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## Effects of Felled Shortleaf Pine (*Pinus Echinata* Mill.) Moisture Loss on Oviposition Preferences and Survival of *Sirex Nigricornis* F. (Hymenoptera: Siricidae)

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EFFECTS OF FELLED SHORTLEAF PINE (*PINUS ECHINATA* MILL.) MOISTURE LOSS  
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ON OVIPOSITION PREFERENCES AND SURVIVAL OF *SIREX NIGRICORNIS* F.  
(HYMENOPTERA: SIRICIDAE)

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

By

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Bachelor of Science in Zoology, 2010

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## **ABSTRACT**

The European woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae) utilizes pine as its host during larval development. Females drill through pine bark to deposit eggs, a symbiotic fungus, *Amylostereum*, and phytotoxic mucus into the tree. In their native range, these insects are not viewed as primary pests because they attack dead or dying trees. Over the last century, this woodwasp has been accidentally introduced into several countries in the southern hemisphere. Some regions have incurred millions of dollars in damage to large plantations of the widely planted pine species, radiata pine (*Pinus radiata* D. Don). *Sirex noctilio* was discovered in northeastern United States and Canada in 2004. Prior studies have focused on damage done to pine stands in the southern hemisphere and, because those pines are not native, these studies may not be applicable to native pines in the U.S. The southeastern U.S. contains millions of hectares of possibly susceptible pine trees and, thus it is advisable to study the native Arkansas woodwasp, *S. nigricornis* F., (as a species with similar biology) in preparation for a possible invasion by its exotic counterpart. The objectives of this research were to 1) examine how shortleaf pine (*Pinus echinata* Mill.) logs (bolts) in moderate drought conditions of Arkansas lose moisture over time, and 2) determine oviposition preferences of *Sirex nigricornis* females in aging pine bolts. To complete these objectives, shortleaf pines were felled and moisture content was measured over a period of 45 days. Moisture content results were used to create parameters for oviposition choice experiments. After a cross-sectional cut was made, the most moisture loss occurs within 3-4 cm of bolt ends while the center of the bolt stays consistent during this time period. Females prefer to oviposit in recently cut bolts. Using these results, trap tree methods can be altered to create more efficient methods of siricid capture and laboratory rearing.

**Keywords:** *Sirex noctilio*, shortleaf pine, moisture content, oviposition preference

This thesis is approved for recommendation  
to the Graduate Council.

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## **DEDICATION**

To my grandparents, Robert and May Hartshorn, who I miss every day.

## TABLE OF CONTENTS

	Page
Introduction	1
Chapter 1 – Literature Review	
Introduction	3
Biology of Siricidae	4
Associated Fungi	7
Natural Enemies	11
Pine Defenses	19
Detection, Monitoring and Management	22
Objectives	25
Figures	26
Bibliography	27
Chapter 2 – Decline in moisture content of shortleaf pine ( <i>Pinus echinata</i> ) bolts and its effects on oviposition behavior of <i>Sirex nigricornis</i> (Hymenoptera: Siricidae) females	
Abstract	37
Introduction	39
Materials and Methods	42
Results	47
Discussion and Conclusions	51
Figures	53
Acknowledgements	60
Bibliography	61
Conclusion	64
Future Research	65
Bibliography	67

## INTRODUCTION

The European woodwasp (*Sirex noctilio* F., Hymenoptera: Siricidae) has been accidentally introduced into several countries in the southern hemisphere, many of which have seen millions of dollars in damage to commercial pine stands after its establishment. This woodwasp is known to kill healthy, vigorous pines outside of its native range of Eurasia and North Africa. However, in areas where severe damage has occurred – the Southern hemisphere – pines are not native and, therefore, natural enemies which keep these secondary pests at low levels are also not native.

Since the introduction of *S. noctilio* into the United States and Canada in 2004, there has been much concern about the potential impact of this invasive on our native pines, native woodwasps, and their natural enemies. In contrast to the Southern Hemisphere, North America, has many native pine species as well as an array of natural enemies and other pine associates (e.g. long-horned beetles, metallic wood-boring beetles etc.) These co-evolved relationships will likely have an effect on the establishment of *S. noctilio* and, therefore, its impact on the U.S.

The southeastern United States has approximately 54 million of hectares of commercial pine stands which are important to both the economy and culture of the southeast. If the invasive woodwasp, if it is a successful colonizer southern pine forests, their impact could be significant. Thus, it is important to study our native woodwasps as well as their interactions with pines and pine insects to prepare for the introduction and management of *S. noctilio*.

This thesis contains two chapters: the first chapter consists of a compilation of previous literature describing the biology and ecology of *S. noctilio* and its natural enemies. The second chapter includes two experiments to examine the relationships between our native *Sirex* and their pine hosts. First, moisture loss of felled pine logs (bolts) was measured to examine how moisture status changes over time in felled pines. Secondly, mating pairs were exposed to these

aging pine bolts to evaluate how their oviposition behavior changes in relation to differing ages and the moisture statuses of bolts. These bolts were later dissected to evaluate the development of *Sirex* larvae within rearing bolts held in the laboratory.

## LITERATURE REVIEW

Insects in the family Siricidae are broad-waisted wasps whose larvae develop in the xylem of trees. The genus *Sirex* consists of woodwasps that insert their ovipositor through pine bark and deposit eggs, along with a symbiotic wood-rot fungus, and a phytotoxic mucus into xylem tissue. In the case of the single pine-infesting woodwasp species native to Arkansas, *S. nigricornis* F. (Hymenoptera: Siricidae), the associated fungus is *Amylostereum chailletii* (Fries) Boidin (Slippers et al., 2012). *Sirex nigricornis* F. typically colonizes trees that are dead or dying, thus these insects are considered secondary pests.

That is not the case, however, for the European woodwasp, *Sirex noctilio* F., which is well documented as a serious pest when introduced outside its native range (Madden, 1975; 1977; Neumann & Minko, 1981). Since its discovery in North America (New York and Ontario) in 2004 (Hoebeke et al., 2005), much concern has been raised, especially by those involved in the timber industry, about its potential to spread throughout the United States. Wood damage and pine mortality occurs in the presence of a combination of phytotoxic mucus, symbiotic fungus, and long, winding galleries created by larvae within the sapwood in stressed pines.

The flight period of *S. noctilio* in Eurasia occurs during the summer (July-August), when tree metabolism is at its lowest point of the year. Their flight period in regions of the Southern Hemisphere begins in the late summer (December – February) with a peak in early fall. That period overlaps with an increase in growth and cell differentiation of *Pinus radiata* D. Don, and therefore, a lack of resin production (Lorio, 1988, Spradbery, 1973). These differences in the cycles of insect flight period and tree growth between Australasia and Eurasia may contribute to the severity of *Sirex noctilio* infestations in Australia and New Zealand, while the woodwasp remains a non-pest species in Eurasia.

Existing literature contains little information about the biology and ecology of *S. nigricornis*, its natural enemies, or how tree characteristics affect females' oviposition behavior and survival. Having information on these complex interactions would allow for the more efficient use of trap trees which are monitoring devices used to detect *Sirex* as well as lure them away from healthy, more valuable trees (Madden, 1988). In addition, knowledge of log (bolt) conditions' effects on siricid survival and fecundity could shed light on a complex issue scientists face: manipulating bolts in the laboratory and attempting to extrapolate that information to whole field trials.

Pine is central to the economy and culture of the southeast (Elliott II et al., 2007), and it is thus imperative that we prepare for the establishment of a possibly dangerous invasive insect by examining interactions among pines, woodwasps, and their associates. Information gathered from the literature about *S. noctilio* will be used to illustrate the biology and ecology of species native to the southeastern United States (Slippers et al., 2012). My objectives were to determine how shortleaf pine and loblolly pine (*Pinus taeda* L.) bolts lose moisture over time under moderate drought conditions in Arkansas and whether *S. nigricornis* females change their oviposition behavior based on a changing moisture regime within pine bolts.

## BIOLOGY OF SIRICIDAE

*Sirex noctilio* F. (Hymenoptera: Siricidae) was chosen for this literature review for several reasons: it has been the focus of extensive research and thus has accumulated a very large knowledge base; it appears to have very similar biology to our native (e.g. development within *Pinus* species, native to temperate climates, production of a phytotoxic mucus) (Smith & Schiff, 2002), and it has been recently introduced into the U.S. In addition, the woodwasp is in association with

very similar species assemblages – i.e. multiple pine hosts (Kirkman et al., 2007; Rouget et al., 2001; Urbietta et al., 2011), many parasitic Hymenoptera, and a sterilizing nematode (Kirk, 1975; Long et al., 2009) – and, therefore, are likely to behave in a similar manner.

Siricid adults are variable in terms of color and body size but can be identified by a short spine at the terminal segment of the abdomen and lack of constriction at the “waist”. Females have a modified ovipositor which is enclosed in a ventral sheath (Schiff et al., 2006). Larvae also have a short spine at the end of their abdomen and different species of *Sirex* larvae are virtually indistinguishable. The presence of this spine in both larval and adult forms explains the common name given to siricids of “horntail” (Wilson & Schiff, 2010).

Morgan (1968) described the basic biology of several species of *Sirex* stating that different species have variable flight times and emergence patterns but the majority of adults emerge within a three to four week period beginning in late summer and continuing through the fall in temperate zones. Keeler (2012) made observations of *S. nigricornis* that agree with these findings and suggests that our native may successfully serve as a proxy species for the study of *S. noctilio*.

Males typically emerge before females and in higher numbers with a large amount of variation in sex ratios between regions, ranging anywhere from 1.2:1 to 32:1 (Slippers et al., 2012). Males then swarm to a light source and wait for females to emerge. Females are facultatively parthenogenetic meaning they can oviposit without mating, and their unfertilized eggs will still develop (Morgan, 1968; Rawlings, 1951). In the case of siricids, unfertilized eggs become males while fertilized eggs become females. This form of parthenogenesis is known as arrhenotoky and is most likely the cause of inconsistent sex ratios among populations, especially at the front of invasions (Ryan & Hurley, 2012).

Several studies (Madden, 1974; 1981; Morgan, 1968) show that females will drill with their ovipositor into pine trees without laying an egg – presumably checking for host suitability – and conversely, can lay multiple eggs in an individual drill hole. The only way to determine if the drill was simply to probe or was to oviposit is by dissecting individual drill holes to examine the number of tunnels branching from that single drill hole and visually confirming the presence of an egg. Madden (1974) describes the patterns of oviposition by *S. noctilio* in trees that have been successfully attacked stating that drill holes are distributed randomly but tend to become uniform over time with increasing density of drills. Conversely, trees which resist attack tend to show an aggregated pattern of drilling by *Sirex* females. Females drill while working their way up one side of the tree, flying back down to the bottom of the same tree, and working their way up another section (Slippers et al., 2012).

Studies cite differing times of *S. noctilio* egg development, with eclosion times ranging from 10-28 days (Madden, 1981; 1988; Morgan, 1968). Development of eggs is largely dependent on temperature, and eclosion times may vary considerably based on the local climate. After eclosion, larvae tunnel into the sapwood creating winding galleries which fall into four distinct patterns – i.e. looped, half-looped, kinked, and reversed – and begin feeding on their symbiotic fungus, *Amylostereum* (Fig. 1) (Madden, 1981).

Mature larvae from the middle portion of the bole are much more abundant, but smaller across all measurement classes (i.e. weight, length, and size of head capsule) and have fewer instars than larvae developing in other sections of the tree. In contrast, larvae in the lower portion of the bole are typically larger – presumably from a lack of intraspecific competition – but less abundant (Hurley et al., 2008). The number of larval instars ranges anywhere from six



to twelve depending on transpiration of the wood, temperature, and other extraneous factors (Madden, 1981).

Moisture content (MC) has been shown to differ along the height of coniferous trees as well as play a very important role in tree defenses (Lorio, 1988, Coutts, 1969a; Talbot, 1977). It has also been shown to be an important factor in the choice of oviposition sites by female *Sirex* and in the development and survival of siricid larvae within the wood. Spradbery and Kirk (1981) state that females prefer to oviposit in bolts of higher MC (up to 135%) but do not discuss in detail how survival is altered by different MC.

Preferential oviposition by females in fresh bolts could also be related to the release of attractive volatiles, which can make up 1% of total volatiles released from a freshly cut tree, and may not always be related to MC (Simpson & McQuilkin, 1976). Larvae often bore deeper into wood that has a lower MC and seem to bore along a 'gradient of moisture content'. Trees experiencing a rapid decline in moisture have exhibited higher *S. noctilio* survival (Morgan, 1968; Morgan & Stewart, 1966). Rawlings (1953) found that a large increase in MC proved to only slow the growth of siricid larvae while Zondag (1959) provides evidence of increased mortality in these high MC situations.

#### ASSOCIATED FUNGI

*Amylostereum* is a genus of filamentous white-rot fungi in the family Amylostereaceae. White rot fungi are characterized by degrading cellulose and lignin simultaneously by oxidative and hydrolysis processes which result in a pale color of the wood (Campbell, 1932; Hudson, 1992). Studies and surveys have failed to provide evidence of fruiting bodies in natural settings outside their native range and, therefore, it is thought that *Amylostereum* relies on clonal reproduction in other regions (Talbot, 1977). *Amylostereum* must kill at least part of a living tree

to allow for successful development of siricid larvae but factors determining the success or failure of inoculations are not fully understood (Coutts & Dolezal, 1966; Gilmour, 1965).

Siricid woodwasps are the only known means of dispersal for this fungus. Siricid females have mycangia, internal sacs first described by Büchner (1928), located at the base of the ovipositor which are designed for transport and storage of fungal spores (Slippers et al., 2003). Oidia – thin-walled spores of some filamentous fungi – are contained within mycangia of female woodwasps. There are three species of *Amylostereum* carried by different female *Sirex* species: i.e. *A. chailletii*, *A. areolatum*, and *A. laevigatum* – of which *A. chailletii* is the species purported to be the sole associate of the native North American *Sirex* (Nielsen et al., 2009; Slippers et al., 2003; Talbot, 1977).

Talbot (1977) stated that the relationship between *Amylostereum* and *Sirex* is obligatory species-specific. However, more recent surveys done by Nielsen et al. (2009) show that multiple species of *Sirex* in New England naturally carry more than one species of *Amylostereum*. Francke-Grossman (1939) had originally suggested that *A. chailletii* and *A. areolatum* may be found in many species of *Sirex* but this suggestion was largely ignored. In addition, *A. areolatum* was initially thought to be introduced into the United States along with *S. noctilio*, but it has since been found in mycangia of native *Sirex* in Maine where there is not yet an established population of *S. noctilio*, indicating that this species was likely already native to the U.S. The presence of *A. areolatum* in Louisiana has also been recorded (Canadian Forestry Service, Sault Ste. Marie, Canada. pers. comm.). This could have serious implications with respect to the ease of establishment by the invasive woodwasp throughout the rest of the U.S. Invasive insects that require a symbiotic fungus for their survival are limited by the successful establishment of their symbiont. A pre-existing, coevolved relationship between the fungus and *Sirex* hosts may allow

for easier establishment of the woodwasp. Current surveys and studies are being conducted in New England and the mid-Atlantic regions to determine the status of *Amylostereum* spp. in the United States (A. Hajek, Cornell University, Ithaca, New York, pers. comm.).

How the fungus has been used by the siricid larvae during development has been widely debated. Büchner (1928) states that siricid larvae do not directly ingest the fungus but ingest the wood after it has been broken down by fungal enzymes. This conclusion was reached by examining frass of larvae and finding only trace amounts of the fungus. However, Müller (1934) states that digestion of the fungus has to take place within the gut of larvae based on his findings of fungus and wood within the gut during dissections, as well as observations of wood gnawing behavior by siricid larvae. Boros (1968) then performed dissections of female larvae leading her to dispute claims by Clark (1933) that fungal carrying mycangia are present within the hind gut of female larvae. These disagreements about the role of fungus and mechanisms of fungal use by larvae make it difficult to determine exactly how it relates to siricid development.

Coutts (1969a; 1969b) describes the effects of the symbiotic fungus and phytotoxic mucus on radiata pine defenses. After inoculating pines with *A. areolatum* and mucus from *S. noctilio* females individually, as well as in combination, he found that, separately, each would stress the tree but not directly kill it. The combination of both, however, can be deadly for even a slightly stressed tree. These tests were performed using a solution containing 50 mL of 1% mucus and 50 mL of “log extract”. When trees were inoculated with both substances simultaneously, the tree was inevitably killed within a few months by the combination causing a sharp decrease in moisture at saturation (MC/S) as well as an accumulation of photosynthate in the leaf.

Having an isolated culture of this symbiotic fungus is essential in completing studies examining *Sirex*-fungal interactions. Thomsen and Harding (2010) describe a procedure that allows for isolation of *Amylostereum* directly from the mycangia of adult females. They suggest using morphological characteristics to identify the fungus. However, allowing the fungus to grow to the point of accurate morphological identification requires at least 6 weeks and obtaining the fungus in pure culture can be challenging. Several approaches can be useful when attempting to identify the strain and origin of these symbiotic fungi. These techniques include isolation of the fungus combined with polymerase chain reaction (PCR) and sequencing, or using vegetative compatibility groups (VCGs) (Vasiliauskas et al., 1998).

Isolation of *Amylostereum* can be difficult due to a number of other genera of wood-rot – or saprophytic – fungi, which also occur in pines. In instances of possible contamination, or difficulties in morphological identification, molecular identification can be a much simpler and objective method to obtain a correct identification of the fungus and typically requires much less time. Nielsen et al. (2009) has developed primer sequences that have been successful in amplifying *Amylostereum* DNA for identification.

When hyphae of two or more strains of fungi are able to fuse, or anastomose, they are considered to be vegetatively compatible, and therefore placed into the same vegetative compatibility group VCG (Leslie, 1993). This ability to fuse can be used to test the relatedness of strains obtained from different areas and, in the case of *Amylostereum*, can determine the origin of fungal strains based on their vegetative compatibility with other, correctly identified, strains (Vasiliauskas et al., 1998).

Using these VCGs, Slippers et al. (2001) found that all *A. areolatum* isolates from South Africa – one of the regions in which *S. noctilio* has caused millions of dollars in damage – were

compatible with each other as well as the South American isolates. This supports previous suggestions that budding – or clonal – reproduction during symbiosis with *Sirex* may be the only form of reproduction for *Amylostereum*. This method of reproduction does not allow for recombination of DNA which would have resulted in genetic differences between strains.

Combining these methods of isolation and identification can allow for a continuous culture of *Amylostereum*. Having a continuous – accurately identified – culture of *Amylostereum* would also allow for the rearing of a parasitic nematode commonly used as a biocontrol agent of *S. noctilio* – *Deladenus siricidicola* (= *Beddingia*) (Bedding).

## NATURAL ENEMIES

Many *Sirex* natural enemies are native to North America including hymenopterous parasitoids, parasitic nematodes, and fungal pathogens (Cameron, 1962). A few of these natural enemies – *Rhyssa persuasoria* L. (Hymenoptera: Ichneumonidae), *Megarhyssa nortoni quebecensis* Cresson (Hymenoptera: Ichneumonidae), *Ibalia leucospoides* Hochenw. (Hymenoptera: Ibalidae), and *Deladenus* (= *Beddingia*) *siricidicola* Bedding (Tylenchida: Neotylenchidae) – have been used very successfully in the control of *Sirex noctilio* in the southern hemisphere (Haugen, 1990; Iede et al., 1998; Taylor, 1978). The main methods of control used as well as their efficacies in different areas of *S. noctilio* infestation will be outlined below.

### *Deladenus siricidicola*

The most widely studied biocontrol agent of *S. noctilio* is the host specific nematode *Deladenus siricidicola* (Bedding). While there are several described strains of the nematode, the most common strain used for control of *S. noctilio*, the Sopron strain, began to lose its virulence due to an extended period of time without being allowed to become parasitic, therefore, was

replaced by the Komona strain which was collected in the early 1990s from the Komona forest in Tasmania (Bedding & Akhurst, 1974; Hurley et al., 2007).

Bedding (1968) first described the biology of *D. siricidicola* stating that the nematode can have two very distinct life cycles, both of which can continue indefinitely depending on their surrounding environment (i.e. within a pine tree or in the presence of a siricid host). Eggs that hatch within wood always begin as mycetophagous, feeding on *Amylostereum* spp., but their life cycle becomes parasitic if they come into contact with conditions indicating the presence of a host larva.

At the point that a fertilized adult female nematode enters the immediate vicinity of a host larva – typically sensing an increase in CO<sub>2</sub> and a decrease in pH – it thrusts a tubular stylet into the cuticle of the larval siricid, sometimes reaching rates of 60 to 200 thrusts per minute. The head and oesophagus initially enter the cuticle quickly and then pause briefly, exploring the inside of the host. After the initial pause, nematodes will enter the host about 20-100 µm at a time while pausing between thrusts until the entire nematode is inside the hemocoel of the larva. Anywhere from one to over 100 nematodes can enter a host but the number of nematodes in a single host is typically between five and twenty (Bedding, 1968).

Unlike their siricid hosts, *Deladenus* females need to mate while outside the larval host, as unfertilized eggs of *D. siricidicola* do not develop. Once inside the host, adult female nematodes' reproductive organs expand, producing thousands of eggs which hatch in 3 to 4 days while inside the parent nematode. After hatching, juvenile nematodes move into the host's haemocoel and migrate to the reproductive organs of the siricid host. Before the end of host pupation, juvenile nematodes have begun to move into the ovaries or testes. Moving to the ovaries results in transportation to a new host tree, but testes are considered a “dead end” for

nematodes as no transportation comes from their colonization. Female siricids then deposit nematodes instead of viable eggs in the wood during oviposition. Once nematodes are deposited in the wood, the free-living life cycle starts again assuming there is *Amylostereum* present (Bedding & Akhurst, 1974; Fenili et al., 2000; Nuttall, 1980; Yu et al., 2009).

Just as MC of the wood plays a very important role in the development of siricid larvae and its symbiotic fungus, it is also critical in the development of the nematode. Once the wood's MC drops below 50%, juvenile nematodes begin to migrate further into the tree (Bedding & Akhurst, 1974). Nematodes have been used as successful biocontrol agents in several parts of the world (Bedding & Akhurst, 1978), however, South Africa has not seen such success. Even with adequate moisture content, success was relatively low, suggesting other factors may also affect parasitism rates (Hurley et al., 2008). What these factors are and their interactions with pines are still under investigation.

Parasitic nematodes are present in native *S. nigricornis* populations throughout Arkansas (Keeler, 2012) but their identity and influence on siricid fecundity are unknown. Siricids have been shown to be completely sterilized by the nematode in previous studies (Bedding & Akhurst, 1974) but dissections performed by Keeler (2012) show that not all females are sterilized and some still have many viable eggs even when infected by the native nematode. During dissections she found several female siricids with nematodes present in the haemocoel surrounding the ovaries but not inside the eggs. When female siricids were dissected in 2009, the percent of eggs parasitized by nematodes ranged from 10-100%. This range was even larger in 2010 with a span of 0-100% of eggs being infected with nematodes. The percent of eggs infected was not significantly different among sites, suggesting large variability even within small geographic areas (Keeler, 2012).

Preliminary testing for presence and abundance of nematodes in pine trees can be performed using the methods described by Haugen and Underdown (1991). In that study, woodchips measuring 6 x 6 x 1.5 cm were removed from pines using a chisel and immersed in 150 mL of tap water in an Erlenmeyer flask. After 24 hours the water was slowly decanted leaving about 20 mL of water filled with debris. The remaining water was poured into a Petri dish and examined for nematodes using a 40x dissecting microscope. This method proved to be successful and was shown to be accurate in predicting the number of siricids infected with nematodes in Australia. However, when this method was attempted using pines from the Ozark National Forest, it did not yield nematodes indicating that this may not be efficient for nematode surveys and detection in this region (J. Hartshorn, unpublished data).

If the methods described above yield a population of nematodes large enough to be reared on plates of isolated *Amylostereum*, methods described by Bedding and Akhurst (1974) can be used to rear these nematodes for release as biological control agents. Bedding and Akhurst (1974) used isolated *Amylostereum areolatum* plated on potato dextrose agar (PDA) successfully to rear nematodes, as the fungus is their only food source while inside a tree. They discovered that, when *Amylostereum* grows on plated agar, it takes approximately 5 days at 22°C for nematodes to mature and mate and they can survive for several weeks in this environment.

Several species of *Ophiostoma* – blue stain fungi – are present in the United States (Six & Paine, 1999; Zanzot et al., 2010) and may outcompete *Amylostereum*, potentially resulting in difficult isolation of the *Sirex* symbiont (Hurley et al., 2012). Having a pure culture of *Amylostereum* allows for the mass rearing of *Deladenus* nematodes for the biological control of *Sirex*. The inability to obtain a pure culture, possibly due to the presence of other, more aggressive fungi, may inhibit these rearing processes. However, if nematode rearing is



successful, they can be very important in population control of *S. noctilio*. After comparing several methods of inoculating nematodes into pine, Bedding and Akhurst (1974) found that a foam-gelatin media is best for inoculating pines with laboratory-reared nematodes when compared to other media such as water or agar.

Nematodes in specific programs have been shown to be very important in the control of *S. noctilio* but there are many barriers to their effective use; i.e. a loss of virulence over time from continuing cultures without allowing them to become parasitic, use of an inoculation technique which may not be appropriate for the region, moisture content of the wood, and the possible incompatibility between populations of *Sirex* (Hurley et al., 2007).

Another common option for control of invasive insects is the use of parasitoids. If selected properly they can be very host specific, often destroy the host or virtually eliminate its ability to reproduce, and can be found almost anywhere (Sweetman, 1963). Parasitoids that are most important to the control of *S. noctilio* populations will be discussed next.

### *Parasitoids*

Reuter (1913) coined the term “parasitoid” to distinguish between parasites and predators. This definition includes insects which ultimately kill their host while parasites live in or on the host but keep it alive. Other definitions have been suggested to include insects which inhabit plants (Price, 1975), and all organisms that complete their development on, and kill, a single animal host (Eggleton & Gaston, 1990). Ulisse Aldrovandi was the first to document a parasitoid emerging from its host in 1602, and the first illustration of a parasitoid was done by Johannes Goedaert in 1662 (Godfray, 1994).

From 1928 to 1968, 11 species of parasitic wasps were reared in New Zealand after being collected from the U.S., Europe, and Asia. The first of which, *Rhyssa persuasoria*, was

introduced beginning in 1928 and collected from England. This release represented the first attempt at controlling *S. noctilio* using a non-native parasitoid and, very soon after, other releases were attempted. *Ibalia leucospoides leucospoides* was introduced several years later and was not successful until 1954 after several introductions. Subsequent introductions included *Megarhyssa nortoni nortoni* from the U.S., *I. leucospoides ensiger* collected from the U.S. but reared in New Zealand, and *R. p. himalayensis* from Pakistan and India (Cameron, 1965; Hurley et al., 2007).

Of the 11 parasitoids released, those previously listed were the most commonly studied parasitoids used in the control of the woodwasp. The final parasitoid mentioned, *R. p. himalayensis*, was not considered successful, however, it is believed that these individuals may have hybridized with *R. p. persuasoria* still making them important possibly in biocontrol. Before the successful introduction of nematodes for control into Tasmania, these parasitoids infected anywhere from 55-80% of siricids (Hurley et al., 2007).

Taylor (1978) suggested that these five parasitoids would differ in efficacy based on local climatic conditions and, therefore, would be complementary to each other, not competitive. Two of these five species occur in the southwestern United States as natives and their ranges and life histories were evaluated by Kirk (1975).

Long et al. (2009) recorded three hymenopterous parasitoids of siricids emerging from *S. noctilio*-infested pines in upstate New York including *I. leucospoides*, *R. lineolata*, and *M. nortoni quebecensis*. *Ibalia leucospoides* was the most abundant parasitoid captured but all three species were found parasitizing native and introduced *Sirex*. Long et al. (2009) also speculate that none of these parasitoids could independently be responsible for keeping *Sirex noctilio* populations low in the northeastern United States.

*Ibalia leucospoides* is an egg-larval parasitoid of siricids. The parasitoid female lays its eggs within the siricid egg and feeds internally. When the parasitoid larva reaches the third instar, it exits the host and feeds externally. Fourth stage larvae pupate within the tree, but outside the siricid host, and emerge from the tree as adults. Their life cycle typically takes three years in New Zealand (Rawlings, 1951). Taylor (1978) stated that *Rhyssa persuasoria* L. and *Megarhyssa nortoni* were responsible for keeping levels of *Sirex noctilio* low in Tasmania. Long et al. (2009) and Taylor (1978) contradict each other's findings in regards to population regulation, however, the differences between their results is most likely due to major dissimilarities between Tasmania and North America with respect to the presence of natural enemies of *S. noctilio*, climate, and native species composition. These parasitoid species are present as natives in Arkansas, but their effects on native *Sirex* populations are not known (Keeler, 2012).

#### *Facultative Predators and Competitors*

Native *Sirex* larvae feed in the xylem of pine trees, and the very common pine associates, long-horned beetles (Coleoptera: Cerambycidae) – specifically the pine sawyers *Monochamus titillator* F. and *M. carolinensis* Olivier – feed in the phloem as early instars, and in xylem as later stage larvae. Stressed or damaged pine trees are often a limited resource, so it is likely that these insect species will inhabit the same host at some point during their life cycles. Cerambycids may have an effect on survival of siricid larvae much like they do on bark beetles. In a recent study done by Ryan (2011) it was discovered that siricid emergence was lower in beetle-infested trees, and average prothorax width of *Sirex* was larger in beetle-infested pines. The mechanisms behind these variations are not understood.

Walsh and Linit (1985) state that *M. carolinensis* oviposits more often in the middle to upper portion of the bole and aggregates oviposition pits around whorls of branches. The bottom portion of the bole was largely ignored by *M. carolinensis* because the bark is too thick for them to chew an oviposition pit. It has also been suggested that *M. carolinensis*, *M. titillator*, and *Acanthocinus nodosus* F. inhabit different heights of the tree to allow for successful resource distribution (Dodds et al., 2002). Siricid oviposition sites are most commonly encountered starting at 3 m and dropping off around 17 m. Distribution of oviposition sites by these facultative predators may have an effect on siricid oviposition and development.

Dodds et al. (2001) studied some interactions between larval cerambycids and larval bark beetles (Coleoptera: Scolytidae) and found that, in laboratory experiments, 74% of *Ips calligraphus* was attacked when in the presence of *M. carolinensis*. Of those attacked, 85% was killed and, of those killed, 76% was ingested. *M. carolinensis* larvae also exhibited high levels of cannibalism when in the presence of other *M. carolinensis* larvae. These studies suggest and aggressive behavior of *Monochamus* in pine and their tendency to attack other members of the pine-phloem feeding guild. If this aggressive behavior were to continue into the xylem-inhabiting stage, this could indicate a possible negative interaction with *Sirex* in the xylem during later instars of *Monochamus*.

Whether these interactions are inhibitory or complementary – allowing for quicker death of the tree or resulting in higher competition and mortality of *Sirex* – is unknown. Several species of wood-boring insects are present within a single tree and can cause damage to wood by girdling, tunneling, or transmission of pathogens and diseases. How pines respond to these different types of injury is discussed in the following section of this literature review.

## PINE DEFENSES

The major components of pine defenses have been divided into primary and secondary forms, also referred to as constitutive and induced (Berryman, 1988). Vertical and radial preformed resin ducts contribute to the primary defense of pines which is the initial response to attack or injury. Secondary responses include both biochemical and cellular changes after attack by an insect which can serve to encapsulate individuals (Cook & Hain, 1988; Paine et al., 1997). Many factors combine to make a tree more susceptible to attack by insects; these include a high density of trees in a single area, senescence of trees, possible root disease, a lack of natural enemies, and wood damage from natural disasters such as lightning, wind, or ice storms (Mattson et al., 1988). Various authors cite different limiting factors as the main cause of stress in trees. Major factors that are most commonly cited include water and nutrient availability, and abundance of suitable host material (Lorio, 1978; Lorio et al., 1982; Rudinsky, 1962). While these factors most likely act together to create stressful situations for defenses to be overcome by insects and pathogens, Sharpe & Wu (1985) and Sharpe et al. (1985) express concern over the differentiation between the different kinds of stress and how they relate to each other.

Lorio (1988) defines several models which may be used to describe how plants, specifically woody plants, defend themselves against attack by herbivores and wood-borers. He states that, while these processes appear too complex to untangle, they are actually very regular and predictable physiological changes within the tree that may allow us to determine the susceptibility of a tree to an insect or pathogen at any given time. There are several theories that have been suggested to explain how trees allocate nutrients, photosynthates, and water.

The growth-differentiation theory was developed to expand on the notion that a mathematical formula calculating the carbohydrate:nitrogen ratio can determine how plants

develop. The growth-differentiation theory places tissue development into three categories: cell division, cell enlargement, and cell differentiation. Under optimal conditions, cell division and enlargement will dominate over differentiation. How nutrients are allocated during times of stress also changes depending on the limiting factor. During times of optimal temperature, oxygen, and sugars – and water as a limiting factor – nutrients are allocated towards proteins and cellulose while continuing cell growth. During times of overall moderate stress, energy is focused on differentiation of cells to create secondary metabolites. When water stress is severe and photosynthates are limited, both growth and differentiation are slowed. This is only a small representation of a wide array of possible circumstances to illustrate the complexity of tree development (Loomis 1932).

The plant apparency theory presented by Feeny (1976) describes how plant characteristics may make them more, or less, susceptible to attack by borers and herbivores. These characteristics may be size, growth form, or relative abundance within the community. He describes the culmination of these characteristics as the “likelihood of discovery”. This likelihood may also be different depending on the part of the plant. Flowers, for instance, may be very apparent to attract pollinators while leaves and stems may be less apparent to protect against herbivores. Apparency of the plant may also be a determining factor in how it allocates nutrients to growth or defense.

Unapparent plants tend to contain compounds that, in small concentrations, can still be very effective. These compounds are toxic to non-adapted enemies and can serve to deter harmful behavior. However, these compounds may also allow for quick adaptation by their associates and, after a short period of time, have little effect on enemies’ fitness or growth. Meanwhile, apparent plants’ compounds are needed in much higher concentrations to be

effective against enemies. However, these compounds may not allow for such quick adaptation as those described earlier. These plants tend to have low nutritive value for herbivores (Berryman, 1986; Feeny, 1976; Rhoades & Cates, 1976).

Seasonality is also important in determining a tree's susceptibility to an insect or pathogen. Resin duct formation is seasonal; during the spring months, trees may be very susceptible to insect attack while they are placing a large demand on photosynthates for growth instead of defense. Conversely, during summer months, trees may be more resistant due to nutrients being allocated for defense purposes (Blanche et al., 1992). Kozlowski (1969) describes this seasonality of tree physiology as a "window of vulnerability" during which time an insect or pathogen may successfully attack and overcome a tree's defenses.

Insect evolution to cope with these seasonal changes in tree physiology is evident in bark beetle behavior. Southern pine beetles have "pioneer" individuals that disperse in the spring to attack these susceptible trees while in the summer, when trees' defenses are at their maximum, re-emerging adults and new generations stay within a small "spot" continuing attack on already stressed trees. After this period of tree defense, new "pioneer" beetles begin host searching again in the fall (Coulson & Klepzig, 2011).

This seasonality of resin flow may also have an effect on siricid survival and fitness. As described previously, native siricids emerge in the fall in the southeastern U.S. (Keeler, 2012) while *S. noctilio* in the northeast begins emerging in the summer (Long et al., 2009). If *S. noctilio*'s summer emergence pattern continues after successfully spreading to the Southeast, the differences in seasonality (i.e. longer, hotter summers and shorter winters) in southern pine defenses may have a direct effect on the survival and fitness of individuals.

## DETECTION, MONITORING, AND MANAGEMENT

There are several steps to take before one can determine the most effective method for detection, monitoring, and control of any insect. The first, and often most difficult, is to define the term “risk”. The meaning is different depending on the observer and, therefore, many forms of risk mapping are needed depending on the individual map user. Often, in terms of forest entomology, risk deals with tree mortality, or growth and volume loss. After risk is defined, insects or diseases to be analyzed should be determined. Sometimes this information is already known based on previous introductions. Areas at risk are then defined which is often determined by the region’s climate and abundance of suitable host material (Lewis, 2002).

Geographic information systems (GIS) are very powerful tools in risk mapping and have been used in mapping hazards of potential of forest fires, diseases of humans, tracking bird habitats and much more (Castro & Chuvieco, 1998; Daniel et al., 1998; Gobeil & Villard, 2002). Layers are created using a single parameter (e.g. area of hardwoods, slope etc.) and added one layer at a time to the GIS model. The final map consists of many layers, each with a geographic characteristic indicating an area that is susceptible to the organism being analyzed. Models can be developed for individual animals using data collected from surveys or by calculating parameters from estimates.

The USDA Forest Service released a risk map in 2006 (Fig. 2) with a detailed image of the likelihood of establishment by *S. noctilio*. This model was created using *S. noctilio* life history information; this consisted of its native climate, host species, and *Sirex* life cycle. In addition to being in a favorable climate, there are millions of hectares of pine, the host of *S. noctilio*, in the southeast (Elliott II et al., 2007). Using this information, areas of the United



States with suitable hosts and a similar climate to the native range of *S. noctilio* were identified as ‘at risk’.

Another method of risk mapping, CLIMEX models, makes the fundamental assumption that species’ distribution is based solely on climate. These models may use experiments or previous literature to determine climatic parameters, or can estimate these parameters, the first method being the preferred (Keller et al., 2009). Using a CLIMEX model, Carnegie et al. (2006) predicted that *S. noctilio* could survive and establish throughout much of the United States, including the southeast, the Appalachian Mountains, and the northeast.

Dinkins (2011) performed experiments to determine host preferences of *S. noctilio* that included three pines, two of which are native to the southeastern U.S. Pines included in these experiments were Virginia pine and loblolly pine (*Pinus virginiana* Mill. and *Pinus taeda* L. respectively). Scots pine (*Pinus sylvestris* L.) was included as a positive control because it is a native host tree of *S. noctilio* (Kirkman et al., 2007). There was a trend for *S. noctilio* to drill more in *P. virginiana* than *P. taeda* but there were no significant differences among the two native species. *Sirex noctilio* females were found walking on larger bolts (18-26 cm diameter) 23 times more than smaller bolts (11-17.8 cm diameter).

The results of those studies contradict those of Madden (1975) and Neumann & Minko (1981) who observed *S. noctilio* attacking smaller diameter trees. However, it was noted that these smaller diameter trees were often in overstocked stands, stressing them and limiting growth and also making them more susceptible to insect attack. Dinkins (2011) also states that *S. noctilio* in the laboratory may be more attracted to the higher amount of volatiles being released from larger diameter bolts in a smaller area. Host preferences of female *S. noctilio* determined by her study will allow for the more effective use of trap trees.

Trap trees are felled to attract specific insects depending on the type of tree felled and possible lures used. In many cases, they are strategically placed to draw insects away from healthy, more valuable trees but can also be used to concentrate populations to a specific area. There are many examples of trap trees being used effectively in different systems for control and monitoring purposes (Lanier & Jones, 1985; Lister et al., 1976; O'Callaghan et al., 1980; Prokopy et al., 2003). In the case of the native *Sirex*, trees are felled to attract adults for capture and laboratory rearing.

Trap trees can be created in ways other than felling. Other methods include damaging the crown, girdling the tree, or treating it with herbicide. Neumann et al. (1982) described techniques for early detection of *S. noctilio* including herbicidal treatment of pines in Victoria with Dicamba™ – an organochlorine compound. Treating trees with an herbicide allows for the stressing – and possibly death – of the tree, creating an attractive environment for siricids. In their study, spring treatment with herbicide resulted in the highest incidence of *S. noctilio* attack and was considered the best way to detect the pest in that area. Several methods of capture, including trap trees, funnel traps or Lindgren traps along with lures, should be examined to determine the most effective way to monitor for the pest in the southeast. While methods have been refined to make use of herbicides to create effective trap trees, methods of mechanical felling to create trap trees has not been examined as thoroughly (Dodds 2007).

Regardless of the method used to monitor for *Sirex noctilio*, management practices should be strictly followed to prevent this insect from becoming a pest in the southeastern U.S. Common silvicultural practices, such as yearly thinning, have been shown to be very effective at controlling levels of *S. noctilio* (Bordeaux & Dean, 2012; Hurley et al., 2007). It should, however, be reiterated that these studies focused on regions in the Southern Hemisphere and,

therefore, different methods of monitoring and control should be studied for use in the Northern Hemisphere. Political, social, and economic situations often complicate the use of silvicultural practices, so a multifaceted approach will be required to have a successful monitoring and control program for *S. noctilio* in the Southeast (Slippers et al., 2012).

## **OBJECTIVES**

The main objective of my thesis was to examine oviposition behavior of female *Sirex* in relation to pine bolt age and moisture status. Moisture content is alleged to be the main driving force behind oviposition and survival of siricids in field conditions (Coutts & Dolezal, 1965; Dye et al., 2008; Madden, 1981; Morgan & Stewart, 1966). To examine possible effects on the oviposition behavior of siricid females and survival of larvae in relation to differing moisture contents, two experiments were conducted and will be described in the following chapter. With this thesis I intend to answer these questions: 1) how do shortleaf pine bolts lose moisture over time and 2) how does moisture content of the wood affect oviposition behavior and survival of *Sirex*? This information is important to better understand survival of siricids in the laboratory and to create more efficient trap trees for capture in the field.

## FIGURES

Fig. 1. Patterns created during larval development within the xylem tissues of pine trees (Madden, 1974).

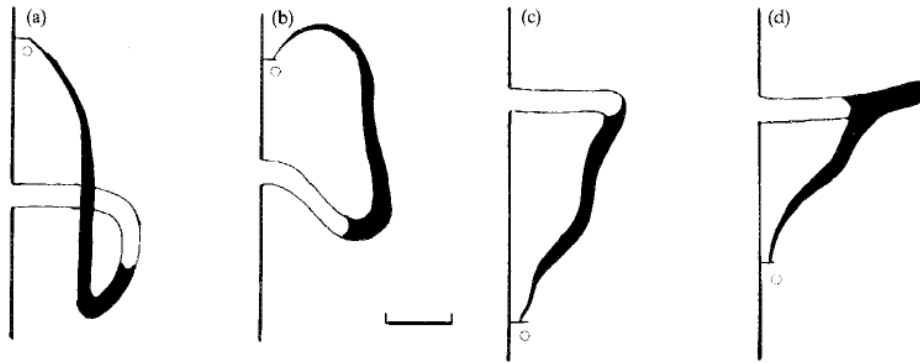
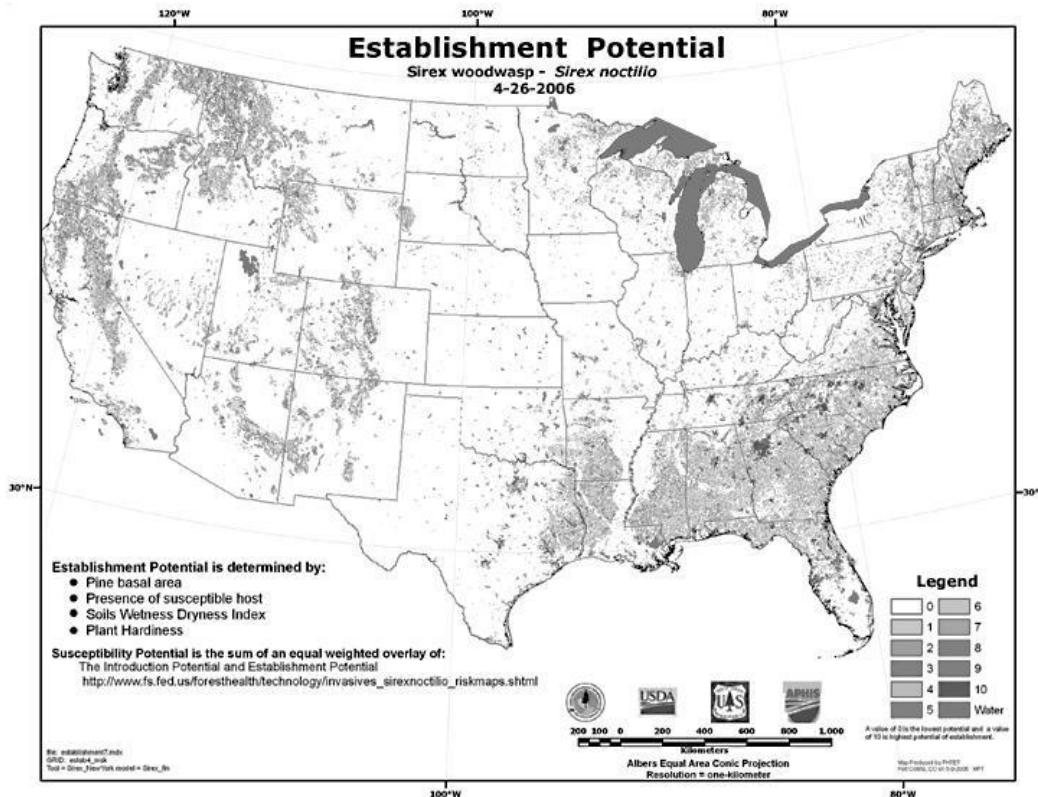


Fig. 1. Types of siricid gallery: (a) looped; (b) half looped; (c) kinked; (d) reversed. ○ Oviposition site. Scale line, 1 cm. After Rafes (1960).

Fig. 2. Risk map of the United States showing areas most likely to become infested with *S. noctilio*. Forest Health Technology Enterprise Team. ([http://www.fs.fed.us/foresthealth/technology/pdfs/sirexestablishment\\_surface.pdf](http://www.fs.fed.us/foresthealth/technology/pdfs/sirexestablishment_surface.pdf))



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**DECLINE IN MOISTURE CONTENT OF SHORTLEAF PINE (*PINUS ECHINATA* MILL.) BOLTS AND ITS EFFECTS ON OVIPOSITION BEHAVIOR AND SURVIVAL OF *SIREX NIGRICORNIS* F. (HYMENOPTERA: SIRICIDAE)**

**ABSTRACT**

The European woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae), was first discovered in the United States and Canada in 2004 and is currently spreading throughout the northeastern U.S. In some areas of the Southern Hemisphere it has become a primary pest of commercially grown, exotic pines and has caused extensive damage. The objectives of this study were to examine moisture content (MC) and moisture loss of felled pine logs, and evaluate the effects of varying moisture content on oviposition behavior and survival of *S. nigricornis*. Five shortleaf pine trees (*Pinus echinata* Mill.) were felled in August of 2012 and cut into four, 75 cm long logs (bolts), and wrapped in a coarse mesh to prevent insects from colonizing bolts. Bolts were separated into four 15-day treatment levels and held in the field during moderate drought conditions. The final set of bolts was left in the field for 45 days. Bolt cross-sections, each 3-4 cm thick, from each end as well as the center, were weighed and dried to calculate moisture content. Data were compared among treatments as well as three positions (bottom, top, center) along the length of the bolt. Average moisture content dropped significantly over time, leveling off after 30 days, with most of the water loss occurring at each end. Bolts' centers stayed consistently at, or above, 80% MC even after 45 days of exposure to dry field conditions. Six additional pines were felled and each cut into five bolts, 75 cm in length, that were randomly separated into three treatment levels (0, 15, 30 days old). Mating pairs of *S. nigricornis* were exposed these bolts and their drilling behavior was observed. Consistently high MC in the center of a 75 cm bolt suggests that the center of a felled tree several meters in length likely contains a high MC weeks after felling.

Females consistently drilled into fresh bolts indicating that, currently, trap trees may be felled too early to efficiently capture ovipositing females for laboratory rearing. These results indicate that trap tree, or rearing bolt, felling methods may be altered to enhance laboratory rearing of adult siricids.

Keywords: *Sirex noctilio*, trap tree, rearing bolt, moisture content, oviposition behavior



## INTRODUCTION

### *Siricidae*

Siricid woodwasp (Hymenoptera: Siricidae) females oviposit under the bark of trees and larvae develop within the xylem tissue. Wasps in the genus *Sirex* L. inject a phytotoxic mucus as well as a symbiotic fungus, *Amylostereum* Boidin (Russulales: Amylostereaceae) into the tree during oviposition. After egg eclosion, larvae create long, winding galleries throughout the xylem while feeding on their symbiotic fungus (Madden, 1974). Pine mortality and damage to wood due to *S. noctilio* is often caused by a combination of larval galleries, *Amylostereum*, and phytotoxic mucus. In the native range of *S. noctilio*, this damage usually occurs in dead or dying trees which makes them secondary pests in those regions (Coutts, 1965; Madden, 1974; Morgan, 1968). In some areas of accidental introduction and successful establishment of *S. noctilio*, it has caused severe damage and mortality to commercial exotic pine stands of the Southern Hemisphere (Dodds & De Groot, 2012).

The European woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae) is native to Eurasia and north Africa where it is not known to be a primary pest (Coutts, 1965; Morgan & Stewart, 1966). It has been introduced into several countries in the southern hemisphere beginning with New Zealand as early as 1900 (Kitching & Jones, 1981). However, it remained undetected there until an outbreak occurred in the 1940's which was most likely triggered by several years of severe drought (Coutts, 1965). Following the accidental establishment in New Zealand and Australia, it has since spread to much of the southern hemisphere including Tasmania, Brazil, Chile, Uruguay, and South Africa (Hurley et al., 2007).

*Sirex noctilio* was discovered in the United States and Canada in 2004 (Hoebeke et al., 2005) and is spreading throughout much of the northeast including parts of Michigan,

Pennsylvania, Ohio, and Vermont (Dodds & De Groot, 2012). Dinkins (2011) performed host preference experiments using several southeastern pine species, including loblolly pine (*Pinus taeda* L.), which is a species native to Arkansas, which was found to be very attractive to *S. noctilio* females. The other pine species native to Arkansas, shortleaf pine (*Pinus echinata* Mill.) was not included in those studies. However, this attraction does not indicate whether females were ovipositing or checking for host suitability. The next step in the process of examining host preferences is to evaluate *Sirex* oviposition behavior in the presence of these attractive pine species.

Keeler (2012) describes the biology and phenology of the only siricid woodwasp native to Arkansas, *Sirex nigricornis* F. Her research suggests that *S. nigricornis* may be used as a proxy species to predict how *S. noctilio* may behave if, or when, it reaches the southeast. Both species have similar flight patterns, closely related species, and are native to similar, temperate climates.

#### *Moisture Content*

Moisture content (MC) of wood and its relation to the defenses of pine has been well documented (Christiansen et al., 1987; Reid, 1961; Waring & Pitman, 1985). The relationship between MC and tree defenses is very complicated; a severe lack of moisture as well as an overabundance of moisture can result in low resin flow, making trees more susceptible to injury. However, moderate moisture stress can increase resin production, making pines more resistant to insect attack (Lorio, 1988). The fine balance between these levels of moisture stress make it unclear whether drought during the life of tree may affect how it dries after felling.

While MC is important to tree defenses, it is also important to *Sirex* biology in many ways. The wood needs to be at least partially dry (40-75% oven dry weight is ideal) to allow for

the development of larvae, and must be relatively dry to allow for the development of the woodwasps' symbiotic fungus, *Amylostereum*. The negative effects of high moisture content (70% and higher oven dry weight) and resin chemicals on the successful development of *Amylostereum* have been well documented (Dye et al., 2008; Madden, 1974; Talbot, 1977).

Madden (1974) describes the oviposition behavior of females in a field setting and gives examples of external forces that may alter this behavior. He describes MC as a major driving factor behind *S. noctilio* oviposition. Logs (bolts) with a higher MC (above 80%) were more likely to contain single drill holes indicating that females were only probing bolts. On the other hand, bolts with lower (40-50%) MC tended to have a higher incidence of drill holes containing multiple tunnels indicating that females were ovipositing and not simply probing. However, at very low MC (below 20%) no eggs were laid by females (Coutts & Dolezal, 1965).

#### *Trap Tree Methods*

Trap trees are those which are felled, or mechanically damaged, to attract certain insects. This attraction may be done to control populations, concentrate individuals to a certain area (Lister et al., 1976; Nagel et al., 1957), or to capture adults for laboratory rearing (Scott & Berryman, 1971) as is the case with our native *Sirex* species.

Previous experiments showed optimal timing for chemical damage and mechanical girdling of pines to effectively attract *Sirex* females (Zylstra et al., 2010). However, other studies examining felled trees for capture and rearing have failed to provide repeatable, effective methods that may be appropriate in the southeastern United States (Dodds, 2007, Hajek et al., 2008). Correctly defining the balance between high and low MC to create an attractive trap tree that also allows for successful development of larvae is difficult yet essential in performing laboratory experiments and rearing.

## *Objectives*

The main objective of this study was to examine changes in moisture content of pine rearing bolts and oviposition behavior of *S. nigricornis* in relation to different-aged pine. These studies aim to address two central questions: 1) how do shortleaf pine bolts lose moisture over time and 2) do female *Sirex nigricornis* show a preference for oviposition in certain ages and moisture regimes of pine bolts? Two experiments were performed to answer these questions and will be discussed in the following sections.

## MATERIALS AND METHODS

### *1. Moisture Loss in Pine Bolts*

#### *1.1 Tree and Bolt Collection*

Five shortleaf pine trees 7-10 cm DBH (diameter breast height) were felled on August 15<sup>th</sup> 2011 at the University of Arkansas Agricultural Research Station. Trees were chosen based on shared characteristics; i.e. bark thickness, height and number of branches, and DBH. Each of the five pines was cut into four logs (bolts), 75 cm in length, resulting in a total of 20 bolts. Bolts were randomly separated into four treatments. Each treatment consisted of bolts being left in the field for a period of 15 days. This resulted in an initial set of five bolts being cut and weighed immediately after felling. The second set was left in the field for 15 days, then cut and weighed. The third set was left in the field for 30 days and the final set was left in the field for a total of 45 days. Each set of treatment bolts was wrapped in a standard, coarse, fiberglass window mesh, closed at each end with zip ties. Bolts wrapped in mesh were laid longitudinally, slightly elevated from the ground using two 5.0 cm x 10.2 cm x 30.0 cm (standard 2 x 4 in) boards at each end of each set of bolts to avoid fungal infection.

#### *1.2 Experimental Design*

Moisture content was calculated by measuring the wet weight of wood at the end of each treatment period and the dry weight after 48 hours in a vacuum oven at 100°C. The following equation illustrates how these two measurements are applied to obtain a percent MC (de Groot et al., 2006):

$$\text{MC \%} = \left[ \frac{\text{Wet Weight (g)} - \text{Dry Weight (g)}}{\text{Dry Weight (g)}} \right] \times 100$$

To obtain a range of moisture contents for the same bolt, three cross-sectional cuts of each bolt – top, bottom, and center – were measured. Each cross-sectional cut was 3-4 cm thick and cut using a band saw. This was performed on five bolts per replicate at four treatment dates resulting in a total of 60 cross-sections.

### *1.3 Data Analysis*

All data were analyzed using R (R Development Core Team, 2010). To determine the most appropriate statistical analysis, the distributions of these percent MC data were examined. Data were not normal and were thus transformed using log, ArcSin, and a Box-Cox transformation test (Southwood & Henderson, 2000). These transformations failed to normalize data, thus, these data were ranked for future analyses. After ranking data, a one-way ANOVA was performed to test significance among treatment levels of days left in the field and MC, as well as the position of each cross-sectional cut and MC (Sokal & Rohlf, 1995). These tests examine statistical significance among treatment levels but do not explain which levels are significantly different from each other. Significance of both tests permitted separation of means using a Tukey's HSD test.

A Tukey's HSD test reveals that the bottom and top cross-sectional cuts had significantly lower MC than the center cuts. To determine if the bottom and top data were significantly

different from each other across treatments, a pairwise t-test was performed to evaluate each treatment level as containing five replications (each individual bolt), each having two responses: bottom and top. The extremely low MC of bolt ends suggested that these low values may have altered the original analysis. To determine if similar results would be obtained without the low MC of the bottom and top cross-sectional cuts, these positions were removed from the data and a one-way ANOVA was performed examining only the center cross-sectional cut data in relation to days left in the field.

Average MC of the center cross-sectional cuts did not significantly decrease over time which suggested that these data may have altered the original ANOVA, leading to less statistical significance. To examine this alternative possibility, the center MC data were removed and a one-way ANOVA was performed examining only the bottom and top MC data over time.

## 2. *Oviposition Choice Tests*

### 2.1 *Sirex Collection*

Several shortleaf and loblolly pine trees were felled in the Ozark-St. Francis and Ouachita National Forests beginning in mid-September continuing through October of 2010 to attract emerging adults. *Sirex* females had the opportunity to oviposit in the trap trees throughout fall of 2010. These trees were collected the following spring and summer of 2011, cut into 75 cm bolts and placed in plastic rearing containers in a covered, but open outdoor shed. This was done to ensure that the development and survival of siricid larvae were not compromised by differences between field and laboratory conditions. Rearing containers were checked daily, numbers of both sexes were recorded, and live siricids were brought into the laboratory. Immediately following extraction of siricid adults, randomly selected male/female pairs were placed in

individual cages for the following choice test experiment. Females do not need fertilization by a male to produce eggs, thus mating was not controlled for.

## 2.2 *Experimental Design*

This split-block experiment designated siricid mating pairs as the blocking factor to account for any individual variation among randomly selected individuals. Cages were designated as treatments and each cage contained three bolts of discrete ages which were considered sub-treatments. Preliminary research showed a steady decline in MC over a 45 day period when exposed to dry field conditions and these results were used to assign MC parameters to bolts that were used to examine possible oviposition preferences of *S. nigricornis* females. These prior studies showed that, after 30 days in dry field conditions, MC levels of felled pine bolts did not continue to drop and, therefore, no treatment level past 30 days was included.

An additional set of two shortleaf pines were felled in mid-September 2011, approximately 30 days prior to expected emergence (30 day bolts). Two more pines were felled 15 days later in early October (15 day bolts) with a final set of two trees was felled 15 days later in mid-October (0 day bolts). Each tree was cut into five 75 cm long bolts immediately after felling resulting in 10 replications per treatment level. These felling events were performed at the times suggested by Dodds (2007) for *S. noctilio* and adjusting them to climatic conditions of Arkansas.

Three bolts, one from each sub-treatment, were exposed to a single siricid male/female pair in a cage within the laboratory. The bolts had average MC of 75, 68, and 50% respectively based on previous experiments. There were 10 replications (i.e. cages) resulting in a total of 30 bolts. Mating pairs typically lived for 7-10 days, and females were observed consistently

ovipositing throughout the duration of their adult life. Adults were held in a photoperiod of 16:8 D:L to mimic local late fall conditions.

After death of the females, bolts were removed and siricid drill holes were counted under a dissecting microscope and marked with a permanent marker or grease pen for later examination. Bolts were held at a constant temperature of 29°C, within the optimal range (25-33°C) for siricid development (Madden, 1981). Adult emergence began in February 2012 and both sexes were observed emerging which indicates successful mating occurred.

### 2.3 *Log Dissections*

Emergence was monitored beginning in February 2012 and continuing until mid-April at which point there had been several weeks without additional emergence. Bark around each drill hole, which had been previously marked, was carefully peeled away. Drill holes were dissected using a wood-carving tool kit and drill holes were slowly excavated until the xylem was encountered. Presence of multiple tunnels, egg cases, and siricid development was recorded.

After bark removal and drill hole dissection, a band saw was used to cut 0.5-1 cm longitudinal sections along the length of the bolt. If any sign of insect development was encountered (e.g. galleries, frass, dead or live larvae etc.), the width of the cut was reduced to ensure all developing insects would be detected. Stage and condition of all insects within the wood was recorded and insects were stored at -15°C.

Drill holes on the surface of the bark are not necessarily an indication of oviposition – females may be testing for host suitability. Multiple tunnels stemming from a single drill hole indicate successful oviposition. Drill holes that branch into multiple tunnels typically have n-1 eggs (n=number of tunnels), while single tunnels do not contain eggs (Coutts and Dolezal, 1969). Coutts (1965) describes “oviposition patterns” as a calculation of proportions of single, double,



triple, and quadruple tunnels to total drill holes. Total number of drill holes in a single bolt was divided by the number of double, triple, or quadruple tunnels found during drill hole excavation to obtain *S. nigricornis* oviposition patterns.

#### 2.4 Data Analysis

Data were analyzed using R (R Development Core Team, 2010). To determine the most appropriate statistical test, distribution of drill hole raw data was examined. These data were non-normal, thus a log transformation was used (Sokal & Rohlf, 1995) to normalize the data. Data were then analyzed using a one-way ANOVA and overall significance allowed for the separation of means using a Tukey's HSD test.

Oviposition patterns of multiple tunnels to drill holes were also non-normal. Both log and ArcSin transformations failed to normalize data, thus proportion values were ranked before performing statistical analysis. A one-way ANOVA was performed to test significance among treatment days on ranked proportion data. Polynomial curves were also fit to determine if relationships existed between number of drills per bolt and proportion of multiple tunnels to drills.

## RESULTS

### 1. Moisture Loss

Moisture content averages of 0, 15, 30, and 45 day-old bolts were 73.4, 68.3, 50.6, and 52.7% respectively with standard errors of 4.4, 5.5, 6.0, and 6.2 respectively. Moisture content was significantly different among treatment levels of days left in the field (Fig. 1;  $p=0.018^*$ ). This level of significance allowed for the separation of means using a Tukey's HSD test. There were significant differences between 0 and 30, and 0 and 45 day treatments ( $p=0.030^*$ ,  $0.050^*$  respectively). The 15 day treatment was not significantly different from 0, 30, or 45 day

treatments ( $p=0.920, 0.120, 0.210$  respectively). There was no statistical significance between the 30 and 45 day bolts ( $p=0.990$ ).

Moisture content of the center, bottom, and top cross-sectional cuts averaged 84.8, 52.0, and 47.0% respectively with standard errors of 2.4, 3.9, and 4.3 respectively (Fig. 2). The center position was significantly different from the bottom ( $p<0.0001^*$ ) and top cross-sectional cuts ( $p<0.0001^*$ ). The bottom and top positions were not significantly different from each other ( $p=0.600$ ). There is a 5% difference between the bottom and top cross-sectional cuts when averaging all MC data. This difference may have been significant if the variation caused by the center position was removed.

To further illustrate that there were no significant differences between the top and bottom cross-sectional cuts, the data were re-analyzed excluding the center cross-sectional cut data. Data were evaluated as a single sample (each bolt) with two responses: bottom and top and, across all treatment levels – 0, 15, 30, and 45 days – there were no statistically significant differences ( $p=0.38$ ). There was more variation in the 0-day bolts but variation decreased over time with very little variation in the 45 day treatment (Fig. 3).

The top and bottom cross-sectional cuts had a consistently lower MC than the center cross-sections which remained fairly high even after 45 days in dry field conditions. A one-way ANOVA was performed to determine if the average MC of the center cross-sectional cut decreased significantly over time and results indicated that moisture in the center was constant throughout all treatments (Fig. 4;  $p=0.0945$ ). Center cross-section MC averaged 81.1, 94.4, 79.4, and 84.4% from 0, 15, 30, and 45 day bolts respectively. Standard errors for these treatments were 9.7, 13.2, 7.5, and 5.7 for 0, 15, 30, and 45 day-old bolts.

The original analysis (Fig. 1) indicated a decreasing trend in average MC. However, center cross-sectional cuts remained at, or above, 80% MC (Fig. 2) suggesting that these data may have skewed our original interpretation.

Center cross-section data were removed and a one-way ANOVA was performed to the remaining bottom and top data which showed an overall significance ( $p < 0.0001^*$ ) and sharp decreases in MC after 30 days in dry field conditions and leveling off after that (Fig. 5). A Tukey's HSD test was used to test for significance among treatment days using only these bottom and top cross-sectional data. Means of the top and bottom cross-sections across treatments dates were 69.5, 73.4, 36.3, and 36.8% with standard errors of 19.0, 24.2, 10.6, and 7.3 for 0, 15, 30, and 45 day-old bolts respectively. The 0 and 30 day treatments, and the 0 and 45 day treatments were significantly different (both  $p < 0.0001^*$ ), as they were in the original analysis. Significance was also observed between 15 and 30 day treatments ( $p = 0.005^*$ ), and 15 and 45 day treatments ( $p = 0.010^*$ ). The 30 and 45 day treatments, and 0 and 15 day treatments were not significantly different from each other ( $p = 0.996$ ,  $0.060$  respectively).

## 2. *Oviposition Choice Tests*

Averages of siricid drill holes were statistically significant among treatment levels ( $p = 0.00084^*$ ) which allowed for the separation of means using a Tukey's HSD test. Zero day-old bolts averaged 44.3 drill holes, 15 day-old bolts averaged 18.1 drills, and 30 day-old bolts averaged 4.4 drills with standard errors of 13.7, 8.4, and 0.5 respectively. The 0 and 30 day treatments were significantly different from each other ( $p = 0.010^*$ ) while 0 and 15, and 15 and 30 day treatments were not (Fig. 6;  $p = 0.130$ ,  $0.560$  respectively).

After dissection of bolts to obtain "oviposition patterns" for *S. nigricornis*, proportions of multiple tunnels to drill holes were calculated and compared among treatment levels. The most

recently felled bolts (0 days) had the highest proportion of multiple tunnels per drill (0.29). The 15 day-old bolts averaged a proportion of 0.2 and the 30 day-old bolts had a proportion of 0.1. Standard errors for all were 0.1. However, these decreasing proportions among all treatment levels were not statistically significant from each other. To examine the relationship between multiple tunnels and drill holes, curves were fitted to these proportion data. There was no statistical significance using a linear, quadratic, or cubic curve. However, the trend that emerged was that the highest proportion of multiple tunnels to drill holes was seen in bolts with a median amount of drill holes (~20 holes). Bolts with very high numbers of drills rarely had those with multiple tunnels and bolts with very low numbers of drills also had few with multiple tunnels. Eggs were found just below the bark but, in every instance, were dry and had not survived (Fig. 7).

### 3. Log Dissections

A total of 33 siricid eggs were found in six 0 day-old bolts while no siricid larvae, pupae, or adults were present. One 0 day-old bolt contained an *Ips* sp. adult and an *Ips* sp. larva. These insects were not alive or intact to obtain a definite identification. Only four *Sirex* eggs were recorded from the 15 day-old bolts. However, a total of 36 siricid larvae were present but none were alive. A single 15 day-old bolt contained two dead *Sirex* pupae and a single, live *Monochamus farate* adult. The only adult siricids (5 total) were recovered from the 15 day-old bolts. This same bolt was infected with blue-stain fungi. A total of four siricid eggs were recorded from the 30 day-old bolts but these eggs were dried out and not developing. Several buprestid (Coleoptera) were encountered and almost every 30 day-old bolt was infected with blue-stain.

## DISCUSSION AND CONCLUSIONS

Moisture content has been noted as being important in both tree defenses as well as in the development of siricid larvae, their symbiotic fungus, and associated nematodes (Madden, 1981). *Sirex* trap trees in Arkansas are felled by adjusting Dodds (2007) methods for creating *S. noctilio* trap trees to conditions common to the southeastern U.S. How differing levels of moisture in pine bolts in the southeastern U.S. affect the survival and fecundity of female *S. nigricornis* is unknown.

Shortleaf pine bolts felled in mid-August of 2011 at the University of Arkansas Agricultural Research Station, 75 cm in length, had a consistently high MC near the center of the bolt at, or around, 80% even after being left in moderate drought conditions for over 30 days. This suggests that a felled tree, kept whole, that is several meters in length, would also have a high MC even after weeks left in dry field conditions.

Current trap tree methods propose felling whole trees for siricid capture a month prior to expected emergence (Dodds, 2007) and our results suggest that there may not be a significant change in MC in the center of the tree – where *Sirex* females concentrate their oviposition (Madden, 1974) – to require this early felling. Females significantly preferred to oviposit in the most recently cut, 0 day-old bolts. In addition, insect development was only recorded in 0 day-old and 15 day-old bolts. No development or emergence was observed during dissection of the 30 day-old bolts. This suggests that the timing of tree felling, and bolt cutting, may not be critical in the attraction of adults.

If felling of trees, or trees which are cut into bolts, can be delayed until emergence of adults is observed, monitoring can continue into the fall to focus time, energy, and funds on areas with definite siricid populations. Therefore, areas lacking a definite population may be ignored

so as to not waste resources on regions that will not contain a high enough population of adults for laboratory rearing and experiments.

More efficient trap tree methods are necessary for successful monitoring and experimentation. There have been no concrete solutions for mechanically creating trap trees that can then be used for laboratory rearing and experiments. Results from these studies point to timing and length of rearing material (i.e. felled pines or bolts) as major factors in attracting female *Sirex* in field settings. Trap tree methods may differ in various regions of the United States as well.

In addition to refining trap tree methods in a field setting, methods of laboratory rearing should also be examined. Laboratory rearing is essential to maintain a continuous culture of insects. Laboratory cultures allow more experiments to be performed and with more replications. However, these methods may be more difficult to define because they vary based on many factors including space available, local humidity, and temperature. Many trees, when left in field conditions, can quickly become infected with fungi, as was observed in the log dissections of this study. In cases where laboratory space is not large enough for fast siricid development, rearing material may be left outdoors to allow for normal development of larvae. However, this does not allow for more than a single generation a year.

Researchers will most likely need to make adjustments in the techniques employed to obtain data that can be used to address their objectives. These adjustments may include time spent in the field, money spent on monitoring, or working with fewer insect generations per year. However, even small changes in these areas may lead to an exponential increase in capture and rearing efficiency.

## FIGURES

Fig. 1. Average moisture content (%) of five logs per treatment level of days left in the field. Horizontal lines within gray boxes indicate means. Vertical lines above and below boxes indicate maximum and minimum values respectively. Diamonds above and below boxes indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles.  $p=0.018^*$ .  $df=3$ .  $n=20$ .

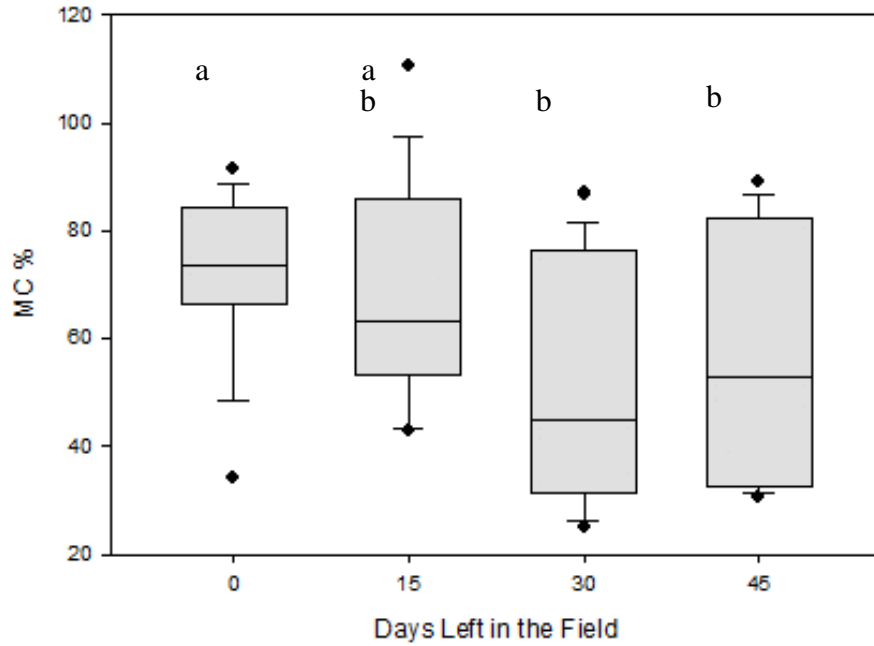


Fig. 2. Average moisture content (%) of 20 logs among three treatment levels of cross-sectional slabs taken at the bottom, center and top of each log. Horizontal lines within gray boxes indicate means. Vertical lines above and below boxes indicate maximum and minimum values respectively. Diamonds above and below boxes indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles.  $p < 0.0001^*$ .  $df = 2$ .  $n = 60$ .

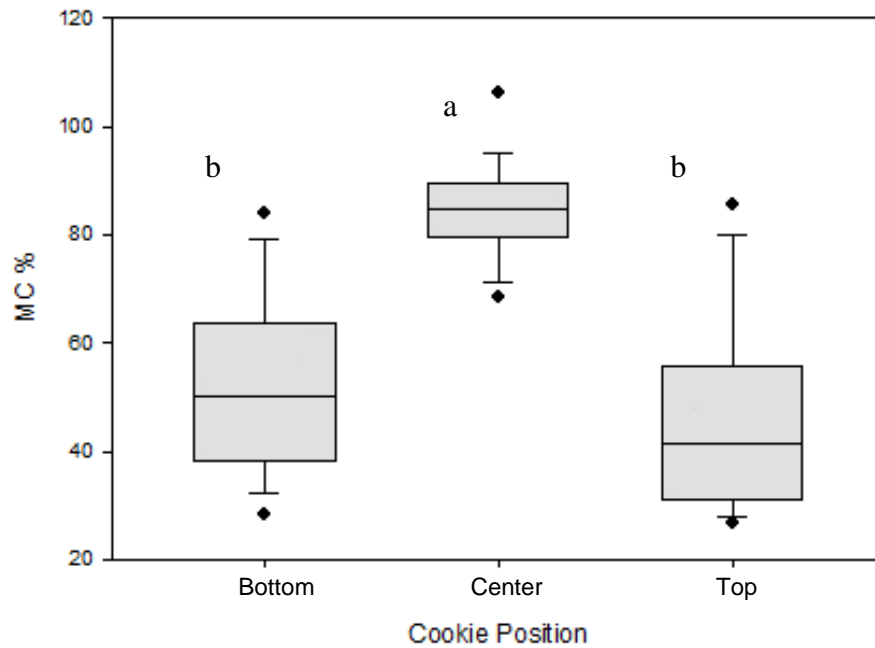




Fig. 3. Moisture content (%) of bottom (●) and top (○) cross-sectional cuts from 20 logs among four treatment levels of days left in the field. Each circle indicates a single moisture measurement from a log.  $p=0.38$ .  $df=3$ .  $n=40$ .

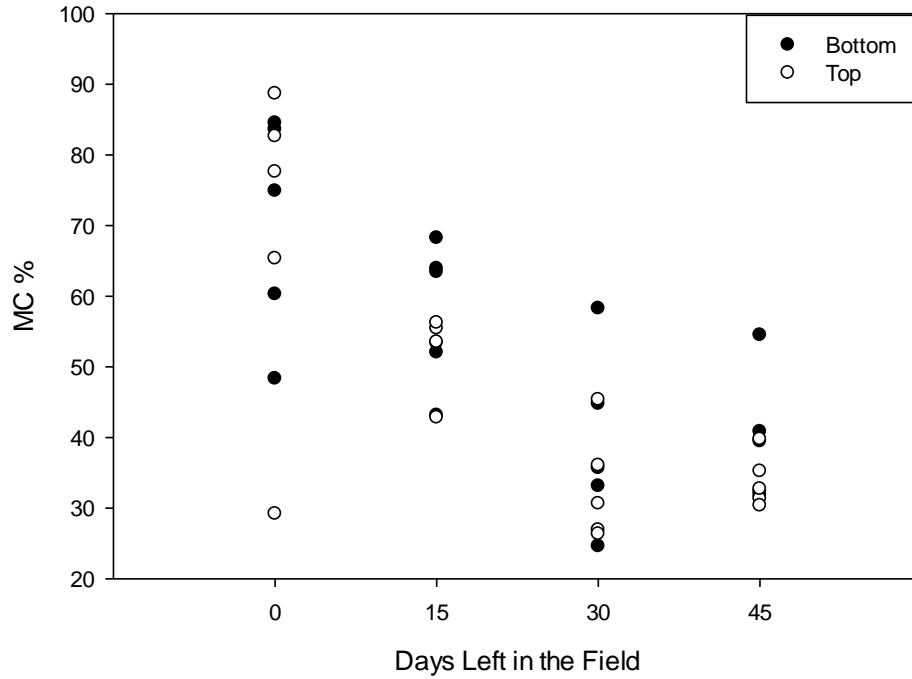


Fig. 4. Average moisture content (%) of center cross-sectional cuts among treatment of days left in the field. Horizontal lines within gray boxes indicate means. Vertical lines above and below boxes indicate maximum and minimum values respectively.  $p=0.0945$ .  $df=1$ .  $n=20$ .

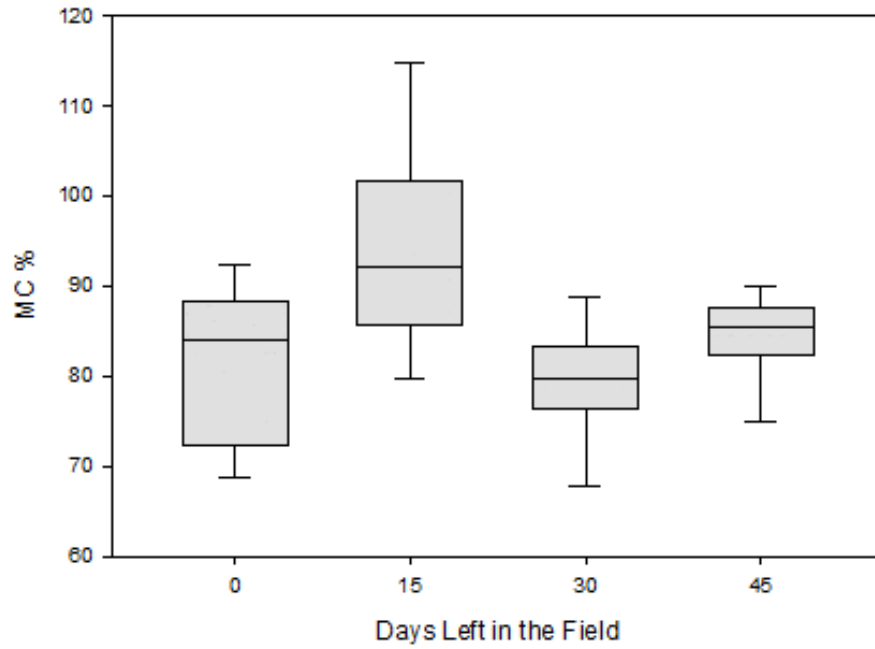


Fig. 5. Average moisture content (%) of bottom and top cross-sectional cuts from 20 logs, five per treatment level, among four treatment levels of days left in the field. Horizontal lines within gray boxes indicate means. Vertical lines above and below boxes indicate maximum and minimum values respectively. Diamonds above and below boxes indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles.  $p=1.4e-07$ .  $df=3$ .  $n=40$ .

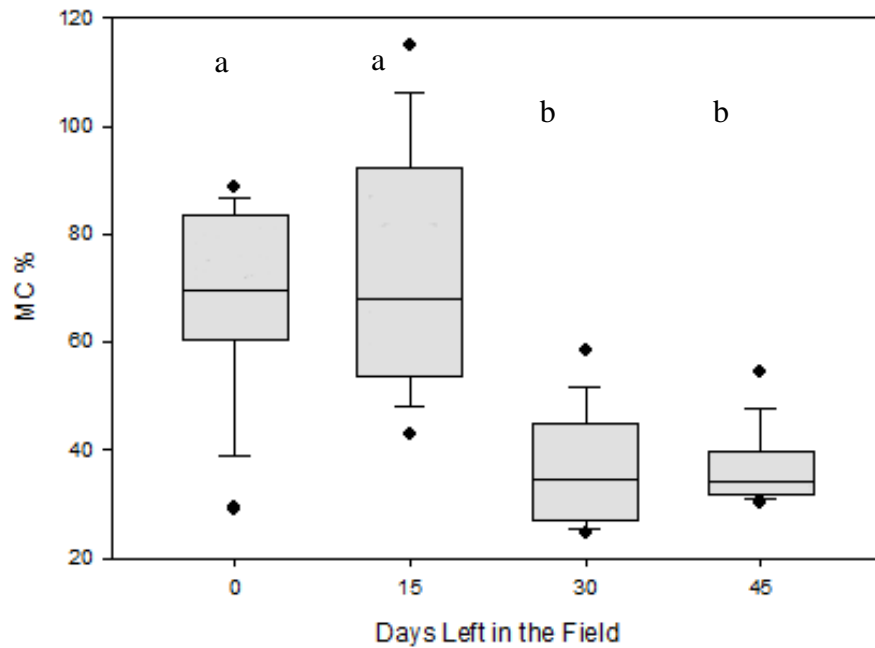


Fig. 6. Average number of siricid drill holes from 10 logs among three treatment levels of days left in the field. Horizontal lines within gray boxes indicate means. Vertical lines above and below boxes indicate maximum and minimum values respectively. Diamonds above and below boxes indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles.  $p=0.00084^*$ .  $df=1$ .  $n=30$ .

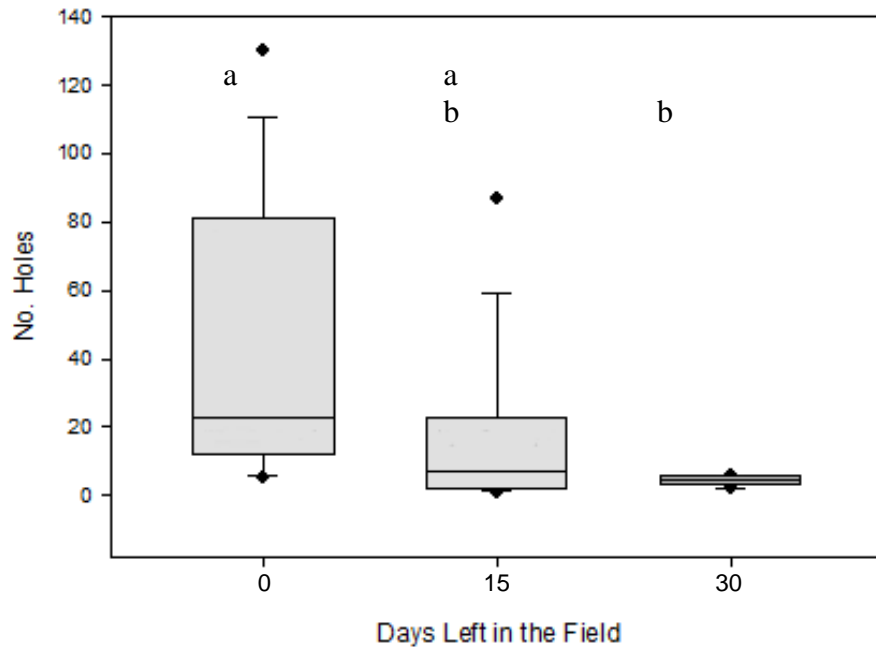
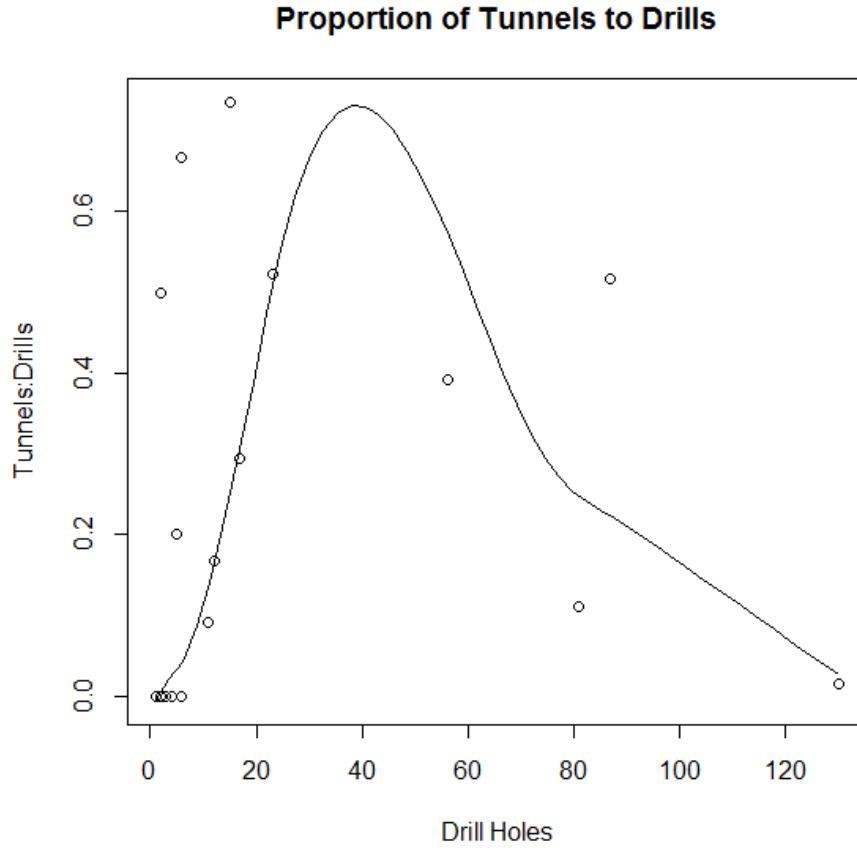


Fig. 7. Proportion of multiple tunnels over drill holes made by siricid females. Open circles indicate a single data point (one log). Y-axis is calculated by dividing the total number of multiple tunnels stemming from a single drill hole by the total number of drill holes in that bolt.



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## CONCLUSIONS

Research has been performed to test the efficacy of herbicidal poisoning in the creation of trap trees for *S. noctilio*, but mechanical felling of pines has not been as thoroughly investigated (Dodds, 2007). Having defined methods for insect capture and rearing is necessary to accomplish laboratory experiments. Many times experiments cannot be completed to their full capacity due to a lack of replications, often caused by the need for defined rearing procedures. This is especially important in the case of siricids due to their short life span and short emergence period.

My research has provided information that can be used to alter current trap tree methods. However, whether altered methods are efficient in a field setting is still unknown. Field trials to test multiple methods of trap tree felling should be performed to compare combinations of bolt ages and lengths to determine the best technique to, not only attract females, but also allow for successful larval development. However, efficiency of trap tree felling is only one aspect of laboratory rearing and experiments.

Once the most efficient combination of age and bolt length is determined, methods of laboratory rearing can then be refined. Moisture loss of bolts appears to be an extremely important factor in maintaining a continuous culture of *Sirex* (Madden 1981). Using information gathered from previous and current studies for the successful rearing of siricids is complicated by a general lack of knowledge about our native woodwasps' life histories and natural enemies. Temperature and humidity varies greatly by region, complicating the rearing process, and more research is needed to determine optimum laboratory settings to fully rear siricids.

## FUTURE RESEARCH

An important issue not addressed in this study is how MC changes across the entire length of a felled shortleaf pine bolt. Moisture levels were only measured at three positions in each bolt and, therefore, much of the bolt was not taken into account in these data. To investigate this further, pine bolts should have cross-sections cut every 3-4 cm along the entire length of the bolt, resulting in approximately 25 cross-sectional cuts per bolt. Obtaining enough replications to allow for justifiable implications from this study would be extremely labor intensive and, thus, could not be undertaken in the short time allowed for this experiment.

These results suggested that felling may be delayed until observed siricid emergence. The length of rearing material was also suggested to possibly alter females' oviposition preferences. To examine these suggestions further, two experiments should be performed.

First, several sites should be monitored for siricid activity beginning in late summer and continuing through the fall. Each site should contain a different method of trap tree, or bolt, felling which would consist of combinations of bolt lengths and ages, as well as trees with and without crowns. Johnson and Zingg (1969) found that, of all combinations examined, Douglas-fir trees with the greatest proportion of intact crown and the least amount of shade had the greatest water loss. They also state that trees with limbs removed may actually gain water over time. These experiments would determine if these drying patterns also apply to *Pinus* spp.

In addition to obtaining a complete moisture loss curve of felled pine bolts, detailed information of siricid development and survival in these varying conditions should also be examined. To determine how development differs in relation to different combinations of bolt ages and lengths, trees and bolts should be destructively sampled at consistent intervals. Using

this sampling method, mortality rates and causes of this mortality can be determined and studied in greater detail.

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