) to determine the effect of lure placement on modified funnel traps on capturing efficiency of large woodboring beetles. Creating trap-logs at the peak flight of siricids (early November) and using fresh pine billets with an intercept panel trap were the most efficient methods for trapping native siricids and their hymenopteran parasitoids. A modified funnel trap with lures hanging on the inside of the trap maximized the catches and diversity of cerambycid and elaterid beetles.

INDEX WORDS: Buprestidae, Cerambycidae, Elateridae, hymenopteran parasitoids, *Pinus* spp., semiochemical lures, Siricidae, Southeastern U.S., trapping, woodboring insects

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BSFR., University of Georgia, 2009

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2012

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August 2012

DEDICATION

This thesis is dedicated to my amazing family, loving friends, and dedicated major professor.

ACKNOWLEDGEMENTS

I would like thank the entire Forest Entomology lab from the Warnell School of Forestry and Natural Resources for being constant supporters and inspirations to me throughout my Masters. Kamal Gandhi, my advisor, for being a wonderful woman, mentor, and supporter, I thank you! Thanks to Dr. Daniel Miller, for being a great supporter and provider of information to my projects! Thanks also to the USDA Forest Service and the Georgia Forestry Commission for providing assistance and equipment. I would also like to thank my parents, Renny and Jacqueline Barnes and my sister Ashley Barnes for being 100% supportive throughout this degree. Thanks to my brother, Chandler Barnes, for giving me the encouragement from above that was imperative to my finishing this degree. I also have to thank my cat, Ilo, for always making me smile! Finally I would like to thank my funding sources, including The Warnell School of Forestry and Natural Resources and the USDA Forest Service for providing financial support.

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CHAPTER 1

THESIS INTRODUCTION

1.1 Introduction

The timber industry in the southeastern United States (U.S.) is vital to the economy of the region. The southeastern U.S. produces ~ 58% of total timber production in the U.S. and 16% of the world's timber (Wear and Greis 2002). Overall, there are 86 million ha of forested land in the region of which 81 million ha is considered commercial timber (USDA Forest Service 2004). Georgia alone has around 10 million ha of forested land (about two-thirds of the state's land), with loblolly pine (*Pinus taeda* L.) as the dominant softwood tree, present on 2.7 million ha (Harper et al. 2004). In addition to being economically important, the southeastern forests provide critical ecological functions such as enhanced water quality, fertile soils, carbon sequestration, and erosion regulation (Noss 1996). For example, pine forests in the Southeast can accumulate almost 1 metric ton of carbon per acre every year (Birdsey 1996). The longleaf (*P. palustris* Mill) pine forests of southeastern coastal plain are considered one of the most diverse ecosystems in the world (Landers et al 1995), containing almost a quarter of all the plant species found in North America (Stein et al 2000). Hence, it is critical to maintain forest processes and health in the southeastern forests, for many societal and ecological benefits.

Currently, there are many threats to forest health in the southeastern region including urbanization, low-density housing developments, climate change, wildfire suppression, pollution, habitat loss, and fragmentation (Stein et al. 2005). The greatest risk to southeastern forests is the rise in human population, not only in the big cities but also the expansion of many small towns in rural areas (Wear and Greis 2002). Though population growth is touted as the greatest risk to forested areas, native and non-native invasive insects also play an extremely important role in forest health. A rise in human population levels leads to an increase in imports and global trade, which is the main route for introduction and establishment of non-native species. These non-native insects and diseases have drastically altered forested ecosystems through their impacts on the formation of canopy gaps, increased amounts of coarse woody debris, and alterations to biogeochemical cycling (Gandhi and Herms 2010). Millions of dollars are spent each year to eradicate and control non-native species, and to restore the original forests before their introduction (Wear and Greis 2002).

Native subcortical insects, and especially forest woodboring insects (Buprestidae, Cerambycidae, Elateridae, Siricidae) usually colonize dying or stressed trees, and degrade the quality of the wood by creating large holes and tunnels through the phloem and xylem (Vallentgood 1991). This group also assists in the overall decomposition of coarse-woody debris in the forest (Dajoz 2000). In the non-native range, woodboring insects can directly contribute to tree decline and mortality. During 1985-2005 in the U.S., 25 new non-native bark and woodboring beetles were intercepted at various ports of entry (Haack 2006), notable being the Asian longhorned beetle [*Anoplophora glabripennis* (Motschulsky)] and emerald ash borer [*Agrilus planipennis* (Fairmaire)]. Examples of non-native woodboring insects in the southeastern forests include redbay ambrosia beetle [*Xyleborus glabratus* (Eichhoff)] with other species such as the European woodwasp [*Sirex noctilio* (Fabricius)] and emerald ash borer slowly expanding their distribution expectedly in the southeastern U.S. It is estimated that if *S. noctilio* enters the southeastern region, it has the potential to cause an upward of $11 billion in pine tree loss (USDA Forest Service 2006). In the southern hemisphere, where *S. noctilio* has caused extensive damage, there are no populations of native siricids (Murphy 1998). In contrast, there are at least 23 known species and subspecies of siricids in North America (Schiff et al. 2006). It is possible that native siricids along with their hymenopteran parasitoids may exert some competitive and control pressure on populations of *S. noctilio* (Taylor 1976). Hence, understanding the population and community structure of native siricids and their hymenopteran parasitoids in the southeastern region will be important before *S. noctilio* arrives in this region.

Non-native insects are typically transported on commodities such as solid wood packing material (Haack 2006), and enter via various ports of entry in the U.S. The Southeast has the 2nd highest trafficked seaport in the U.S. along with the world's busiest airport (Atlanta Hartsfield) and one which receives the world's 2nd most cargo shipments by volume (Georgia Power 2010, Georgia Public Broadcasting 2011, Global Airport Cities 2011). Due to the high risk of exotic insect introductions in the southeastern region, surveys are conducted annually to identify and manage for new exotic species (Dodds et al. 2010). Cooperative agricultural pest surveys (CAPS) are conducted annually in all 50 states, and from 1985-2002 these surveys collected over 7,400 species of plant pests, many of which could have become established (USDA APHIS 2005). These surveys are typically done using a multiple funnel trap or an intercept panel trap baited with various semiochemical lures, such as monoterpenes and ethanol that attract the insects (Lindgren 1983, Chénier and Philogène 1989, Czokajilo et al. 2001, Allison et al. 2004). Many studies have assessed the different trapping methods including different trap-type combinations of monoterpenes, and placement of traps. At present, we have little information about how lure placement on the trap may optimize trap catches of woodboring insects to better detect newly introduced species in the region.

1.2 Research Objectives

This thesis is focused on the populations and communities of native and non-native woodboring insects present in pine stands in the southeastern U.S. I have two major research objectives, which are: 1) to assess the efficacy of various trapping techniques for surveying of native siricids (woodwasps) and their hymenopteran parasitoids in southeastern pine (*Pinus* spp.) stands; and 2) to determine the effect of lure placement on modified funnel traps on capturing efficiency of large woodboring beetles. Chapter 2 deals with the species complex of native siricids and their hymenopteran parasitoids in the southeastern U.S. along with the trapping efficacy of various trapping techniques for capturing these two taxa. Chapter 3 deals with trapping efficacy of lure placement on a modified 10-unit Lindgren funnel trap on woodboring beetles in the southeast U.S. The overall goal of my thesis to provide a better understanding of trapping methods for woodboring insects for their use as biocontrol agents, and for the early detection and eradication of non-native species in pine-dominated landscapes.

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CHAPTER 2

EVALUATION OF CAPTURE TECHNIQUES FOR NATIVE SIRICIDAE AND THEIR HYMENOPTERAN PARASITOIDS IN SOUTHEASTERN UNITED STATES[1]

[1] B F Barnes, Meeker J R, Johnson C W, Asaro C, Miller D R, and Gandhi K J K. To be submitted to *Agricultural and Forest Entomology*

Abstract

The Eurasian woodwasp, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) is an introduced invasive pest of pines (*Pinus* spp.) in the southern hemisphere, and has been established in North America at least since 2004. While *S. noctilio* has caused pine mortality in its non-native range, its impacts in North America are not well known. In the southeastern U.S. forests, it is possible that *S. noctilio* may have limited impacts on pines, as native siricids and their parasitoids may, respectively, act as natural competitors and biocontrol agents of *S. noctilio*. Our research objectives were to determine the species complex of native siricids and their parasitoids in the southeastern region, especially in Georgia, Virginia, and Louisiana. We also assessed the efficacy of various capture techniques for these two taxa using various combinations of traps (intercept, funnel, and Santé traps), lures (host attractants and bark beetle pheromones), and trap-trees. During the fall of 2009-2011, a total of 2,049 siricids were captured, including *Eriotremex formosanus* (Matsumura), *Sirex nigricornis* (Fabricius), *Tremex columba* (Linneaus), and *Urocerus cressoni* (Norton). Traps captured 440 siricid adults, whereas 1,609 siricids emerged from trap-trees during the three years. A majority of the siricids (76%) in the study were caught in Louisiana where 486 *Ibalia leucospoides ensiger* Norton (a parasitoid) were also reared from trap-trees. Peak flight of native siricids is October in Virginia, and November in Georgia and Louisiana. Commercially available *Sirex* lure alone and *Sirex* lure with ethanol captured 2-5 times greater numbers of siricids than the unbaited trap. There was no difference in siricid trap catches between intercept panel and funnel traps, and no parasitoids were caught in any traps. There was no difference in siricid trap catches between the *Sirex* lure, ethanol, α-pinene, ipsenol, or ipsdienol, or among trap-types. We caught greater numbers of siricids in Louisiana by using fresh pine billets over the *Sirex* lure in funnel traps and creating trap-trees in early rather than late November.

Keywords: *Ibalia leucospoides ensiger* · Native Siricidae · Parasitoids · Semiochemical Lures · *Sirex noctilio* · *Sirex* spp. · Southeastern U.S. · Trapping

2.1. Introduction

The Eurasian woodwasp, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), is an exotic woodboring insect accidentally introduced to North America, where established populations were discovered in 2004 (Haugen and Hoebeke 2005). *Sirex noctilio* is native to Europe, Asia, and North Africa where it is usually a secondary colonizer of damaged and declining conifer trees (Spradberry and Kirk 1978). In contrast, *S. noctilio* is a primary colonizer of conifer trees in non-native habitats, including Australia, New Zealand, South Africa, and South America (Rawlings 1948, Iede 1998, Ciesla 2003). This woodwasp has a wide host range, and while it mainly colonizes pines (*Pinus* spp.), it can occasionally attack tamarack (*Larix* spp.), fir (*Abies* spp.), and spruce (*Picea* spp.) (Carnegie et al. 2006). *Sirex noctilio* can cause tree mortality through a combination of female oviposition activities: deposition of a phytotoxic mucus, spores of a fungal pathogen, [*Amylostereum areolatum* (Fr.)], and eggs oviposited into the tree (Ciesla 2003). The larvae feed primarily on this symbiotic fungus as they burrow through the xylem, growing and developing into pupae and emerging as adults from the tree; this process generally takes one year. North American conifer forests may be impacted by this pest because the native trees may not have host resistance mechanisms to defend themselves against *S. noctilio*.

In North America, *S. noctilio* has been discovered in the Great Lakes region including Connecticut, Michigan, New York, Ohio, Ontario, Pennsylvania, Quebec, and Vermont (USDA-APHIS 2011). Currently, it is hypothesized that if *S. noctilio* is not contained or eradicated, populations may continue to spread and become established in other regions of North America, including parts of the southeastern United States (U.S.)

that have heavy human and commercial traffic. For example, Georgia's port of Savannah is the second highest trafficked seaport in the U.S. for exports and the fourth busiest seaport overall in the country (Georgia Public Broadcasting 2011). Hartsfield-Jackson International Airport in Atlanta receives some of the highest number of cargo shipments in the country and is the world's busiest airport (Georgia Power 2010). The Memphis Airport in Tennessee receives the world's second largest cargo shipments by volume, with couriers having their main hub at the airport. International shipments from around the world are constantly being brought into the southeastern region and distributed around the country (Center for Asian Pacifica Aviation 2011, Global airport cities 2011). With this high volume of commodities being brought into the Southeast region, there is a high risk of S. *noctilio* being introduced and established.

In the southern hemisphere, planted North American pines including Monterey (*P. radiata* D.), loblolly (*P. taeda* L.), slash (*P. elliottii* Eng.), and ponderosa (*P. ponderosa* Doug.), are susceptible to colonization by *S. noctilio* (Carnegie et al. 2006). Forests in the Southeast contain a large volume of southern pines, including loblolly, slash, and shortleaf pine (*P. echinata* Mill). Most of the pine forests in Georgia are plantations that are privately owned with 6.5 and 4.3 million acres of planted and natural pine stands, respectively (Harper et al. 2009). These forests are typically not managed intensively, and therefore, they could be overstocked and prone to pest outbreaks (Ciesla 2003). With the presence of a large number of pine plantations and natural conifer forests in the southeastern region, this area has a high potential for invasion which could lead to economic and ecological damage in the region (Haugen 2002).

Native species of siricids are common in North America as compared to other countries that have been invaded by *S. noctilio* (Long et al. 2009). For example, more than 23 species and subspecies of siricids belonging to the genera *Sirex*, *Tremex*, *Urocerus*, and *Xeris* are present in North America (Schiff et al. 2006). At least ten species have been recorded in the southeastern region, including both native and non-native species (Schiff et al. 2006). Native siricids generally do not attack unstressed trees, and they inject a Basidiomycete fungus and eggs into weakened and/or dying trees. The larvae feed on fungi and/or wood, helping with the decomposition process of coarse-woody debris (Schiff et al. 2006). The larvae can take 1-3 years to develop inside the tree before they pupate and emerge as adults in late summer or fall. Males emerge first, followed by females who fly to suitable trees to mate and lay their eggs. Siricids have a haplo-diploid sex determination system, where the female will produce both females and males if mated but only male progeny if unmated (Schiff et al. 2006). It appears that *S. noctilio* is most problematic in areas that do not have populations of native siricids (Murphy 1998), such as in Australia and South America (Smith 2002). It is possible that native siricids in North America may be able to outcompete *S. noctilio*, either directly or indirectly, and perhaps be able to limit the spread of *S. noctilio* within the region.

In the southern hemisphere, biological control agents such as entomophagous nematodes [*Deladenus siricidicola* (Fr.) Boidin], and hymenopteran parasitoids (Hymenoptera: *Ibalia* spp., *Megarhyssa* spp., and *Rhyssa* spp.) (Families: Ibalidae and Ichneumonidae), have been used in attempts to control *S. noctilio* in non-native ranges (Taylor 1976, Madden 1998). The female parasitic nematode enters the siricid larvae as it is pupating, and the nematode digests the eggs of the female or testes of the male host.

The adult siricid emerges, but instead of ovipositing eggs, the female deposits nematodes into the trees, thus contributing to their spread (Schiff et al. 2006). There are two important groups of hymenopteran parasitoids: idiobionts (generally ectoparasitoids) and koinobionts (generally endoparasitoids) (Askew et al. 1986). Idiobiont parasitoids paralyze their host on the outside, killing or immobilizing it immediately, lay their eggs and then they develop on the host. Koinobiont parasitoids parasitize on the inside of their host, allowing the host to continue to develop before killing it in later stages.

Hymenopteran parasitoids have been an important part of successful control programs for *S. noctilio* in Australia and South America (Madden 1998, Murphy 1998). North America has 21 species and subspecies of hymenopteran parasitoids in three different families (Stephananidae, Ibaliidae, Ichneumonidae) (Schiff et al. 2006). In the southeastern U.S., siricid parasitoid communities have been documented to include 17 different species, including: *Ibalia leucospoides ensiger* (Norton), and *Ibalia anceps* (Say) (Hymenoptera: Ibaliidae), *Rhyssa howdenorum* (Townes), *Rhyssa lineolata* (Kirby), and *Rhyssa persuasoria persuasoria* (Linneaus) (Hymenoptera: Ichneumonidae) (Taylor 1976, Liu 1992, Murphy 1998). Some of the parasitoid species native to the southeastern region, such as *I. leucospoides ensiger*, *R. howdenorum*, and *R. persuasoria* have already been used or tested in other countries as biological control agents for *S. noctilio* (Taylor 1976). A study conducted over 36 years in Australia indicated that along with the entomophagous nematode, *I. leucospoides* was one of the most effective biological control agents for *S. noctilio* (Collett 2009).

At present, relatively little is known about the distribution and ecology of native siricids and their hymenopteran parasitoids in the southeastern pine ecosystem. The last major field collection of siricids and hymenopteran parasitoids in the Southeast was >40 years ago by Kirk (1974). Three siricid species including *S. abottii* Kirby, *S. cyaneus* Fabricius and *S. nigricornis* Fabricius, five parasitoid species *I. leucospoides*, *R. howdenorum, R. lineolata*, *R. persuasoria*, and a kleptoparasite *Pseudorhyssa maculicoxis* Kreich were documented from these forests (Kirk 1974). In a museum survey, Smith et al. (2002) documented 15 siricid and two parasitoid species in the eastern U.S. (east of the Rocky Mountains and south of Canada). Ten of these species have been observed in the Southeast, including *Eriotremex formosanus* (Matsumura), *Tremex columba* (Linneaus), *S. abottii*, *S. areolatus* (Cressoni), *S. cyaneus*, *S. nigricornis* , *S. longicauda* (Middlekauf), *Urocerus albicornis* (Fabricius), *U. cressoni* (Norton), and *U. taxodii* (Ashmead). Although these species have been reported in this region, at present, there is not much known about when, where, and how to catch them. Assessing the current regional species complex of native siricids and parasitoid species may be needed to evaluate whether and how the southeastern pine ecosystems has potential to withstand invasion of *S. noctilio* when it arrives in the region.

In previous studies on woodboring insects, various trap designs and baits have been used, including flight intercept, silhouette interception, sticky, drainpipe, and bucket traps (Chenier 1989, McIntosh 2001). The multiple funnel trap and the intercept panel trap are two commonly used traps for capturing woodboring insects. The multiple funnel trap, designed to imitate the silhouette of a host tree, consists of black plastic funnels aligned vertically that overlap with a wet collection cup at the bottom (Lindgren 1983). The intercept panel trap was originally developed to capture Coleoptera but can also capture a significant numbers of siricids (Czokajilo et al. 2001). The panel trap is made of a light weight, water-proof, corrugated plastic that bisects in the middle to form a 90° angle (Czokajilo et al. 2001). Both the funnel and intercept traps have been used to capture siricids and their parasitoid species in combination with semiochemical lures. Other studies have modified the funnel or panel traps in various ways (e.g., enlarging the funnels and adding a surfactant as a lubricant) to capture woodboring insects (Moorewood 2002, de Groot 2003). Allison et al. (2011) showed that in Louisiana, adding a lubricant to funnel and panel traps did not necessarily increase siricid catches, but it did catch greater numbers of *I. leucospoides.* Woodboring insects are generally attracted to monoterpenes and ethanol that are released by stressed trees. Monoterpenes are defensive compounds and ethanol is a byproduct of anaerobic respiration that is released when the trees are stressed (Allison et al. 2004). A commercial *Sirex* lure that consists of 70% α-pinene and 30% β-pinene was developed to monitor siricid species, as this combination of monoterpenes was shown to elicit greatest antennal response by *S. noctilio* (Simpson and Mcquilken 1976). The ethanol and *Sirex* lures along with the funnel or intercept panel trap are the most common lures and traps used to capture siricids in national survey programs.

In addition to traps and baits, trap-trees have been found to be an extremely efficient way to capture siricids (Madden 1971). Trap-trees are created either by injecting herbicide into live pine trees (chemical girdling) or by cutting the entire tree down to attract siricids (Minko 1981, Dodds et al. 2010). In the northeastern U.S., trap-trees have been shown to be a more effective and reliable survey tool than traps baited with a semiochemical lure, but the timing of creating these trap-trees is extremely important. It has been shown that trees girdled one month before or up to the beginning of peak flight for *S. noctilio* have the most attraction for woodwasps (Spradberry and Kirk 1978, Zylstra et al. 2010). Similar studies on efficacy of trap-trees and trap-types in the southeastern region of the U.S. are currently lacking.

Our two major research objectives were as follows: 1) to determine the species complex of native siricids and their hymenopteran parasitoids in the Appalachian, Piedmont, and Coastal Plain region of the southeastern U.S.; and 2) to assess the efficacy of various trapping techniques (traps and lures, and trap-trees) for capturing these two taxa.

2.2 Methods

2.2.1 Study Sites

In 2009-2010, siricids were sampled in three southeastern regions including the Appalachian, Piedmont, and Coastal Plain regions in three states as follows: 1) Virginia; 2) Georgia; and 3) Louisiana (Figure 1, Table 1). To maximize the catches and diversity of siricid wasps, all trapping took place in either recently disturbed forests, including those that had experienced windstorms, bark beetle attacks, thinning, and ice-storms or overstocked pine forests.

In Virginia, trapping was conducted in 2009 in the Appomattox-Buckingham State Forest (N 37°23'36", W 78°43'45"), which is Virginia's largest state forest encompassing 8,016 ha. The forest is managed by the Virginia Department of Forestry and was previously used as farmland. In 1954, the farm was converted to forested land and is now being used for recreation, wildlife management, water quality protection, and timber production (Virginia Department of Forestry 2011). Trapping of siricids took place at three different sites in the Forest. Site one was a thinned loblolly stand, that contained scattered pine trees that had previously been damaged due to an ice-storm. Site two was a highly overstocked stand dominated by Virginia pine (*P. virginiana* Mill.) in the overstory, with few understory plants. The stand was also experiencing decline due to windstorms that blew down mature Virginia pines, and a few bark beetle infestations (southern pine beetle, *Dendroctonus frontalis* Zimmermann) that were present in the stand. Site three was a heavily thinned *P. echinata* stand; containing *Quercus* spp., *Carya* spp., and *Acer rubrum* Linnaeus saplings in the understory. In site one, trees were cut down for trap-trees and remained in the forest until August 2010, when each tree was cut into 1m sized logs, and transported to Athens Clarke County, Georgia to monitor wasp emergence.

In Georgia, trapping was conducted in 2009 in the Whitehall Experimental forest (N 33°53'12" , W 83°21'42") in Athens Clarke County. Whitehall Forest is a 340 ha experimental forest in the Piedmont region of Georgia. The sampled site in Whitehall Forest was a natural pine forest dominated by loblolly pines intermixed with various *Quercus* spp., *Carya* spp., and *Liquidamber styraciflua* L. Trapping was also conducted in the Bartram Educational Forest (33°06'43"N, 83°12'40"W), an 856 ha land tract located in the Baldwin State Forest in Baldwin County. In 1990, 80% of the Bartram forest was transformed from farmland into seed orchards (American Dreams Inc. 2011). The study site within Bartram Forest was a 15-20 year old planted slash pine stand, with few understory plants.

In Georgia, trapping was conducted in 2010 in two sites each, in Jackson County (N 34°07'36", W 83°35'25") and Morgan County (N 31°35'17", W 83°28'21"). We focused on saw mills, timber mills, and lumber yards in an effort to increase the number of siricid captures, since there was a large volume and type of coarse-woody debris as suitable habitats for siricids in these areas. The first site was a pine mill that manufactured wood products, such as flooring, sheathing, and structured panels. Sampling was conducted in a mixed pine/hardwood stand located about 800 m away from the manufacturing site. The stand was dominated by loblolly pine with an understory of *Quercus* spp. and *Carya* spp. The second site was used as a log storage area where the logs were brought, stored, and then picked up for transfer to another location. Traps were set up on the border of the facility in mixed hardwoods. The third site manufactures plywood made out of various pine species. This site had a consistent wood pile that was constantly being moved into the manufacturing plant and replaced by new loads of pine trees. Traps were placed in two different locations on this site that were ~800 m apart. The fourth site was a hardwood chip mill that had various hardwoods brought into and manufactured on site. Traps were placed in two different locations on this site that were ~ 600 m apart.

In Louisiana, trapping was conducted in 2009 and 2010 in Grant Parish in the Catahoula Ranger District of the Kisatchie National Forest (N 31°57'56", W 92°38'24"). In 2009, the study was conducted in a 43 ha site composed of loblolly, shortleaf, and longleaf pines in the overstory. The stand was last thinned in October 2008 with the average diameter at breast height (DBH) of the remaining sawtimber was 38 cm. In 2010, trapping was conducted within days of commercial sawtimber thinning in a 67 year-old, 26 ha sized mixed loblolly and longleaf pine stand. Further, twelve trap-trees were created in 2009 on a loblolly pine plantation, approximately 25 years old and located adjacent to a pine sawmill that produces plywood. All of the trees remained in the field until August of 2010 after which trees were cut and logs were moved into screen tents under a covered structure, so that logs were exposed to ambient temperatures but, largely shaded from the sun.

2.2.2 Sampling of Siricid Wasps

2.2.2.1 Sampling with Traps and Lures

Sampling of siricids was conducted in the fall season (September-December) of each year during their peak flight times. In 2009, trapping took place in Virginia, Georgia, and Louisiana. In each site, 30 intercept panel traps (Alpha Scents, Inc., Portland, Oregon) were hung 25 m apart along a linear transect. Intercept panel traps are 106 X 40 cm in height and width, consisting of two black panels made out of corrugated plastic bisecting in the middle at right angles, with a top and bottom, and wet collection cup. In Georgia and Virginia, all traps were hung between two trees, with the trap cup 1-1.5 m off the ground. Traps in Louisiana were hung on 2.4 m tall metal poles, with the collection cup about 1.5 m off the ground. Intercept panel traps were placed on 10 different transects with three traps per transect. Transects were spaced >50 m apart to reduce adjacent trapping effects. Each intercept panel trap had one of the following lures: 1) *Sirex* ultra high release (UHR) lure (70:30 α-pinene: β- pinene, Synergy Semiochemicals, Corp. Burnaby, British Columbia, Canada); (2) *Sirex* UHR + 95% ethanol (EtOH) (ConTech Enterprises, Victoria, British Columbia, Canada); and (3) an unbaited, control trap (Table 2). All trap cups contained 7-8 cm of propylene glycol (Peak RV and Marine Anti-freeze, Old World Industries Inc., Northbrook Illinois), to catch and preserve the insects. Lures were replaced after six weeks when they started showing signs of deterioration.

To compare the trap efficiency of different trap-types, we additionally used Lindgren funnel traps in 2009 using the same lure-types as intercept panel traps in the same sites in Georgia. Funnel traps are composed of eight vertically aligned black funnels that overlap with a wet collection cup attached at the bottom (Lindgren 1983) (ConTech Enterprises Inc., Victoria, British Columbia). In 2009, funnel traps were installed in the following sites: 1) Whitehall Forest, where we installed eight transects, with 12 each of funnel and intercept traps and; 2) Bartram Forest, where we installed 12 transects, with 18 each of funnel and intercept traps. Hence, a total of 30 each of funnel and intercept traps were installed. In addition, four Santé canopy insect traps (Santé traps, Lexington Kentucky) were placed in the canopy in the Bartram forest to capture parasitoids. Santé traps were made out of a fine mesh material, about 3 m tall and 1 m wide, with two collection cups attached to the top and bottom of trap. Unbaited Santé traps were placed 25 m apart along a linear transect and about 5 m high in the tree canopy.

To better understand the effectiveness of host attractant and bark beetle lures to catch siricid wasps, traps were established at four different sites in mid-October, 2010 in Georgia. At each site, traps were baited with either: 1) *Sirex* UHR alone (70:30 α-pinene: β-pinene); 2) *Sirex* + EtOH; 3) EtOH alone; and 4) *Sirex* + EtOH + ipsdienol + ipsenol (referred to as SEII hereafter); and 5) an unbaited, control trap (Table 2). Bubble caps with racemic ipsenol and ipsdienol were used [chemical purities >95%, enantiomeric composition of 50:50 (+)/ (-)] (Contech Enterprises Inc.). Traps and transects were installed and operated similarly to studies conducted in 2009. Hence, all four sites contained a total of 40 traps with 20 each of Lindgren funnel and intercept panel traps. Traps were taken down in mid-December, 2010.

To test the effectiveness of host material (freshly cut pine billets) compared to commercially available lures as an attractant for siricids, 30 intercept panel traps were used in 2010 in Kisatchie National Forest in Grant Parish, Louisiana. Ten linear transects were established with three traps in each transect. Each trap had one of the following lures: 1) *Sirex* UHR lure (70:30 α-pinene: β-pinene); 2) fresh pine lure, a nylon mesh (Amber Lumite® screen of mesh size 81 X 81 mesh/cm, and 0.24 m x 0.85 m in size) containing 10-12 split loblolly pine billets (created by quartering 7.5-10 cm diameter pine bolts) and 10-12 pine boughs (including the foliage); and 3) an unbaited, control trap. Traps suspended from 2.4 m tall metal poles, with the collection cup ~1.5 m from the ground, were spaced by 25 m. Collections were made every week from late-October to mid-December, 2010. Pine bag and *Sirex* lures were replaced every three weeks with the *Sirex* lure replaced as necessary. Due to the high numbers of siricid catches from the fresh pine lure, the contents of the lure were analyzed to find the key constituents. Coupled GC-EAD tests revealed three compounds that exhibited both relatively high quantities in the lure and elicited antennal responses in one native siricid *(S. nigricornis*). These compounds included limonene and beta-phellandrene, collectively called dipentene and caryophyllene. The compounds were placed into 5 polyethylene bags, 5 cm in diameter by 4 cm in length, filled with 5-7 ml of dipentene and 1 ml of caryophyllene. Due to limited quantity of dipentene + caryophyllene, the bags were only added to half of the *Sirex* baited lures two weeks into the experiment. The dipentene + caryophyllene lures remained for 15 days and then were removed from the *Sirex* lures.

All traps were emptied every two weeks from October-December in 2009-2010, and sorted. Siricid species were identified using available taxonomic literature (e.g., Schiff et al. 2006). A voucher collection will be deposited in the Georgia Museum of Natural History, University of Georgia, Athens, Georgia.

2.2.2.2 Sampling with Trap-Trees

In 2009, trap-trees were created in Virginia and Louisiana. In Appomattox, Virginia, three loblolly trees were felled in September 2009. The felled trees stayed in the forest for approximately 11 months to allow siricids to complete life-cycle and for parasitoids to attack siricids. In August 2010, prior to emergence of adults, the felled trees were cut into logs and transported directly back to Athens, Georgia. Logs were placed into screen emergence tents sized 3.9 X 2.7 m (Ozark Trail Polyester Dome Screen House). Logs remained in the tents from early September to early January 2010, and were removed from tents after no wasps emerged for a month. The average log length was 100 cm and the average DBH of the trees were 19.3 ± 0.31 cm. Eighteen logs were then chosen randomly based on position of each tree: six logs each from the top, middle, and bottom of each tree. These selected logs were then cut split, to retrieve any larvae or pupae of siricids. Larvae of siricids were distinguished from those of cerambycid and buprestid larvae by their identifiable posterior cornus on the last abdominal segment (Smith and Schiff 2002). Siricid emergence holes were identified based on shape of hole, as siricids create perfectly round holes throughout their galleries.

In Louisiana, four loblolly pine trap-trees were created in late-September 2009 by applying 1 mL of 20% (w/v) aqueous herbicide dicamba [56.8% active ingredient (a.i) is diglycolamine salt; 480 g/L] (Vanquish, Syngenta Crop Protection, Inc., Greensboro, North Carolina) in a distilled water solution into a 6 -7 cm deep, 10 mm diameter pre-drilled hole every 10 cm around the tree's circumference. Holes were approximately 15 cm above ground level and drilled at a downward 45° angle (Neumann et al. 1982; Neumann and Morey 1984). The number of drilled holes ranged from 5-8 per tree and the absolute amount of the a.i per tree ranged from 2 - 3.2 g. On November 3, 2009, four additional loblolly pine trees were felled and cut into logs (up to the crown) and stacked crosswise atop each other adjacent to the intact crown. Logs stacks and crowns for each tree lay inside the stand at least 15 m apart in a linear array (cut, buck, and stacked). This same process was repeated on four additional trees on November 18, 2009, for a total of 8 trees. A single intercept panel trap was suspended from a 2.4 m aluminum pole adjacent to each trap-tree. Traps were emptied every 1-7 days from November-December, 2009.

Trap-trees were left standing until August 2010, when they were felled and bucked up to bolts as small as 7.6 cm in diameter and placed in 3.5 X 3.5 m sized screen tents (one tree per tent) (REI Screen House, Nylon/mesh, Imported). Logs cut from the dicamba trap-trees had an average length of 125.0 ±7.51 cm and an average DBH of 13.5 ± 0.99 cm. The mean length of the logs cut from the eight trees (late and early) were 113.62 ± 3.62 cm and the mean DBH was 11.3 ± 0.44 cm. Siricids and parasitoids were collected weekly from the emergence tents, and were all identified to species-level.

In October 2010, trap trees were created in Georgia's Whitehall forest. Ten loblolly pine trees were felled and cut into logs (up to the crown) and stacked crosswise atop each other adjacent to the intact crown. Log stacks and crowns for each tree lay inside the stand at least 15 m apart in a linear array (cut, buck, and stacked). Trees were left in the stand for one year. In October 2011, all trees were moved into emergence tents where siricids and parasitoids were collected weekly from the tents and were all identified to species-level. The mean heights of the ten trees were 21.31 ± 0.30 m and the mean DBH was 18.3 ± 0.42 cm.

2.2.3 Statistical Analyses

Trap data were standardized to catches per two weeks to account for disturbed traps and variation in sampling period. Similarly, emergence from trap-trees was standardized to m^3 to account for different tree sizes. Standardized trap catch data for each state and emergence data from trap-trees was analyzed for normality and constant variance (PROC Univariate normal, SAS 2010). Data were not normal and failed to meet the requirements for analysis of variance tests (ANOVA). Various transformations failed to achieve normality. Hence, all data were analyzed using nonparametric tests, such as Kruskal-Wallis and Mann-Whitney U tests (Appendix A) for differences in trap- and lure-types for each state separately, and for trap-tree methods in Louisiana (SAS 2010).

2.3 Results

2.3.1 Sampling of Wasps with Traps

A total of 440 siricids (all females) were collected over the two years in the three different states using intercept and funnel traps (Table 3). The majority of catches were of *S. nigricornis* (which includes *S. edwardsii*) (95%), followed by *T. columba* (2%), *E. formosanus* (1.6%), and *U. cressoni* (1.4%). Further, *S. nigricornis*, *U. cressoni*, *T. columba*, and *E. formosanus* were trapped in Virginia; *S. nigricornis* and *U. cressoni* in Georgia, and *S. nigricornis*, *T. columba*, and *E. formosanus* in Louisiana. In Virginia, the majority of the siricids (85%) were caught in traps during September 28- October 29; in Georgia (72%) during October 7 - November 19; and in Louisiana (86%) during October 16 - November 20.

Lure-type was a significant factor for trap catches of siricids in 2009 in Virginia (H_2 = 11.93, P = 0.003), Georgia (H_2 = 8.597, P = 0.014), and Louisiana (H_2 = 8.922, P=0.012) (Figure 2). *Sirex* lure alone and *Sirex* + EtOH lure captured 2-5 times greater numbers of siricids than the unbaited trap (Figure 2). There was no difference in siricid trap catches between intercept and funnel trap in Georgia (P = 0.553). Lure-type was also a significant factor for trap catches in 2010 in Georgia (H_4 = 9.679, P= 0.046). Traps baited with EtOH lure alone caught lower numbers of siricids than the SEII, *Sirex*, and *Sirex*+ EtOH lures (Figure 3). Similar to 2009, there was no difference in trap catches of siricids between intercept and funnel traps (P = 0.451). In Louisiana in 2010, there was a significant difference in siricid trap catches among traps baited with fresh pine billets, *Sirex* lure alone, and unbaited trap (H_2 =18.23, P < 0.001) (Figure 4). Traps with pine billets captured about three times more siricids compared to the other two traps, and traps with *Sirex* lure caught twice as many siricids than the unbaited trap (Figure 4).

2.3.2 Sampling of Wasps with Trap-Trees

A total of 1,609 siricids and 486 parasitoids emerged from trap-trees created in all the three states (Table 3). The only two wasp species that emerged from the trap- trees were *S. nigricornis* and *I. l. ensiger*. A total of 82 *S. nigricornis* (42 males: 40 females) with no parasitoids emerged from trap-trees in 2010 in Virginia. Siricid species emerged from mid-September to early-December, with females peaking mid-October and males with similar numbers emerging from September through late October (Figure 5). Males appeared to emerge sooner than females with females not emerging until the very end of September. Fifteen siricid larvae were found in nine logs that were split open, and all these larvae were found in the same trap-tree.

A total of 299 *S. nigricornis* (141 males: 158 females) emerged from trap-trees in 2010 in Georgia (Table 3). Siricid species emerged from early October until late November, with the peak occurring in early November (Figure 6). Males emerged sooner than females, with peak emergence in early November and those of females in late November (Figure 6).

A total of 1,228 *S. nigricornis* (899 males: 329 females) and 486 *I. l. ensiger* emerged from the trap- trees in 2010 in Louisiana (Table 3). There was a 28% parasitism rate by *I. l. ensiger*. There were significant differences in the total numbers of siricids ($H_2 = 9.27$, $P = 0.091$) and parasitoids ($H_2 = 8.12$, $P = 0.017$) emerging from trap-trees created using Dicamba, and early and late cut (Figure 7). Both of the cut, buck, and stacked (early and late) trees had >14 times more siricid emergences than those trees that were sprayed with Dicamba, with the greatest numbers coming from trees cut early in the siricid flight season. Parasitoid emergences were twice as much when the trees were cut, buck, and stacked early compared to using Dicamba, but there was no difference between cutting the trees in early and late November or between cutting the trees later and using Dicamba (Figure 7). Siricids emerged from early October until mid-December, with males peaking in late October and females peaking in early November (Figure 8). Parasitoids emerged later than the siricids from mid-October till mid-December, with a peak at the end of November.

2.4 Discussion

In our three-year study, we caught four (three native and one exotic) species of siricid woodwasps in Virginia, Georgia, and Louisiana. As there are 22 (*S. edwardsii* and *S. nigricornis* combined to one species) known native species of Siricidae in North America (Schiff et al. 2006), our study indicates that the southeastern region contains at least 20% of the known North American fauna. Virginia is the only state in which we trapped all the four species, with three species in Louisiana, and two in Georgia. There are likely more species present in these states that were not captured in our study. For example, Kirk (1974) reported *S. abbottii* (Kirby) from the same location (Whitehall Forest) in Georgia where traps were operated in our study. It is unlikely the *S. abbotti* is not in Georgia anymore, but rather that the population is more restricted than other native species making the probability of catching *S. abbottii* much smaller.

There appears to be a significantly larger population of *I. l. ensiger* in Louisiana than in the other two states. Similar methods were used in 2009 in Louisiana for trap-trees (cut buck and stack), and in 2010 in Georgia. However, 486 *I. l. ensiger* emerged from trap- trees in Louisiana, whereas only five emerged in Georgia. These results could represent differences in population-levels in these states, and/or differences in host-types, as well as stand structure and composition between the two states. Another possibility is that differences in timing of trap-trees led to the difference as Louisiana trap- trees were created in early November, and in Georgia they were created in early October. Since *I. l. ensiger* parasitizes egg and first instar stage of siricids, we may have missed collecting *I. l. ensiger* in Georgia since the hosts may have already been in an advanced stage by the peak flight of parasitoid species.

In intercept and funnel traps, the majority of siricids were captured in a one-month period in all three states. The earliest catches in 2009 were in Virginia (October), and in Georgia and Louisiana (November). In 2010, 84% of the captures in Georgia were in November, and 55% of catches were in the first two weeks of November in Louisiana. In trap-trees in Virginia, 74% of the emergences occurred in October, in Georgia 54% of emergences occurred in November, and in Louisiana 64% of emergences occurred in October. In studies conducted during May-September in British Columbia and South Dakota, siricids were not captured and/or peaked until August and September, but these studies ended in September. If these studies had continued into the fall, then the peak flight may have been later to what is seen in the Southeast (Mcintosh et al. 2001, Costello et al. 2008). In New York, *S. noctilio* peaked in July while the native siricids emerged later in the fall, with very little emergence overlap between *S. noctilio* and native siricids (Zylstra et al. 2010).

Greater numbers of siricids were caught in baited than unbaited traps in our study. In 2009, there was no difference in siricid trap catches in traps baited with *Sirex* lure alone or *Sirex* lure with ethanol. In Georgia in 2010, ethanol alone did not capture a greater number of native siricids as also reported from the Great Lakes Region (Coyle et al. 2012). Using the *Sirex* lure alone would be preferred, as it would be cheaper and easier than using a combination of lures. However, in 2010 in Louisiana, fresh pine lure clearly had three times greater trap catches than the *Sirex* lure. These results suggest that although *Sirex* lure is better than some of the other tested lures, it may still be missing some important volatile components from the pine trees, and that the bait would likely benefit from the inclusion of these components.

There were no differences in catches of siricids between intercept and funnel traps, although we caught only 74 siricids in Georgia. Studies in Australia have found that intercept traps captured 40% more S. *noctilio* then the Lindgren funnel trap (Bashford 2008). Similar to our study, there were no differences in native siricid catches between the two trap-types in South Dakota (Costello et al. 2008), and for *S. noctilio* in New York (Dodds and de Groot 2012). These regional differences indicate that as compared to the southern hemisphere, intercept panel and funnel traps may capture similar numbers of native siricids and *S. noctilio* in the northern hemisphere.

In Louisiana, more siricids emerged from trees that were not chemically treated as compared to the ones that were treated with dicamba. Further, there was a significant difference in siricid emergence between the early and late cut, bucked, and stacked trap-tree method. Cutting the tree at the peak of siricid flight (early November) captured significantly more siricids and parasitoids than cutting the tree two weeks later. Dicamba is commonly used to create trap logs for monitoring populations of *S. noctilio* elsewhere (Minko 1981), and it is likely that native siricids don't cue in on chemically treated trees as much as *S. noctilio*. Another reason for this trend may be timing as trees were treated with dicamba in late September, which is in the early part of the siricid flight season. In the Southern Hemisphere, trap trees for detection of *S. noctilio* were created 2-3 months before its peak flight period (Neumann et al. 1982), but in North America this is not ideal. This is because there are a large number of bark and woodboring beetles in North America (Mitton et al. 1983, Lingfelter 2007) that may colonize the trees before siricids in the late-summer and exert competition pressure. For example, in New York, it was shown that injecting dicamba into the tree stem one month prior to peak flight or at flight season captured the greatest numbers of *S. noctilio* (Zylstra et al. 2010). Late October to early November is the peak flight period of native siricids, therefore using dicamba in late September could have been too early to sample our native species.

Trap-trees were found to be a more effective way to monitor populations of native siricids and their parasitoids than using traps with semiochemical lures. Overall, eight times more siricids emerged from trap-trees than were caught in funnel and intercept traps. Further, baited traps did not catch any parasitoids of siricids. These results are consistent with previous observations that traps baited with host material are the most attractive to native siricids. Zylstra et al. (2010) found that hanging funnel traps over trap trees did catch *S. noctilio*, but the actual trap-trees themselves had seven times more *S. noctilio* emerging from them than were caught in traps. Another reason could be that as trapping was conducted during the fall, openings of the traps were frequently clogged with leaf and needle litter, possibly preventing insects from being captured in the collection cups. In support of this idea, captures were greater in 2010 than in 2009 in Louisiana when traps were collected once a day as compared to once a week and traps were able to be cleared of debris more often.

2.5 Conclusions

Our study provides information about the distribution, seasonal patterns, and trapping methods for native siricids and their parasitoids in three southeastern regions of U.S. Results indicate that host material (trap-trees or traps baited with pine billets) is optimal for monitoring populations of these two taxa. The optimal time to capture siricids in funnel and intercept traps is in October in Virginia, and November for Georgia and Louisiana. The most opportune time to create trap-trees is in early November for maximizing catches of siricids and parasitoids.

2.6 Acknowledgements

We thank laboratory and field assistance provided by the entire Forest Entomology Laboratory at the University of Georgia, and by Chip Bates, James Johnson, and Eric Mosely from the Georgia Forestry Commission. Financial support for the project was provided by USDA Forest Service *Sirex* grant, and Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia.

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Table 2.1. Geographical locations and site descriptions for study sites in Virginia, Georgia, and Louisiana for trapping of siricid woodwasps and their hymenopteran parasitoids in 2009-2010.

Attributes	**Virginia**	**Georgia**				**Louisiana**
County	Appomattox	Clarke	Baldwin	Jackson	Morgan	Winn
Location	Appomattox Buckingham State Park	Whitehall Forest	Bartram Forest	Pine Mill, Lumber Yard	Pine Mill, Hardwood Mill	Kisatchie National Forest
Region	Appalachian	Piedmont	Piedmont	Piedmont	Piedmont	Coastal Plain
Year of sampling	2009	2009	2009	2010	2010	2009-2010
Latitude and Longitude	N 37°23'36" W 78°43'45"	N 33°53'12" W 83°21'42"	N 33°06'43" W 83°12'40"	N 34°07'36" W 83 35'25"	N 33°35'17" W 83°28'21"	N 31°57'56" W 92°38'24"
Dominant tree species	*P. taeda, P. virginiana, P. echinata*	*P. taeda*	*P. elliottii*	*P. taeda*	*P. taeda*	*P. taeda, P. palustris, P. echinata*
Mean (± SE) temperature (°C) (sampling time)	16.5 ± 6.8	14.3 ± 3.5	16.3 ± 8.8	14.53 ± 4.3	15.5 ± 4.2	18.5 ± 3.4* 18.6 ± 3.6**
Mean monthly rainfall (cm) (sampling time)	12.9 ± 3.6	20.9 ± 3.8	18.6 ± 3.6	8.6 ± 2.2	8.7 ±0.5	19.5 ± 7.0* 8.1 ± 2.8**
Mean growing degree days (sampling time)	187 ± 112.1	292 ± 144.2	382 ± 313.1	342 ± 169.1	374 ± 175.7	406 ± 167* 454 ± 175.8**
Elevation (m)	171	213	117	279	206	25

*Sampling in 2009 **Sampling in 2010

Table 2.2 Semiochemical component, release rates, chemical purity, enantiomer composition, and release device of lures used in funnel and intercept panel traps in Virginia, Georgia, and Louisiana.

Semiochemical Component	Release Rate (mg/d)	Chemical Purity (%)	Enantiomer Composition (±)	Release Device
Ethanol	1000-2000	95	NA	Ultra-High Release
Sirex Lure 70 α-pinene: 30 β-pinene	1500-2500		(-) α-pinene (-) β-pinene	Ultra-High Release
Ipsenol	0.1-0.2	95	50/50	Bubble cap
Ipsdienol	0.1-0.2	95	50/50	Bubble cap

Table 2.3. Siricid species caught in 2009-2010 in traps and emerged from trap trees in Georgia, Virginia and Louisiana baited with different semiochemicals.

	Bait-Type								
Hymenoptera Species	**Unbaited**	***Sirex****	**S+EtOH****	**ETOH***£**	**S+E+I+I°£**	**S+D°°¤**	**Pine°°°¤**	**Trap-Trees**	**Total**
Siricidae									
Sirex nigricornis (Fabricius*)*	41	121	36	1	13	3	203	1609	2027
Urocerus cressoni (Norton*)*	0	2	3	0	0	0	0	0	5
Tremex columba (Linneaus)	1	6	3	0	0	0	0	0	10
Eriotremex formosanus (Matsumura)	1	2	2	0	0	2	0	0	7
Ibalidae									
Ibalia leucospoides ensiger (Norton)	0	0	0	0	0	0	0	486	486
Total	43	131	44	1	13	5	203	2,095	2,535

* *Sirex* lure
** *S* + ETOH: *Sirex* +Ethanol
***ETOH: Ethanol alone
°I: *Sirex* + Ethanol+ Ipsenol + Ipsdienol
°°S+D: *Sirex*+ Dipentene
°°°Pine: Fresh pine lure
£Only used in GA in 2010
¤Only used in Louisiana in 2010

<u>Figure Legend</u>

Figure 2.1. Geographical locations of study sites to sample siricid woodwasps and their hymenopteran parasitoids in 2009-2011 in Virginia, Georgia, and Louisiana.

Figure 2.2. Mean (± SE) number of siricid adults caught per 14 days per trap in (A) Virginia, (B) Georgia, and (C) Louisiana. Trap catches for funnel and intercept panel traps were pooled in Georgia.

Figure 2.3. Mean (± SE) number of siricid adults caught per 14 days per trap in baited (funnel and intercept) traps in Georgia 2010. Baits included- *Sirex*: *Sirex* lure, *Sirex* + EtOH: *Sirex* lure and ethanol, and SEII: Sirex lure+ Ethanol+ Ipsenol+ Ipsdienol.

Figure 2.4. Mean (± SE) number of siricid adults caught per 14 days per trap in baited traps in Louisiana 2010. Baits included- *Sirex: Sirex* lure and Pine Billet: fresh pine billets.

Figure 2.5. Mean (± SE) number of female and male *Sirex nigricornis* emergence from loblolly pine trap-trees in 2010 in Virginia.

Figure 2.6. Female and male siricid adult phenology from trap-trees in 2011 in Georgia.

Figure 2.7. Female and male siricid adults and parasitoid emergence from trees killed with dicamba and by cutting in early and late November in 2010 in Louisiana.

Figure 2.8. Mean (± SE) number of *Sirex nigricornis* and *Ibalia leucospoides ensiger* parasitoid emergence from pines in 2010 in Louisiana.

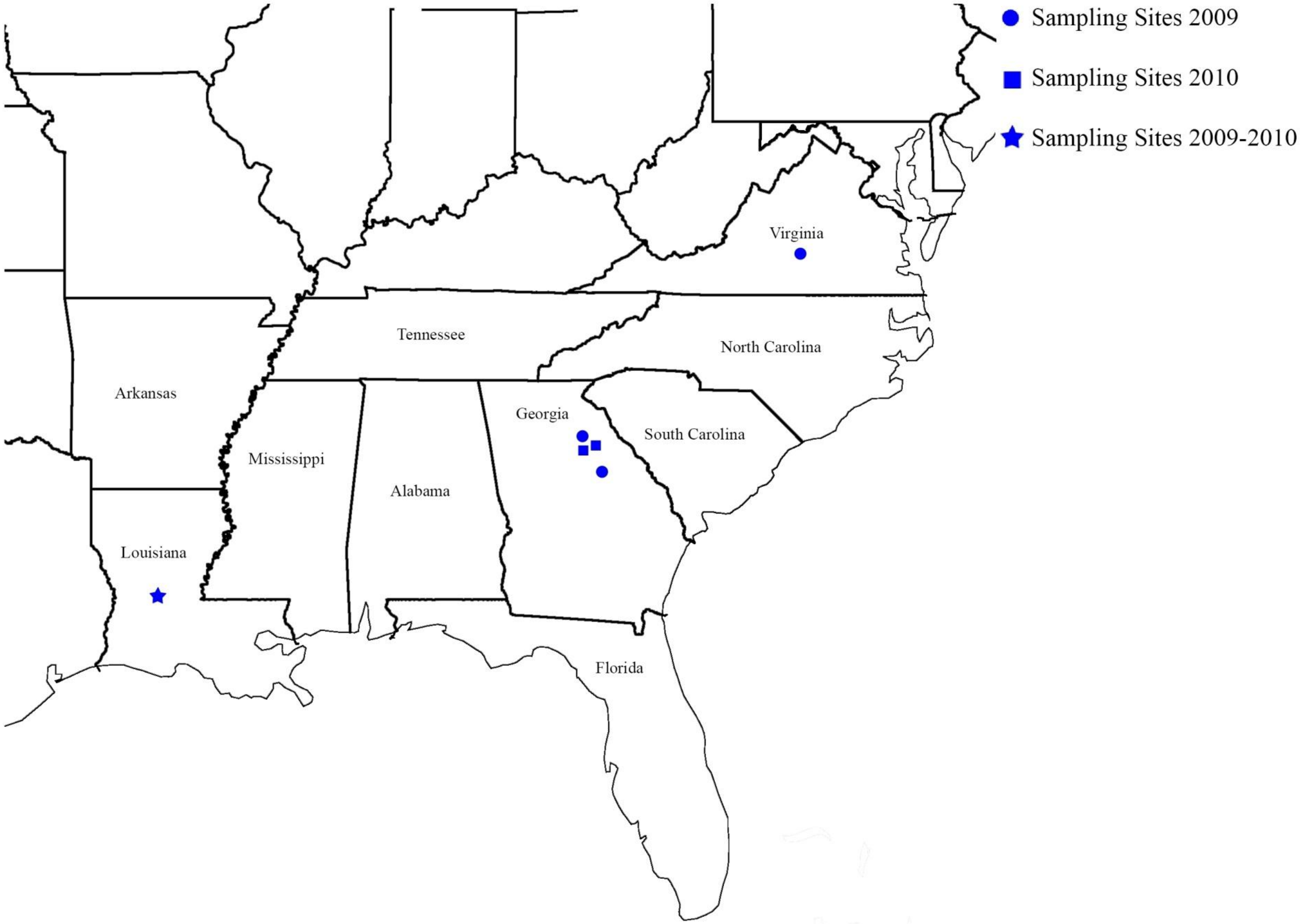

Sampling Sites 2009
Sampling Sites 2010
Sampling Sites 2009-2010
Virginia
Tennessee
North Carolina
Arkansas
Georgia
South Carolina
Mississippi
Alabama
Louisiana
Florida

Figure 2.1

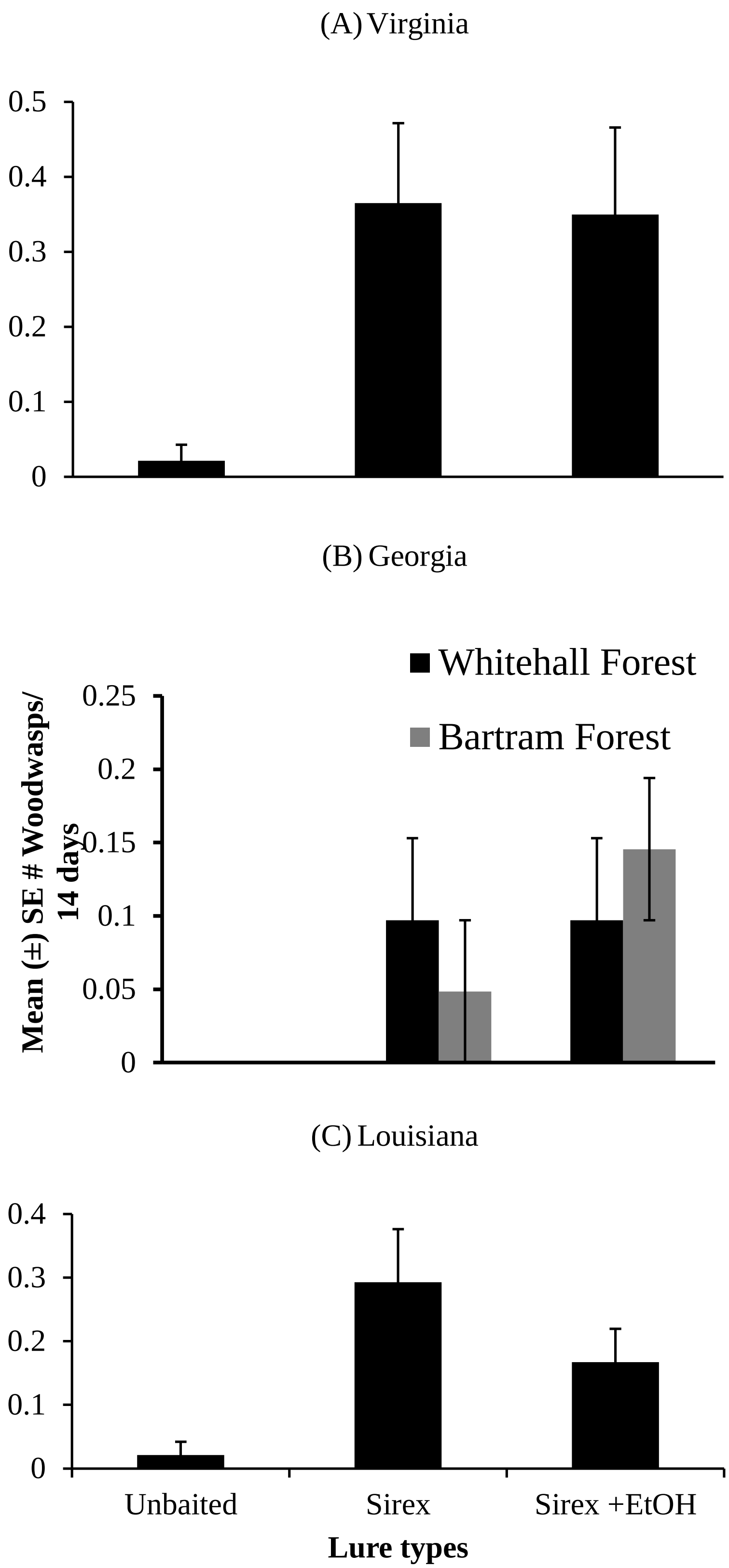

(A) Virginia
0.5
0.4
0.3
0.2
0.1
0
(B) Georgia
Whitehall Forest
Bartram Forest
0.25
0.2
0.15
0.1
0.05
0
Mean (±) SE # Woodwasps/ 14 days
(C) Louisiana
0.4
0.3
0.2
0.1
0
Unbaited
Sirex
Sirex +EtOH
Lure types

Figure 2.2

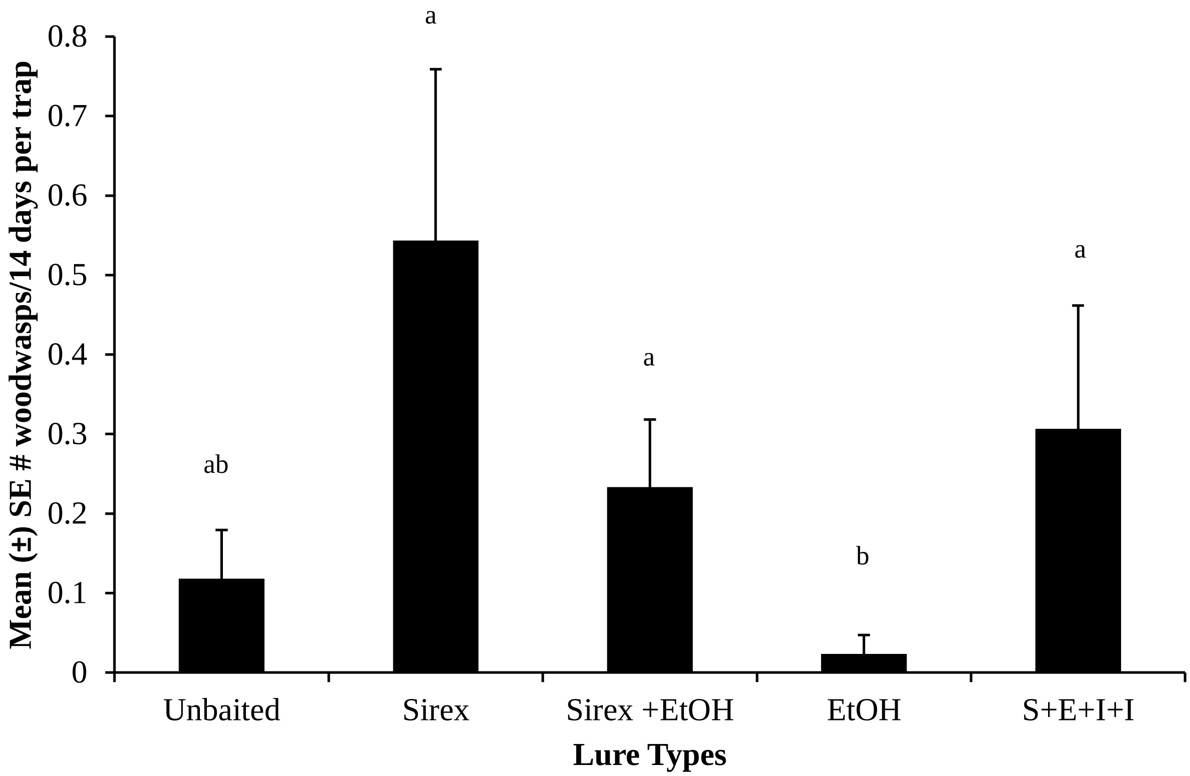

a
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean (±) SE # woodwasps/14 days per trap
a
a
ab
b
Unbaited
Sirex
Sirex +EtOH
EtOH
S+E+I+I
Lure Types

Figure 2.3

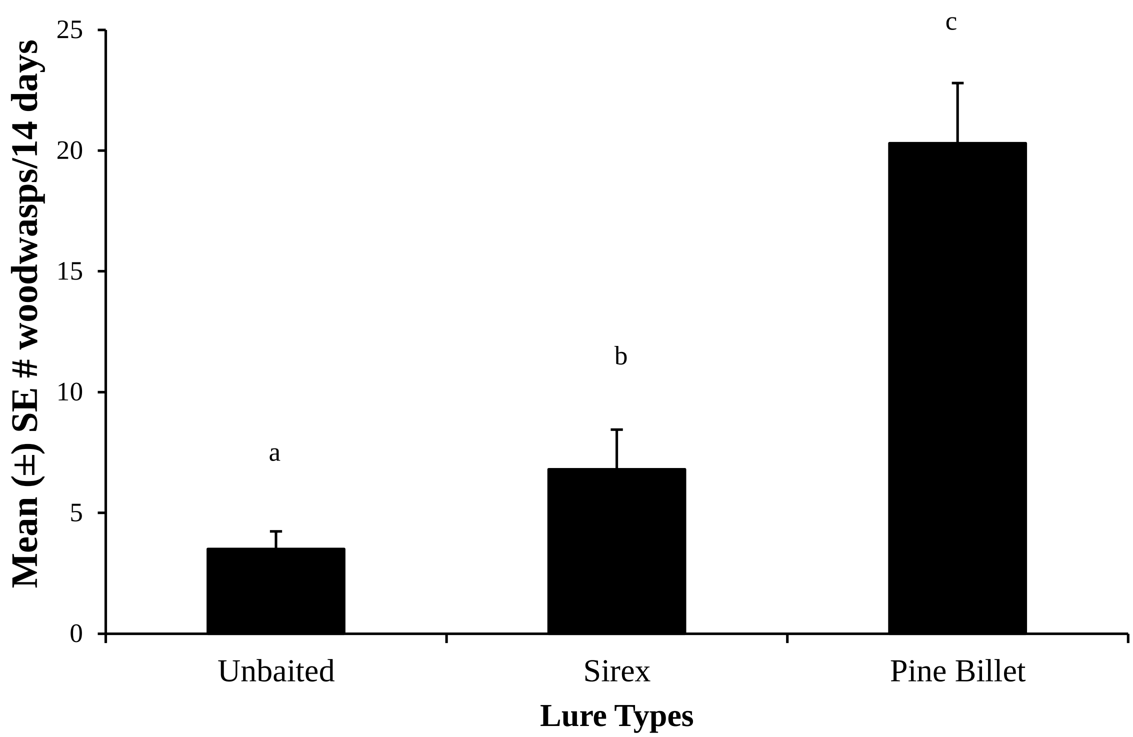

Mean (±) SE # woodwasps/14 days
25
20
15
10
5
0
a
b
c
Unbaited
Sirex
Pine Billet
Lure Types

Figure 2.4

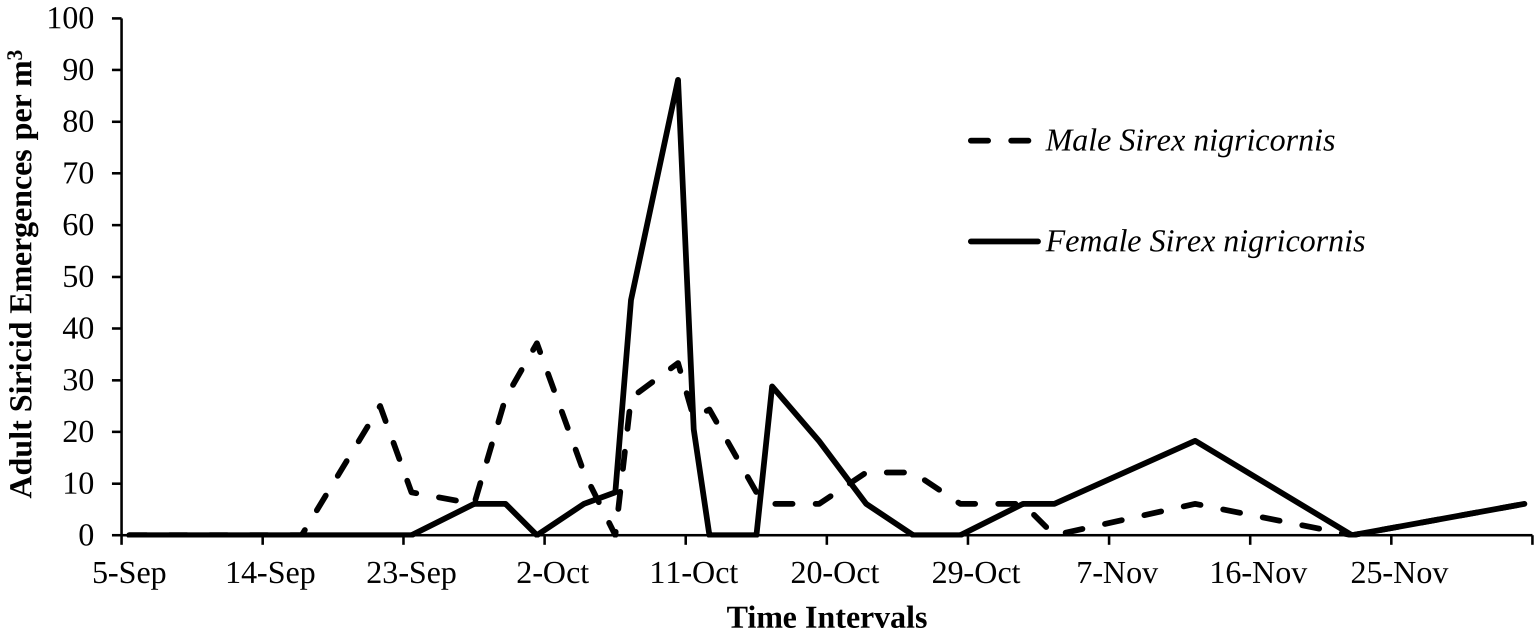
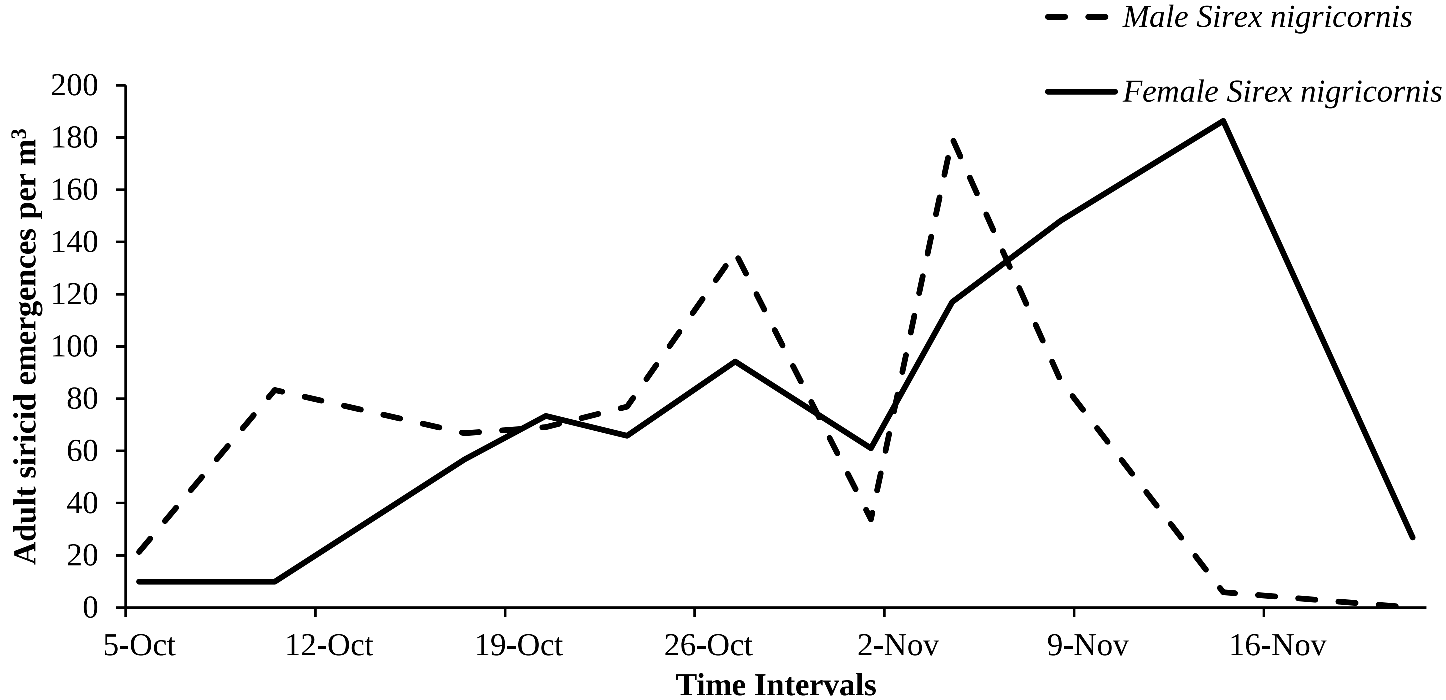

100
90
80
70
60
50
40
30
20
10
0
Adult Siricid Emergences per m3
5-Sep
14-Sep
23-Sep
2-Oct
11-Oct
20-Oct
29-Oct
7-Nov
16-Nov
25-Nov
Time Intervals
Male Sirex nigricornis
Female Sirex nigricornis

Figure 2.5



Male Sirex nigricornis
Female Sirex nigricornis
200
180
160
140
120
100
80
60
40
20
0
Adult siricid emergences per m3
5-Oct
12-Oct
19-Oct
26-Oct
2-Nov
9-Nov
16-Nov
Time Intervals

Figure 2.6

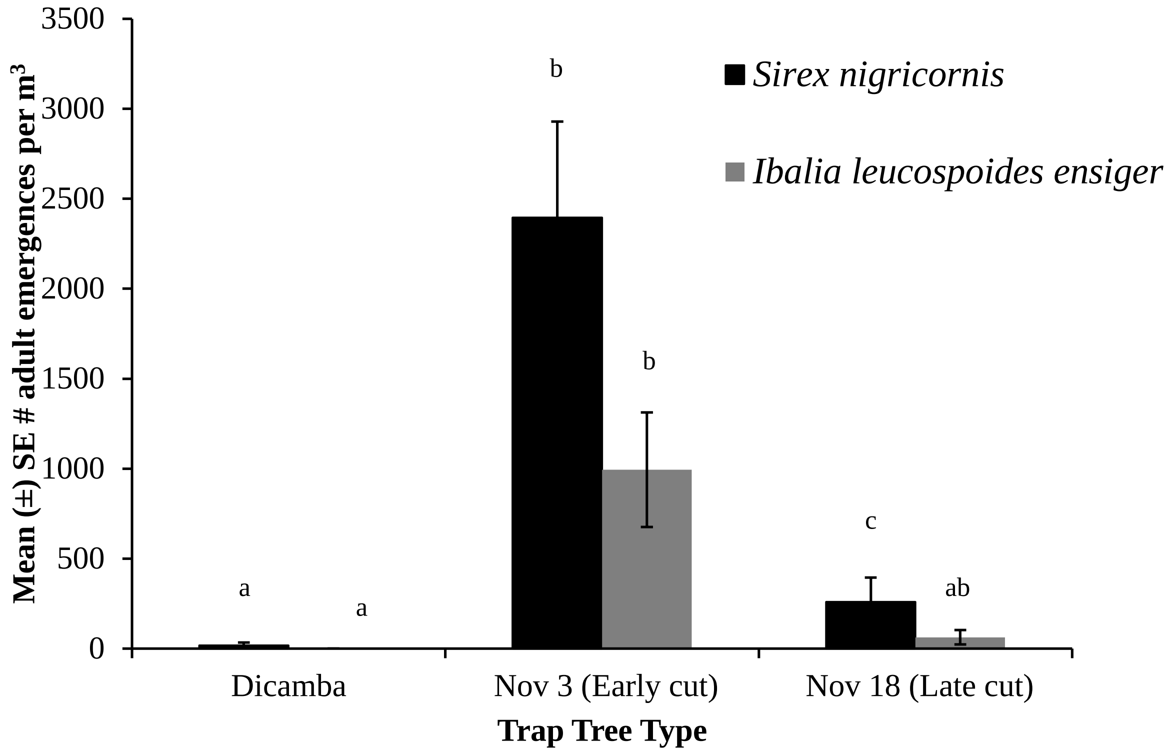

3500
3000
2500
2000
1500
1000
500
0
Mean (±) SE # adult emergences per m³
b
b
a
a
c
ab
Sirex nigricornis
Ibalia leucospoides ensiger
Dicamba
Nov 3 (Early cut)
Nov 18 (Late cut)
Trap Tree Type

Figure 2.7

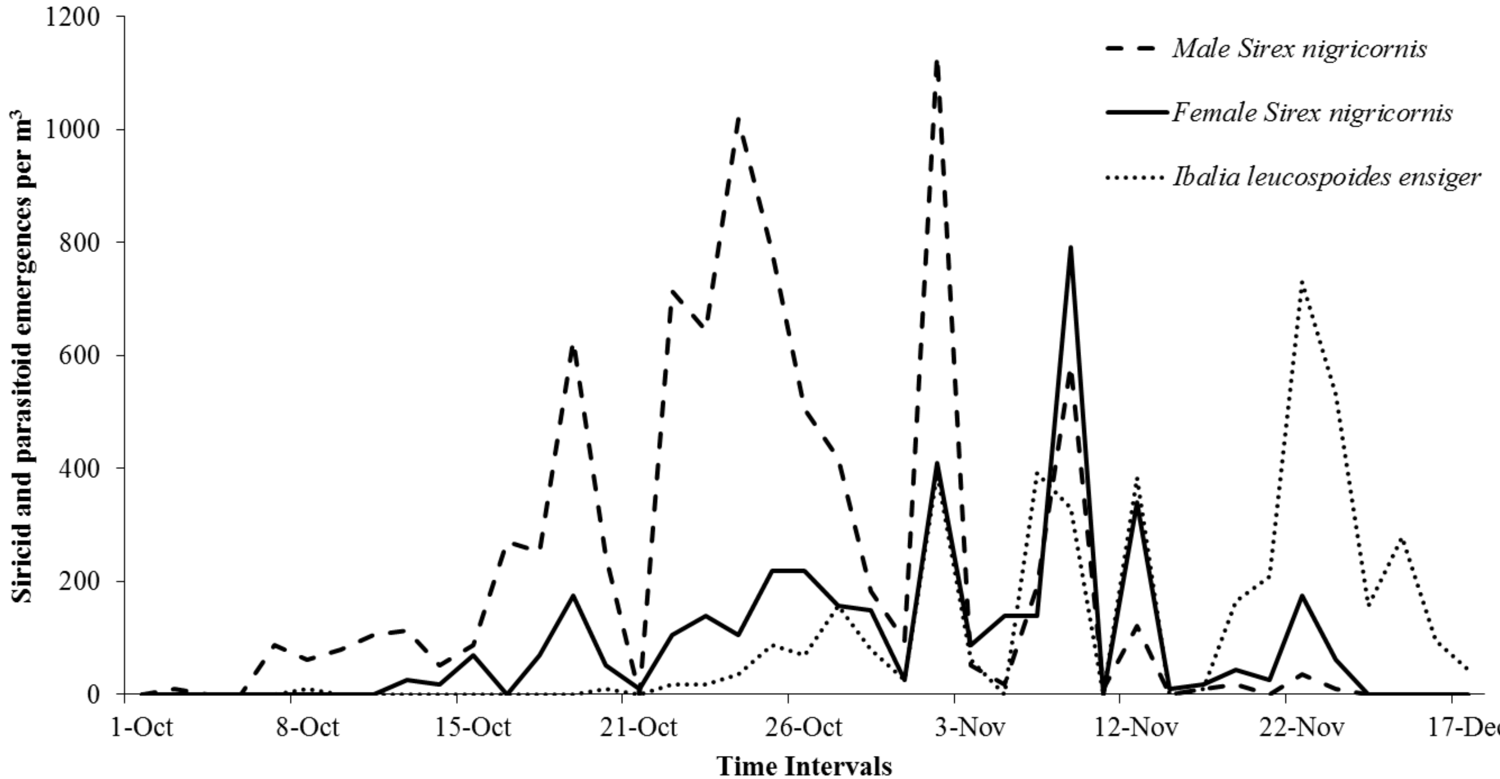

1200
1000
800
600
400
200
0
Siricid and parasitoid emergences per m³
Male Sirex nigricornis
Female Sirex nigricornis
Ibalia leucospoides ensiger
1-Oct
8-Oct
15-Oct
21-Oct
26-Oct
3-Nov
12-Nov
22-Nov
17-Dec
Time Intervals

Figure 2.8

CHAPTER 3

EFFECTS OF LURE PLACEMENT ON TRAPS ON CATCHES OF LARGE WOODBORING BEETLES IN SOUTHERN PINE STAND[1]

[1] B F Barnes, Miller D R, Crowe C M, and Gandhi K J K. To be submitted to Journal of *Economic Entomology*

Abstract

In the United States, surveys of bark and woodboring beetles (Coleoptera: Buprestidae, Cerambycidae, and Curculionidae) are conducted annually to detect new exotic species that can cause ecological and economical damage to forest ecosystems. Typically, Lindgren funnel traps and intercept panel traps baited with various lures are used in these surveys to catch bark and woodboring beetles. Trapping efficiency of these two trap-types have been studied in the past, but little is known about how they could be modified in terms of lure placement to increase the number, and species richness and diversity of captured woodboring beetles. We studied the efficacy of modified funnel traps to maximize catches and species richness of woodboring beetles in southeastern pine stands. During June-August 2010, we established ten replicates of three traps per block in a mature loblolly pine (*Pinus taeda* Linneaus) stand in the Oconee National Forest, Georgia. Traps were all baited with a combination of ultra-high-release ethanol and α-pinene, and racemic ipsenol and ipsdienol. Three trap-types were used as follows: 1) intercept panel trap; 2) modified funnel trap with lures placed on the inside of trap; and 3) modified funnel trap with lures placed on the outside of trap. Funnel traps were modified by increasing the diameter of the center from 5.5 to 12 cm, so that lures could fit in the trap. A total of 2,130 beetles in three different woodboring beetle families (Buprestidae, Cerambycidae, and Elateridae) representing 23 species were captured during the summer. Twice as many beetles were caught in the funnel traps with the lures placed on the inside than the other two trap-types, and these trends were also present at the species-level. None of the beetle species preferred the lures placed on the outside of the funnel traps, and only one elaterid beetle (*Alaus myops* F.) preferred the panel instead of funnel traps. Species richness was highest in funnel traps with lures placed on the inside. Species composition was different between the three different trap-types with funnel traps with the lure placed on the inside and the panel traps present on the opposite end of the hypothetical gradient. We conclude that funnel traps with the lures placed on the inside will likely maximize catch and species richness of woodboring beetles; however different species may be caught in these three trap-types.

Keywords: Cerambycidae, Buprestidae, Elateridae, Intercept panel trap, Lindgren funnel trap, Semiochemical lures, Southeast United States

3.1 Introduction

Woodboring beetles (Coleoptera: Buprestidae, Cerambycidae, and Elateridae) are abundant, species-rich, and as saproxylic insects (those dependent upon dead or decaying wood), are critical components of nutrient cycling within forest ecosystems. There are nearly 718 species of buprestids, 987 species of cerambycids, and 800 species of elaterids in North America (White 1983, Lingfelter 2007). Woodboring beetles typically colonize stressed and declining trees, assist with breakdown of coarse-woody debris, and contribute to overall decomposition within forest stands (Basham and Belyea 1960, Dajoz 2000). However, they are also economically important since their larvae bore through the phloem and xylem (or wood) creating large diameter holes and tunnels which can significantly degrade the quality of forest products (Vallentgoed 1991). Logs from North America that have been attacked by *Monochamus* spp. (Cerambycidae) are not allowed into many countries, as these beetles can vector pinewood nematode, which can kill conifer trees in the non-native range (Cerezke 1977, Dwinell and Nickle 1989).

Exotic woodboring beetles from other continents have already caused significant damage and ecological changes in forest ecosystems throughout North America. Since 1996, Asian longhorned beetle (ALB), *Anoplophora glabripennis* Motschulsky, has killed thousands of maple trees (*Acer* spp.) in the northeastern part of the U.S., and it is estimated that if ALB continues to spread to every urban area in the United States, 35% of total canopy and 30% of trees will be lost (USDA APHIS 2005). Since 2002, emerald ash borer, *Agrilus planipennis* Fairmaire, has killed or weakened tens of millions of ash trees (*Fraxinus* spp.) in 14 states in the Midwest (USDA APHIS 2010).

In the U.S., trapping surveys e.g., Early Detection Rapid Response (EDRR) and Cooperative Agricultural Pest Surveys (CAPS) are conducted annually to identify, delimit, and attempt to eradicate new exotic species in the country (Dodds et al. 2010). A funnel or an intercept panel trap baited with various semiochemical lures are typically used to sample woodboring beetles in these surveys (Jackson et al. 2011). There has been significant research conducted on various trapping methods for cerambycid beetles. Two studies by Moorewood et al. (2002) and Dodds et al. (2002) reported that panel traps caught greater numbers of cerambycids than funnel traps, respectively, in British Columbia and New Hampshire. In contrast, Pajares et al. (2004) found no differences in catches of cerambycid beetles between funnel and intercept traps in southeastern Spain. Other studies focused on the effectiveness of a lubricant (Rain-X, aerosol formulations of Teflon) on the surface of panel and multiple funnel traps, and found that beetle catches were enhanced by addition of a lubricant (de Groot and Nott 2003, Allison et al. 2011). Miller and Duerr (2008) reported that using a wet, instead of dry collection cup in funnel traps increased the likelihood of capturing woodboring beetles.

A large number of woodboring beetles are attracted to volatiles, such as ethanol and monoterpenes, that are emitted from conifer species when they are weakened (Chénier and Philogène 1989). Miller et al. (2011) found that traps baited with α-pinene, ethanol, and racemic ipsenol and ipsdienol is the best lure combination when trying to capture a broad range of saproxylic beetles in the southeastern U.S. Traditionally, these lures are placed on the outside of the funnel trap, which allows for a point-source plume pattern, leaving the trap from only one funnel (Lindgren 1983). Hanging the lures inside of the trap, however, allows for a more dispersed pattern where all pheromones or volatiles are emitted through multiple funnels (Fig. 1). In contrast to trap-types, there have been few studies assessing the lure placement on the individual trap itself. Dodds et al. (2010) indicated that hanging lures above the trap caught significantly fewer cerambycid beetles compared to the most common method of hanging lures on the trap itself. Miller et al. (*unpublished data*) found that large woodboring beetles were captured in greater numbers when lures were placed inside instead of on the outside of funnel traps. In this study, funnel traps with the lures placed on the inside were modified for the lures to fit, with the diameter of the center hole of each funnel being manually increased from 5.5 to 12 cm. The funnel traps with the lures placed on the outside were unmodified. Currently, it is unclear if woodborer catches increased due to either the larger center hole or because of the placement of the lures. We therefore, decided to modify both the trap-types and test if lure placement altered catches of woodboring beetles.

The two major objectives of this one-year study were as follows: 1) to determine the effect of lure placement on modified 10-unit Lindgren funnel traps on the capturing efficiency of woodboring beetles; and 2) to assess the effects of an unmodified panel trap to lures on the inside and lures on the outside of modified Lindgren funnel trap on total catches and diversity of woodboring beetles.

3.2 Methods

3.2.1 Study Sites

The study on woodboring beetles was conducted during June-August 2010 in the Oconee National Forest near Eatonton, Georgia (N 33°19'35", W 83°23'16"). Sampled stands were primarily dominated by *Pinus taeda* Linnaeus as the overstory tree species and the understory by *Ulmus alata* (Michaux), *Cornus florida* (Linnaeus), *Quercus nigra* (Linnaeus) and *Q. falcata* (Michaux). The mean (± SE) age of the trees in the overstory was 34 ± 2.3 years, diameter at breast height (DBH) was 30 ± 2.3 cm, and height was 13.1 ± 0.84 m. Soil consisted of a sandy clay loam, sandy loam mixture. The mean (± SE) precipitation at this site from June-August 2010 was 11.43 ± 4.5 cm, temperature was 28.3 ± 0.32 ºC, and growing degree days were 1,015 ± 22.9 (NOAA 2012).

3.2.2 Beetle Sampling

A randomized complete block design was established in the Oconee National Forest with ten replicates of three traps per block. Traps and blocks were placed 10 m and 50 m apart, respectively. All three traps per block were suspended between two trees by a rope, with the collection cup hanging about 0.5 m off the ground. Two different types of traps were used in the study, including intercept panel traps (Panel) (IPM Technologies, Portland, Oregon) and ten-unit multiple funnel (funnel) traps (ConTech Enterprises, Victoria, British Columbia). Funnel traps were modified by increasing the diameter of the center hole from 5.5 to 12 cm, to make the funnels wide enough for the lures to fit inside. Intercept panel traps measured 106 x 40 cm in height and width, and these traps consisted of two black panels made out of corrugated plastic bisecting in the middle at right angles, with a top and bottom (Czokajlo et al. 2003). Traps were baited with ethanol ultra-high release (UHR) (150ml), (-)-α-Pinene UHR pouches (200 ml), and racemic 50(+): 50(-) ipsenol and ipsdienol bubble caps (ConTech Enterprises). A wet collection cup was attached at the bottom of all the traps that contained 7-8 cm of propylene glycol (Peak RV and Marine Anti-freeze, Old World Industries Inc., Northbrook, Illinois) to kill and partially preserve specimens.

Collections were made every 14 days. All adult specimens were identified using the Illustrated Key to the Longhorned Woodboring Beetles of the Eastern United States (Lingfelter 2007), American Beetles (Bellamey 2002), and Identification and Phylogenetic Characterization of Buprestidae in the Southeastern United States (Hansen 2010). A voucher collection was deposited at the USDA Forest Service, Southern Research Station, Athens, Georgia.

3.2.3 Statistical Analyses

Trap catches were pooled for each trap and analyzed for normality and constant variance (PROC Univariate Normal) for beetle species that had greater than 50 individuals captured (SAS 2007). The trap data were not normal, and therefore, data were transformed using log10 (x +1) after which normality was achieved. All data were analyzed using analysis of variance test (ANOVA) and Tukey's HSD test was used to conduct pairwise comparison among treatment levels for significant factors at $\alpha = 0.05$ (SAS 2007). Nonmetric multidimensional scaling (NMS) was conducted to assess species richness and differences in species composition among trap-types (PC-ORD™ version 5.31) (McCune et al. 2002). Data was first checked for skewness, kurtosis, and coefficient of variance. Final NMS ordination analysis was run using two dimensions, with one run of real data and 250 iterations. An ordination graph was created using mean ($\pm$SE) coordinates for two axes that explained the most variation in the data.

3.3 Results

A total of 2,130 individuals from 23 species in three woodboring beetle families: Buprestidae, Cerambycidae, and Elateridae were captured during this study (Table 1).

Buprestid beetles accounted for 10% of total individuals captured, with 215 individuals and four species. Cerambycid beetles comprised 76% of the total individuals with 1,629 individuals and 17 species caught in traps. Elaterid beetles accounted for 14% of total individuals captured, with 286 individuals and two species (Table 1). Five beetles that had >5% of total catches included *Monochamus titillator* F. complex (982 individuals, cerambycid), *Xylotrechus sagittatus* Germar (339, cerambycid), *Alaus myops* L. (282, elaterid), *Buprestis lineata* F. (156, buprestid), and *Acanthocinus obsoletus* Oliver (130, cerambycid).

Randomized block ANOVA indicated there were twice as many beetles caught in the funnel traps with the lures placed on the inside as compared to the funnel traps with the lures placed on the outside or the panel traps ($F_{2,27} = 20.54$, $P = 0.001$) (Fig. 2). *Monochamus titillator* ($F_{2,27} = 18.15$, $P = 0.001$), *X. sagittatus* ($F_{2,27} = 18.46$, $P = 0.001$), *B. lineata* ($F_{2,27} = 4.04$, $P = 0.0292$), and *A. obsoletus* ($F_{2,27} = 4.58$, $P = 0.019$) were all caught in 2-3 times greater numbers in modified funnel traps with the lures placed on the inside than in funnel traps with lures placed on the outside or in panel traps (Fig. 3 A-D). In contrast, *A. myops* was caught in greater numbers in panel traps rather than either of the modified funnel traps ($F_{2,27} = 6.32$, $P = 0.005$) (Fig. 3 E).

Species richness was greater in funnel traps with lures placed on the inside as compared to the other two trap-types (Fig. 4) ($F_{2,27} = 18.81$, $P = 0.001$). The ordination graph constructed through NMS technique indicated that species composition of woodboring beetles in funnel traps with lures placed on the inside was distinct from those with lures placed on the outside on funnel traps or panel traps (Fig. 5). Interestingly, funnel traps with lures placed on the inside and panel traps were placed on the opposite end of the ordination graph with no overlap. Funnel traps with the lure placed on the inside were in intermediate locations suggesting that they contained a subset of species from each of the other two trap-types.

3.4 Discussion

The southeastern U.S. plays an important role in the timber industry since 58% of all timber in the U.S. and 16% of the world's timber is produced in the region (Wear and Greis 2002). Establishing a trapping system that increases the efficacy of trapping to detect exotic and native large woodboring beetles at low population levels will increase the likelihood of eradicating pockets of large populations of beetles before they become fully established. If an exotic species can be detected early, then the success of eradication increases (Myers et al. 2000): therefore, optimizing catches could potentially reduce the damage to the structure and function of forests in the Southeast U.S. Our results from a one-summer study are consistent with those found by Miller et al. (*unpublished data*) where the trap catches of woodboring beetles were significantly increased by placing the lures on the inside of a Lindgren multiple funnel trap as compared to leaving the lures outside of the trap. Further, we found greater species richness and distinct species composition of woodboring beetles in modified funnel traps with lures placed on the inside as compared to the other two trap types.

Four species showed a significant preference for modified funnel traps with lures placed on the inside: *X. sagittatus*, *M. titillator* complex, *A. obsoletus*, and *B. lineata.* Previous studies in the Southeast U.S. show varying results for captures of most abundant beetles in different trap-types. For example, Miller et al. (2011) found that *M. titillator* complex showed a preference for panel traps over the multiple funnel traps, whereas *X. sagittatus*, *A. obsoletus*, and *B. lineata* did not show a preference for funnel or panel traps. Dodds et al. (2010) found that *X. sagittatus* preferred the panel trap over the Lindgren multiple funnel trap. In contrast, these four species were consistently caught in greater numbers in funnel traps with lures on the inside than in the intercept panel traps with direct implications for monitoring of populations of these species, at least in the southeastern region.

Similar to trap catches, species richness of woodboring beetles was also greatest in funnel traps with the lures placed on the inside and lowest in the panel traps. Further, species composition of funnel traps with the lures placed on the inside was distinct indicating that we caught different beetle species in this trap-type. Most of the other studies conducted on trapping efficiency have rarely reported species richness, diversity, or composition (Morewood et al. 2001, de Groot and Nott 2003, Dodds 2011). Considering that intercept panel traps were placed at the opposite end of the ordination graph than funnel traps with lures placed on the inside, monitoring protocols may want to consider using both these trap-types to capture different types of beetle species.

A major drawback to this study is that we did not catch any exotic woodboring beetle species. We argue that our results may still be applicable to exotic species, since a number of them (e.g., emerald ash borer and Asian long-horned beetle) follow attractants, such as ethanol and monoterpenes to find their tree host (Crook et al. 2008, Nehme et al. 2009). Future studies may need to be conducted in the native range of exotic beetle species e.g., in Asia and South and Central America with which we have major commerce and import of products (US Census Bureau 2012). This information then could be useful to forest managers in their trapping surveys to increase the likelihood of capturing a potentially devastating beetle species before it has becomes established in North America.

3.5 Acknowledgements

We thank the USDA Forest Service, Southern Research Station and the Warnell School of Forestry and Natural Resources, University of Georgia for funding for this project. We also thank personnel in the Forest Entomology Lab at the Warnell School of Forestry and Natural Resources for all of their support and assistance during this project.

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Table 3.1. Total trap catches and species of woodboring beetles in Oconee National Forest, Eatonton, Georgia.

Beetle Family	Species	Trap-Type Funnel (IN)	Funnel (OUT)	Panel	Total Numbers
Buprestidae					
	Buprestis consularis (Gory)	14	17	10	41
	Buprestis lineata (F.)	75	44	37	156
	Chalcophora virginiensis (Drury)	3	4	4	11
	Dicerca obscura (F.)	6	1	0	7
Cerambycidae					
	Acanthocinus nodosus (F.)	10	3	1	14
	Acanthocinus obsoletus (Olivier)	79	25	26	130
	Aegomorphus modestus (Gyllenhal)	0	1	0	1
	Arhopalus rusticus (LeConte)	17	10	7	34
	Astylopsis arcuatus (LeConte)	13	13	6	32
	Astylopsis sexguttata (Say)	16	1	8	25
	Curius dentatus (Newman)	5	1	1	7
	Elaphidion mucronatum (Say)	7	2	2	11
	Enaphalodes atomarius (Drury)	1	0	0	1
	Monochamus titillator complex (F.)	550	239	193	982
	Neoclytus mucronatus (F.)	5	0	1	6
	Neoclytus scutellaris (Oliver)	3	1	0	4
	Prionus imbricornis (L.)	2	6	1	9
	Prionus pocularis (Dalman)	10	6	5	21
	Typocerus lunulatus (Swederus)	0	1	0	1
	Xylotrechus colonus (F.)	6	5	1	12
	Xylotrechus sagittatus (Germar)	177	73	89	339
Elateridae					
	Alaus myops (F.)	84	70	128	282
	Alaus oculatus (L.)	3	0	1	4
Total Numbers		1,086	523	521	2,130
Total Species Richness		21	20	18	

<u>Figure Legend</u>

Figure 3.1. Images of the modified funnel traps with lures placed on the inside (IN), outside (OUT), and an intercept panel trap.

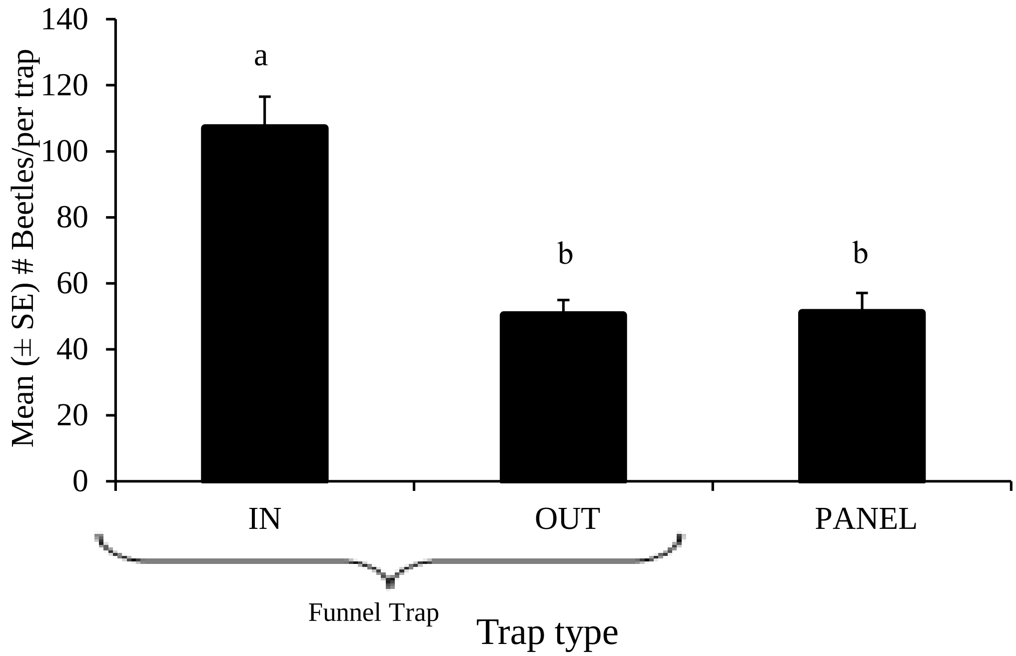
Figure 3.2. Mean (± SE) number of large woodboring beetles in all (N=30) traps in Oconee National Forest, Eatonton, Georgia.

Figure 3.3. Mean (± SE) of most commonly caught beetle species including (A) *Monochamus titillator* complex (B) *Xylotrechus saggitattus* (C) *Acanthocinus obsoletus* (D*) Buprestis lineata* and (E) *Alaus myops* caught in the modified funnel traps with lures placed on the inside (IN), outside (OUT), and an intercept panel trap.

Figure 3.4. Species richness of woodboring beetles in the modified funnel traps with lures placed on the inside (IN), outside (OUT), and an intercept panel trap.

Figure 3.5. Non-metric Multidimensional Scaling graph showing the differences in species composition between modified funnel traps with the lures placed on the inside (IN), lures placed on the outside (OUT), and panel traps.

Figure 3.1



140
120
100
80
60
40
20
0
Mean (± SE) # Beetles/per trap
a
b
b
IN
OUT
PANEL
Funnel Trap
Trap type

Figure 3.2

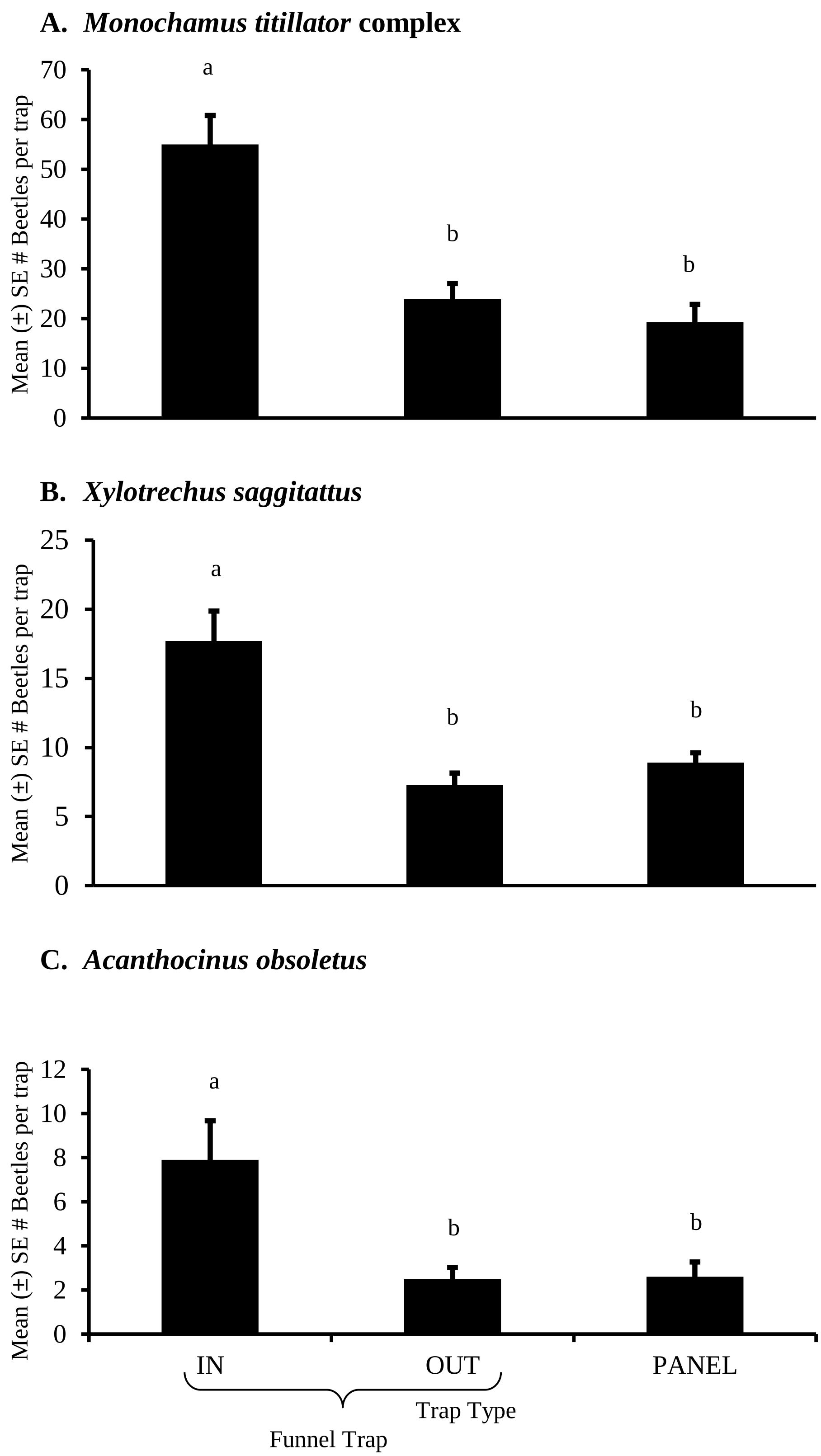
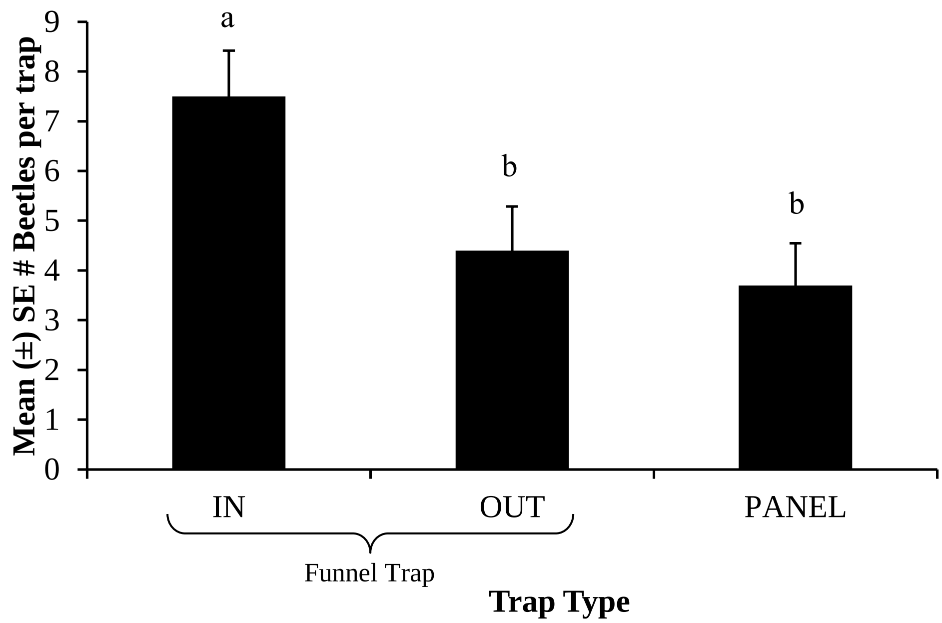

A. Monochamus titillator complex
Mean (±) SE # Beetles per trap
70
60
50
40
30
20
10
0
a
b
b
B. Xylotrechus saggitattus
Mean (±) SE # Beetles per trap
25
20
15
10
5
0
a
b
b
C. Acanthocinus obsoletus
Mean (±) SE # Beetles per trap
12
10
8
6
4
2
0
a
b
b
IN
OUT
PANEL
Trap Type
Funnel Trap

Figure 3.3

D. Buprestis lineata



E. *Alaus myops*

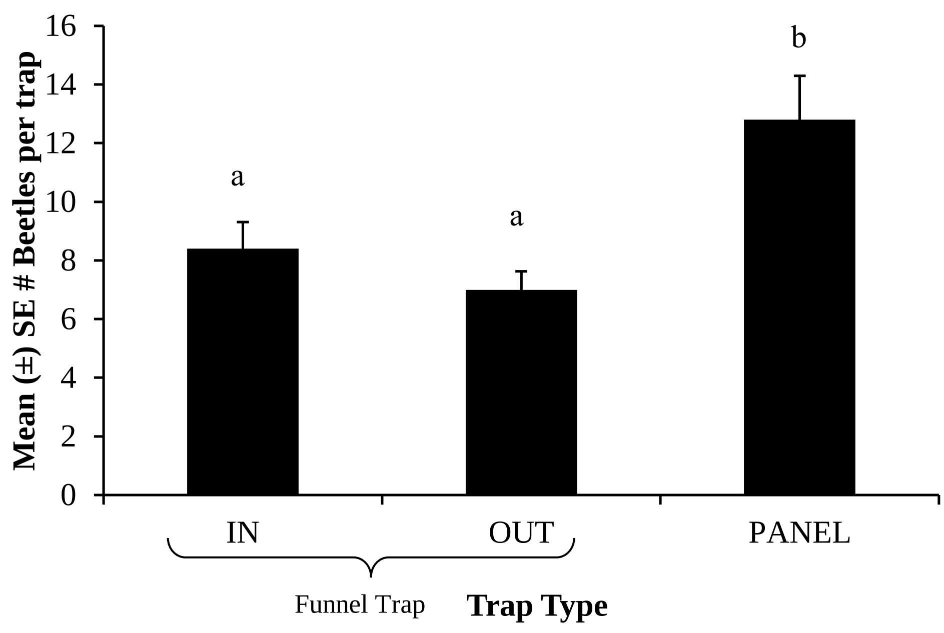
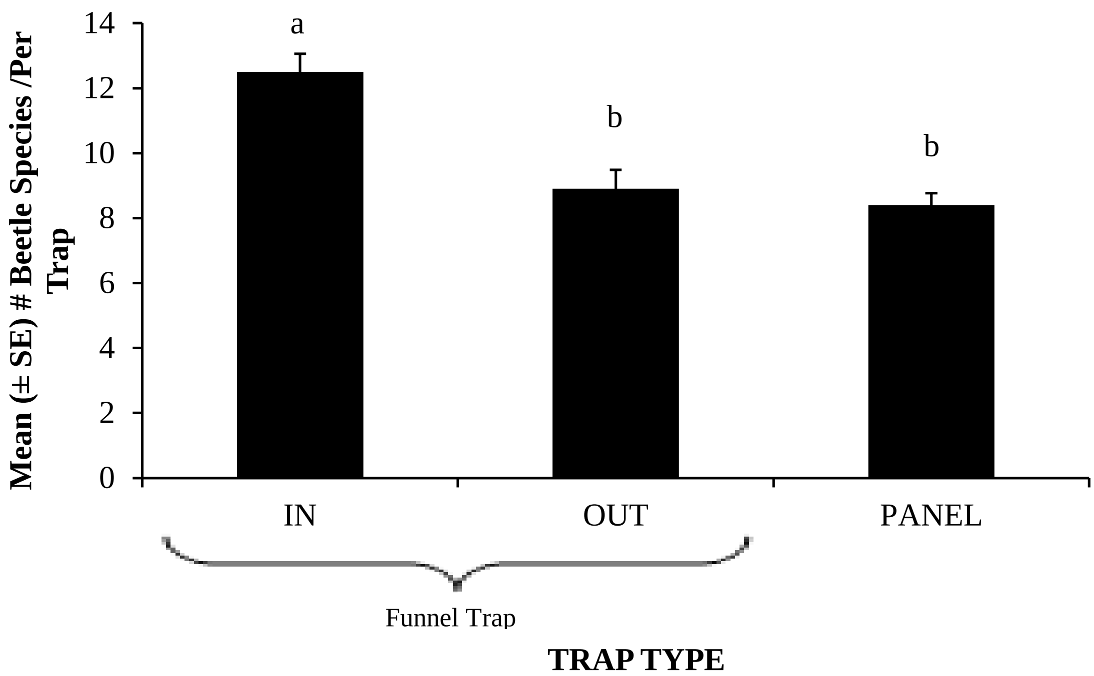
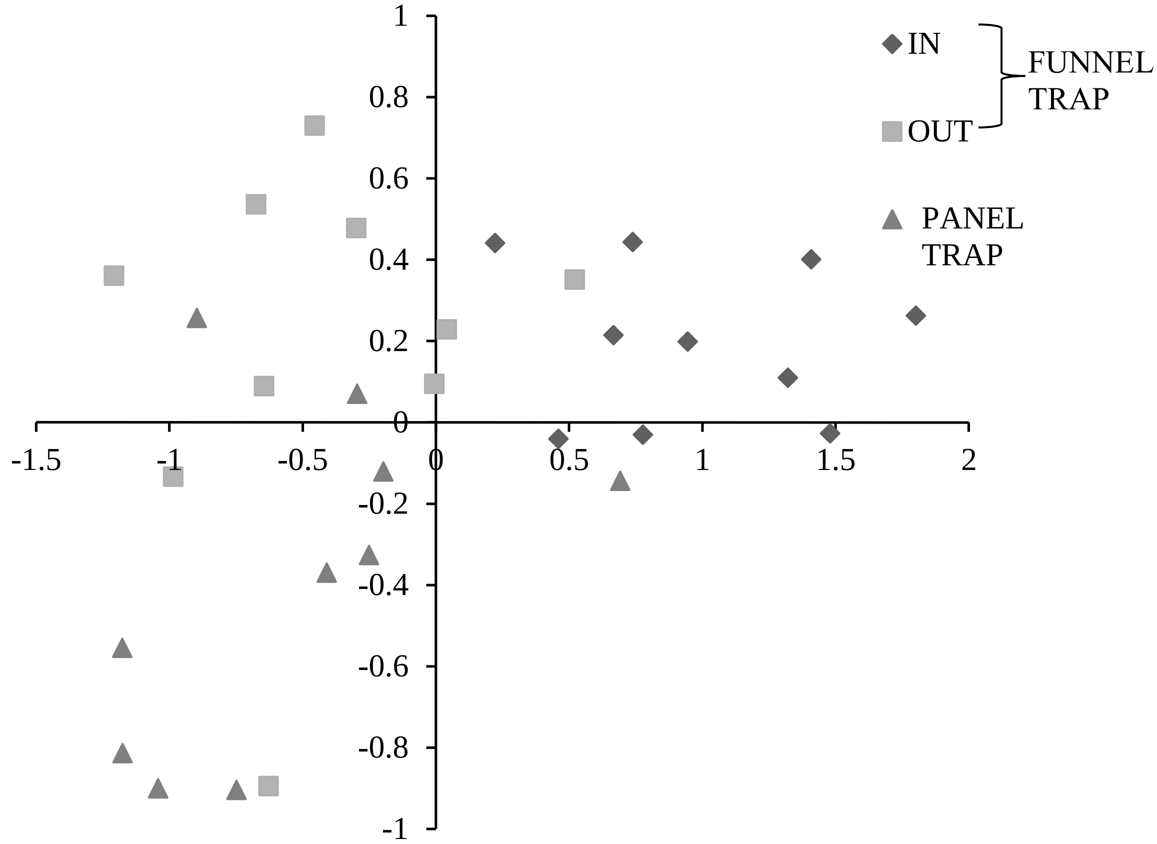


Figure 3.3



Mean (± SE) # Beetle Species /Per Trap
14
12
10
8
6
4
2
0
a
b
b
IN
OUT
PANEL
Funnel Trap
TRAP TYPE

Figure 3. 4



IN
OUT
FUNNEL TRAP
PANEL TRAP
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.5
-1
-0.5
0
0.5
1
1.5
2

Figure 3.5

CHAPTER 4

THESIS CONCLUSION

4.1 **Thesis Conclusion**

Woodboring insects have the potential to be devastating to forest stands, especially in the southeastern region of the United States where the timber industry compromises such a large part of the economy (Weir and Greis 2002). Understanding the most efficient way to trap for these insects, and having knowledge about existing native woodborers, as well as natural biological control agents may assist in preventing exotic insect establishment and spread. If exotic insects are detected before establishment occurs, then the success of eradicating the insect increases significantly before they can cause substantial damage to ecosystems (Myers et al. 2000).

The results of Chapter 2 indicate that there is an established population of native siricids and hymenopteran parasitoids in the Southeast. The most efficient way to trap for these two taxa is by using trap-trees, but siricids may also be caught using intercept or funnel traps. For both of these trapping techniques, timing is extremely important to maximize catches, making sure that traps are established during the peak months that adults are flying . The *Sirex* lure has shown to be effective at catching siricids, but in Louisiana using fresh pine billets proved to be three times more effective. Hence, if a quick and easy survey is needed, using intercept or funnel traps with fresh pine billets would be more effective than creating trap-trees that have to stay in the forest for a year before woodwasp emergence occurs. Future studies may focus on using consistent methods for wasp sampling between states and years. For example, establishing traps at the same time, using identical lures in all sites, and creating and establishing trap-trees using the same methods. Using inconsistent methodology as necessitated by trying to find optimal trapping methods prevented us from making direct comparisons of populations and communities of woodwasps and parasitoids between the three states.

The results of Chapter 3 were similar to a previous study conducted by Miller et al. (*unpublished data*). Placing the semiochemical lures inside of modified multiple funnel traps doubled the number of catches of large woodboring beetles compared to hanging them on the outside or using an intercept panel trap. Four woodboring beetle species, *Monochamus titillator* complex F, *Xylotrechus sagittatus* Germar, *Buprestis lineata* F., and *Acanthocinus obsoletus* Oliver had significantly greater catches in multiple funnel traps with lures placed on the inside, whereas in previous studies these four species had higher catches in the intercept panel trap or had no preference for either trap. Species richness was also higher in the funnel traps with lures placed on the inside and species composition was different between the three different trap-types. Future work may assess beetle trap catches with the lures placed on the inside of a modified multiple funnel trap for bark beetles using their pheromone baits, siricids using the *sirex* lure, and trapping for non-native species in their native geographic range.

Overall, my thesis enhanced ecological knowledge about populations and communities of our native siricids, their hymenopteran parasitoids, and woodboring beetles in the southern pine ecosystems. Results indicate that host materials are the best attractant for monitoring populations of woodwasps and their parasitoids. Funnel traps with lures inside of the traps will enhance catches and species diversity of woodboring beetles, but traps with lures placed on the outside will catch a different assemblage of woodboring beetles.

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USDA APHIS. 2003. 7 CFR Part 301 – Domestic quarantine notices. Subpart – emerald ash borer. Federal Register, 14 October 2003, 68: 59 088 – 59 0.—what is this number?

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Appendix A

Formulae for Non-parametric Kruskal Wallis Test

$$K = (N-1)\frac{\sum_{i=1}^{g} n_i(\bar{r}_{i\cdot} - \bar{r})^2}{\sum_{i=1}^{g}\sum_{j=1}^{n_i}(r_{ij} - \bar{r})^2},$$

- n_i is the number of observations in group i
- r_{ij} is the rank (among all observations) of observation j from group i
- N is the total number of observations across all groups
- $\bar{r}_{i\cdot} = \frac{\sum_{j=1}^{n_i} r_{ij}}{n_i}$,
- $\bar{r} = \frac{1}{2}(N+1)$ is the average of all the r_{ij}.