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FLIGHT PERIOD AND SPECIES COMPOSITION OF *SIREX* (HYMENOPTERA:
SIRICIDAE) AND ASSOCIATED *DELADENUS* (NEMATODA: NEOTYLENCHIDAE)
WITHIN ARKANSAS PINE FORESTS

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SIRICIDAE) AND ASSOCIATED *DELADENUS* (NEMATODA: NEOTYLENCHIDAE)
WITHIN ARKANSAS PINE FORESTS

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

By

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Bowling Green State University
Bachelor of Science in Environmental Science, 2009

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ABSTRACT

The European woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae), which is a known destructive pest of pine in the southern hemisphere was recently discovered in the eastern United States. Before we can understand how *S. noctilio* may affect pine forests throughout the United States, we need a better understanding of native *Sirex* and the role they play in the ecosystem. The objectives of this research were to 1) determine species composition and flight period for native *Sirex*; 2) confirm presence of parasitic nematodes, *Deladenus* (Nematoda: Neotylenchidae), within adult *Sirex*; 3) investigate *Deladenus* parasitism rates; 4) verify the number of *Deladenus* species found using molecular techniques. To accomplish these goals, panel traps were erected within pine forests in three ecologically distinct regions of Arkansas: Ozark Mountains, Ouachita Mountains, and Gulf Coastal Plain. Specimens collected in these traps were identified and later dissected for presence of *Deladenus*. One species of *Sirex* was collected, *Sirex nigricornis* F., and a single species of *Deladenus* was found in *S. nigricornis* specimens from all three regions.

Key words: invasive monitoring, flight period, Siricidae, *Sirex noctilio*, *Deladenus*,

This thesis is approved for recommendation
to the Graduate Council.

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DEDICATION

To my grandparents, Daniel and Janet Sellers, who always believed in me.

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CHAPTER ONE

LITERATURE REVIEW

Arkansas Forest Regions

Arkansas is comprised of six physiographic regions: Ozark Mountains, Ouachita Mountains, Arkansas River Valley, West Gulf Coastal Plain, Mississippi Alluvial Plain and Crowley's Ridge (Robison and Allen 1995). Each region has its own set of distinctive features, giving the state a wide range of ecological habitats. Among these six regions are three with extensive pine or pine-hardwood forests: Ozark Mountains, Ouachita Mountains and Gulf Coastal Plain. Despite all having a pine component, each area is unique.

The Ozark region not only includes Arkansas but also Missouri, Oklahoma and Kansas, an area of 34.3 million acres (The Nature Conservancy 2003). The Ozarks were formed by a geological uplift causing rock outcroppings throughout the region to be comprised of stacked layers of rock (Robinson and Allen 1995). Although not definitively proven, the Ozark Mountains are believed to be the oldest continuously exposed land mass in North America. The area has never been glaciated making it a refuge for a variety of organisms during major glacial events (The Nature Conservancy 2003). The Ozarks are considered the westernmost region of eastern deciduous forest. Within the forest, shortleaf pine is naturally the dominant pine (Paananen and Wilde 1999). Frequent low-intensity surface fires have been important in the development of Ozark forests. According to fire-scar analysis these areas have been burned for hundreds of years (Engbring et al. 2008) causing the habitat to be a mix of forest, woodlands and glades (Stambaugh and Guyette 2006). Today due to fire suppression, the Ozarks are primarily made up of closed canopy forests, although there are also extensive tall grass prairies (Schroeder 1981) and glade communities (Nelson and Ladd 1980).

The Ouachita Mountains are a belt approximately 60 miles wide and 120 miles long, spanning east-central Arkansas and south-western Oklahoma (Robison and Allen 1995). The

Ouachitas were the result of an orogeny, the collision of a continental and an oceanic plate causing the upward thrust of the mountains (Robinson and Allen 1995). This event can be seen in outcroppings of rock, which are twisted vertically toward the sky (Robinson and Allen 1995). The Ouachitas trend east to west (Foti and Bukenhofer 1998), resulting in mesic forest occurring on high elevation north facing slopes, a unique feature for a mountain range in North America (Shepard and Burbrink 2009). The Ouachita Mountains are covered in dense pine-hardwood forest (Foti and Bukenhofer 1998) with the northern area predominantly shortleaf pine, and with loblolly found in the southernmost region (Paananen and Wilde 1999). Together the Ozarks and Ouachita regions comprise the only highland in midcontinental North America and the only significant topographic relief between the Appalachians and the Rockies (The Nature Conservancy 2003).

The Gulf Coastal Plain is characterized by its lowlands and swamps (Robinson and Allen 1995). The Western Gulf Coastal States consisted mainly of old-growth pine forests up to the early 20th century (Bragg 2002). Before logging was initiated in the forests of southern Arkansas, there was a mix of habitats. Some pine areas were open, with older trees, while others were more of a closed canopy forest with a mixture of loblolly and shortleaf pine and hardwoods (Bragg and Heitzman 2009). These older forests have now been transformed into even aged and single species pine plantations (Bragg and Heitzman 2009).

Not only is pine important ecologically throughout Arkansas, but it is also crucial for the economy of the state. Of the 33 million acres in Arkansas, 18 million acres is timberland. Timber and poultry are toe to toe as Arkansas' number one cash crop (Reynolds 2003). Arkansas pine production is valued at over 1 billion dollars annually and is one of thirteen states that make up the "southern pine belt", which provides fifteen percent of the world's lumber (Reynolds 2003,

Borchert et al. 2007). Seventeen percent of the state's workforce is employed in the forest industry (Hughes 1998).

Siricidae Biology

Siricidae (Order: Hymenoptera) is in the suborder "Symphyta" (sawflies and woodwasps), which are characterized by a "broad waist" (the thorax broadly joined to the abdomen), as opposed to the "constricted waist" (constriction between the mesosoma and metasoma) of Apocrita. Unlike the rest of Symphyta, which have blade like ovipositors for inserting into plant leaves and stems, siricids have an ovipositor modified for inserting into wood. Siricidae are widespread in deciduous and coniferous forests of the northern hemisphere. However, none are native to Australia or South America and only two species are Afrotropical (Smith and Schiff 2002). Worldwide, there are eleven genera within Siricidae with approximately 100 species. Five of these genera and twenty-three species are found in North America (Schiff et al. 2006). There are two subfamilies within Siricidae, Tremicinae and Siricinae, which are distinguished by host preference. Tremicinae are associated with hardwoods, and Siricinae are associated with conifers, with species of *Pinus* being the major hosts for Siricinae (Benson 1943, Talbot 1977, Schiff et al. 2006). Most siricids attack only dead or dying trees with most species preferring windblown timber and decayed logs and a few preferring standing dead trees (Stillwell 1966, Spradbery and Kirk 1981). Many species are attracted to disturbance, such as forest fires (Smith and Schiff 2002).

Although many species of siricids have been studied sporadically, *Sirex noctilio* is the most well-known and widely studied due to its pest status in various countries. Most life-cycle and biology examples given in this review focus on *S. noctilio* and although the basic life-cycle

is assumed to be similar throughout siricids, in many cases it is unknown whether all species follow the same biological patterns.

Siricids can be identified by an anal spine, or cornus, which is retained in all life stages and gives the family the common name, horntails. The adult females can be distinguished from the males by a ventral ovipositor in a sheath directly below the cornus. An unusual feature of siricid adults is the extreme variation in body size even among the same species and sex (Schiff et al. 2006). Siricid larvae are whitish and cylindrical with three pairs of vestigial thoracic legs, toothed mandibles and the cornus (Morgan 1968). Larvae are morphologically indistinguishable from other siricid species (Wilson and Schiff 2010). The female larvae can be distinguished from the males by a pair of hypopleural organs situated on the sides of the first abdominal segment (Rawlings 1953). These structures contain hyphae of a fungal symbiont (Parkin 1942, Rawlings 1951). The eggs of siricids are elongate oval and a white to cream color, except in *Tremex columba* L., which has black eggs (Morgan 1968).

All siricids have a unique life cycle involving an important fungal symbiosis. Buchner (1928) discovered a paired glandular structure at the base of the ovipositor in female siricids, later named mycangia, which leads to the reproductive tract. Inside the mycangia, he found fungal arthrospores and determined the fungus was a Basidiomycete or wood rotting fungus. Fungal spores were later found not only in the mycangia but also in oviposition sites and larval tunnels, suggesting siricids introduce fungal spores into the wood during oviposition (Cartwright 1938). The relationship between the fungus and siricids is a highly evolved mutualism. Through this mutualism, the fungus becomes highly mobile and is inoculated into fresh hosts (Gilmour 1965, Slippers et al. 2003), while providing a suitable environment and nutrients for developing siricids within the host tree (Franke-Grosmann 1939, Madden and Coutts 1979, Kukor and

Martin 1983). *Xeris* is the only genus of Siricidae without a fungal symbiont. However, the fungus is still needed for survival. *Xeris* females oviposit in trees previously colonized by other siricid species and utilize the already present fungus (Spradbery 1973, Bedding and Akhurst 1978, Gilbertson 1984, Smith and Schiff 2002).

Talbot (1964) described the fungus associated with siricids as a wood-rotting fungus in the genus *Amylostereum* (Amylostereaceae). Previously, the associated fungus was thought to be in the genus *Stereum* (Francke-Grosmann 1939, Rawlings 1948, Stillwell 1960). Siricidae throughout the world carry one of three *Amylostereum* species, *A. chailletii* (Pers.:Fr.) Boid., *A. areolatum* (Fr.) Boid., or *A. laevigatum* (Fr.) Boid. Boidin (Slippers et al 2000, Slippers et al. 2003). The relationship between the *Amylostereum* species and the siricid species is shown to be species-specific for the siricid (Talbot 1977, Tabata and Abe 1997). For example, native North American *Sirex* species are considered to be associated with *A. chailletii*, while *Sirex noctilio* F. is associated with *A. areolatum* (Gaut 1969, Bedding and Akhurst 1978, Smith and Schiff 2002). However, since *S. noctilio* was introduced to the United States, the native *S. nigricornis* F. (formerly *Sirex edwardsii* Brullé) (Goulet 2012) has been found carrying *A. areolatum*, which may indicate the associations are not as species specific as originally thought (Nielsen et al. 2009).

Not only do siricids oviposit a symbiotic fungus into the tree, but paired glands in the female siricid secrete colorless “mucus” which is oviposited with *Amylostereum* (Coutts 1969b). This substance is a protein-mucopolysaccharide complex and is found in all siricids (Boros 1968, Wong and Crowden 1976). However, the mucus found in *S. noctilio* is the only siricid mucus found to cause pathological symptoms (Spradbery 1973).

Due to their neck flexibility, adult siricids create round emergence holes, which are distinguishable from emergence holes made by other wood boring insects by the circular shape and smooth edges (Morgan and Stewart 1966). Males tend to emerge earlier than females (Morgan 1968, Spradbery and Kirk 1978). Upon emergence, *Sirex* males fly into the tree tops where the females join them to mate (Chrystal 1928, Morgan and Stewart 1966, Smith and Schiff 2002). A contact pheromone in *S. noctilio* produced by the female has been isolated. However, how siricid males and females find each other long range is still unknown (Böröczky et al. 2009). Females are believed to be attracted to and seek out the males but it is unknown whether females are attracted by pheromones, sound or visual cues (Dolezal 1967).

Siricid females are facultatively parthenogenetic, which allows them to produce viable eggs without mating (Rawlings 1951, Rawlings 1953). All the males are haploid having only one set of chromosomes and females are diploid having two sets of chromosomes. Unfertilized females produce males, and fertilized females can produce either males or females (Morgan 1968). During favorable forest conditions the sex ratio of males to females is close to one, but during unfavorable conditions males surpass females even as high as 20:1 (Morgan and Stewart 1966). Unmated *S. noctilio* females are found to be less active and oviposit fewer eggs than mated females and similar behavior would be expected in other siricids (Madden 1974). Egg number at emergence has not yet been documented for most species, but *S. noctilio* can have anywhere between 30 and 450 eggs and larger females have more eggs than small females (Rawlings 1953, Madden 1974).

Although oviposition activity peaks on warm, sunny days, a few siricids can even be found ovipositing in the rain (Chrystal 1928, Morgan 1968). Oviposition behavior is also controlled by the properties of the tree's phloem such as moisture content and vigor (Madden

1968). The female *Sirex* assesses the suitability of the tree and regulates egg-laying according to the osmotic pressure of the tree. Healthy, dominant trees with high osmotic pressure are rarely attacked (Madden 1974, Coutts and Dolezal 1965). *Sirex noctilio* has been observed drilling two tunnels into the tree, ovipositing an egg into the first tunnel and fungal spores into the second (Rawlings 1948, Coutts and Dolezal 1969). Other species such as *Urocerus gigas* F. and *Sirex cyaneus* F. have been observed ovipositing both eggs and fungus into a single tunnel (Chrystal 1930). Due to the stimulation of resin flow by the fungus (Titze and Stahl 1970, Kile and Turnbull 1974b), separate tunnels could keep the developing egg from being flooded with resin (Coutts and Dolezal 1969).

Eggs normally hatch within 14 to 28 days, though *Sirex noctilio* eggs have been found dormant 10 weeks after oviposition (Morgan 1968, Madden 1981). In *S. noctilio*, egg development has been found to be temperature dependent. However, fungal growth always precedes egg development and the threshold temperature for the fungus and the egg are the same. Fungal growth may also be limited by high moisture content in the wood. These factors suggest by-products by the fungus may also play a role in initiation of embryogenesis (Madden 1981).

To develop, larvae require the successful establishment of the symbiotic fungus, *Amylostereum* (Gilmour 1965). The larvae are unable to digest the wood on their own and when ingested, the fungal symbiont supplies the larvae with essential digestive enzymes needed to digest cellulose (Gilmour 1965, Kukor and Martin 1983). Immediately after eclosion, the larvae begin constructing a tunnel at a right angle to the original oviposition tunnel, eventually turning toward the heartwood (Chrystal 1928, Morgan 1968). Although the number of larval instars can vary, the average is seven (Morgan and Stewart 1966). Larvae even of the same species can take one to three years to emerge as adults depending on climate (Talbot 1964, Morgan 1968).

Sirex noctilio

Sirex noctilio is a siricid native to Europe, Asia and North Africa where it is considered a secondary pest, only attacking dead or dying conifers (Smith 1979, Schiff et al. 2006). However, when introduced into areas in the southern hemisphere, *S. noctilio* began attacking healthy pines (Smith and Schiff 2002). The majority of the pines attacked in the southern hemisphere are North American pine species, such as *Pinus resinosa* Ait., *P. strobus* L., and *P. banksiana* Ait. (Dodds et al. 2010). *Sirex noctilio* has caused extensive damage in pine plantations in areas of New Zealand, Australia, Uruguay, Argentina, Brazil, South Africa and Chile (Bain 2005).

In September 2004, an adult female *S. noctilio* was caught in a funnel trap in Oswego County, New York (Hoebeke et al. 2005). By mid November 2005, 85 *S. noctilio* females were caught in the state (de Groot et al. 2006). Trapping surveys indicated *S. noctilio* was established over a large area, including west-central New York, northern Pennsylvania, eastern Michigan, northern Vermont, southern Ontario, and western Quebec suggesting it was present in North America prior to initial detection (Dodds et al. 2010). So far, *S. noctilio* is attacking stressed and dead trees and is not causing excessive damage in North America (Dodds et al. 2010). However, it would appear to have the potential to become an economical and ecological problem.

Sirex noctilio has the ability to quickly spread beyond the eastern United States through both natural and artificial means. The risk of natural dispersal is high because *S. noctilio* has high reproductive potential and mobile adults (Haugen 1991, Ciesla 2003, Borchert et al. 2007). Female adults are able to fly up to 50 kilometers, although body mass can affect dispersal. Dispersal through human mediation is even more likely through logging activity and movement of firewood (Borchert et al. 2007). Economic risk from *S. noctilio* is also considered high due to

the importance of softwood production in the United States and Canada (Haugen 1991, Borchert et al. 2007, Yemshanov et al. 2009).

Why is *S. noctilio* such a threat to North American pines? A combined effect of the symbiotic fungus, *Amylostereum areolatum* and toxic mucus produced by the female allows *S. noctilio* to become a tree killer at high densities. Death of host trees was first thought to be caused by fungal growth alone restricting the sap supply to the crown (Rawlings 1948). However, a *S. noctilio* attack can cause crown dieback to begin within 5 to 10 days, which is too soon for sap blockage to be the only cause (Coutts 1968). Further studies suggest neither the *A. areolatum* nor the mucus is able to kill a tree on their own and they must work together to overcome tree defense (Vaartaja and King 1964, Coutts 1969b, Titze and Stahl 1970). The early symptoms seen can be attributed to the mucus, which immediately causes tissue desiccation and collapse of cells in the phloem along with changes in respiration, lessening the tree's initial resistance to the fungus (Coutts 1969b, Fong and Crowden 1973). The fungus subsequently restricts the sap supply to the crown by drying out the sapwood (Coutts 1969a, Coutts 1970, Kile and Turnbull 1974a).

Despite the ability of *S. noctilio* to attack healthy pines, suppressed trees in unthinned stands and drought stressed trees are the most susceptible to attack. Under stress, a tree will release volatile materials due to an increase in bark tissue permeability and these volatiles along with the release of water vapor by the tree attract *S. noctilio* and the probability of the tree being attacked increases (Madden 1977). Stands with an age of 10 to 25 years are most susceptible (Haugen et al. 1990). Outbreaks of *S. noctilio* tend to occur in conditions that are adverse to pines, such as prolonged drought and in overstocked stands and can be triggered by weather related damage (Carnegie et al. 2005). *Sirex noctilio* has also been found to exhibit strong spatial

aggregation during the early stages of colonization, which could increase the probability of outbreak and allow *S. noctilio* to attack healthy trees (Corley et al. 2007). In New Zealand, overstocked stands, a severe drought throughout the region and a wide spanning forest fire led to a sudden *S. noctilio* outbreak in 1946 (Elliot 1976). By 1949, *S. noctilio* had killed 30% of pines in 600,000 acres of forest (Rawlings 1948).

Closely monitoring areas of potential introduction and infested areas for spread of *S. noctilio* is important. The first symptoms of attack are needle wilt, followed by a yellowing of the foliage which eventually turns to a brick red color. Die-off from large trees can be spotted from the air. Examining the bark surface for resin beading and round exit holes can confirm the attack is from *S. noctilio* and not other woodborers (Eldridge and Taylor 1989).

Success of control programs throughout the southern hemisphere has hinged on the use of three management tactics: silviculture, parasitoid release and inoculation of parasitic nematodes. Properly maintained stands have a low incidence of *S. noctilio* attack. Restricting thinning to times outside the flight season, quickly salvaging any weather damaged trees (wind, lightning), and timing thinning for sustained vigor all can greatly reduce the risk of *S. noctilio* establishing within a stand (Neumann et al. 1987, Eldridge and Taylor 1989). After extensive parasitoid collections from the U.S., Europe, and Asia, eleven species of parasitoids have been used for biological control of *S. noctilio* (Cameron 1965, Nuttall 1989). Of these, *Ibalia leucospoides* (Hochenwarth), *Rhyssa persuasoria persuasoria* L., and *Megarhyssa nortoni* (Cresson) have been the most successful (Taylor 1978, Neumann et al. 1987, Carnegie et al. 2005). Proper inoculation of parasitic nematodes into infested stands can cause close to 99% parasitism and the collapse of the outbreak within a couple years of inoculation (Bedding 1979).

By closely following a well developed monitoring and control program, South Africa has experienced little tree mortality, with losses never exceeding 4% within a pine stand (Tribe and Cillie 2004). However, when monitoring and control programs are not strictly followed, *S. noctilio* will quickly become a severe problem. Introduced into Victoria, Australia in the early 1970s with little impact, *S. noctilio* was ignored when found in the “Green Triangle” of southwest Victoria in 1979. Monitoring was not properly executed, silvicultural techniques were not used and nematode introductions were too sparse to get successful establishment (Haugen 1990). By 1987, *S. noctilio* had killed 1.8 million trees and another 3 million the following year (Haugen and Underdown 1990, Bedding and Iedes 2005).

Use of parasitic nematodes in the control of *S. noctilio*

Nematodes associated with siricids were first discovered within specimens of *S. noctilio* in New Zealand (Zondag 1962). These nematodes were placed in the genus *Deladenus* (= *Beddingia*) Bedding (Nematoda: Neotylenchidae) and seven species have been described. Each species has a complex lifecycle involving a mycetophagous or fungal feeding cycle and a parasitic cycle. Both cycles are able to continue indefinitely without the intervention of the other (Bedding 1972, Bedding 2009).

Although literature has focused on the biology of *Deladenus siricidicola* Bedding, the basic biology is similar in all seven species. During the mycetophagous life cycle, *Deladenus* live within the trees and feed on the *Amylostereum* fungi deposited by female siricids. When the wood is dry, nematodes can be found anywhere with growing fungus: tracheids, between the wood and bark, host galleries and resin canals. However, nematodes rarely migrate throughout the tree until moisture content is below 50% (Bedding 1972). *Deladenus* are fungal specific,

except *D. wilsoni* Bedding which will feed and reproduce on both *A. areolatum* and *A. chailletii*. *Deladenus siricidicola* is restricted to *A. areolatum*, while the other five are all specific to *A. chailletii* (Bedding and Akhurst 1978).

Mycetophagous males produce amoeboid spermatozoa which are transferred to the female. Within a few hours, females produce eggs which hatch in 4 to 5 days. Juveniles immediately begin feeding on the fungus and take about 7 days to mature into mycetophagous adults. This cycle can continue indefinitely with cultures maintained as long as 4 years without intervention from a siricid host (Bedding 1972). Adult mycetophagous females will live several weeks and produce more than 1,000 eggs in ideal conditions. When females become old, they retain up to 20 eggs within the oviduct and upon death juveniles rupture the cuticle and emerge from the parent (Bedding 1972).

If eggs or young juveniles come into contact with the conditions surrounding a host larva, high CO₂ and low pH, they will grow into infective adults. The infective adult females are often bright green with an elongated stylet, and infective males have small, round spermatozoa instead of the amoeboid spermatozoa found in mycetophagous males (Bedding 1972, Bedding 1979, Bedding 2009). The infective female is so morphologically distinct from the mycetophagous female, they were thought to be in separate families when initially discovered (Bedding 2009). Infective males will not mate with mycetophagous females even in the absence of mycetophagous males and infective females (Bedding 1972).

Only infective fertilized females can enter a siricid larva. Using the tubular stylet, the female thrusts in and out of the siricid cuticle, and penetrates the cuticle within 30 seconds to 6 minutes. The female will fully enter the larva anywhere from 4 to 60 minutes after the initial thrusts (Bedding 1972). A characteristic melanized spot develops on the host cuticle where the

nematode entered. Female nematodes will enter anywhere on the host body. However, most entries are at the end of segments and the posterior end is preferred. Anywhere from 1 to 100 nematodes may enter a single siricid larva but the average is between 5 and 20 (Bedding 1972). After a few days inside the haemocoel of the host, the nematode sheds its cuticle revealing a body surface covered in microvilli, which are used in the absorption of food (Riding 1970, Bedding and Iedes 2005). The nematode will reach full size in the haemocoel of the host within a few weeks. However, its reproductive system remains unchanged until host pupation. Eight days after host pupation, the nematode reproductive system is fully developed and is three quarters the length of the nematode (Bedding 1972). Three to four days after development of the reproductive system, the eggs hatch and juveniles are released via the vulva. Toward the end of host pupation, the nematode has become packed with juveniles causing the oviduct to rupture and juveniles to emerge through the body surface of the nematode. Juveniles immediately migrate from the haemocoel to the host's reproductive organs (Bedding 1972).

The release timing of juvenile nematodes greatly influences the effect on the host and can depend on strain of nematode and host species. For example in *S. noctilio*, *D. siricidicola* enters the ovaries well before the end of pupation causing suppression of the ovaries and a reduction in egg number and size. Normally, all host eggs contain juvenile nematodes, sterilizing the female *S. noctilio* (Bedding 1972, Bedding 2009). In *S. juvencus* L. and *S. cyaneus*, juvenile *D. siricidicola* are not released until right before host pupation and the ovaries and eggs are fully developed. However, juveniles still enter all the eggs before host emergence (Bedding 1972). In *S. nitobei* Matsumura, juveniles are not released until after the host emerges and no eggs contain nematodes and all juveniles are located in the ovaries and oviducts (Bedding 1972). In *Xeris*

spectrum L., ovaries and eggs are fully developed and juveniles only enter 10-30% of the eggs and many are found in the ovaries (Bedding 1972).

Although eggs containing nematodes are not viable, the presence of nematodes does not affect the oviposition behavior of the siricid. Parasitized females readily oviposit symbiotic fungus along with eggs packed with juvenile nematodes (Bedding 2009). The nematodes introduced by the siricid can breed rapidly within the tree due to their mycetophagous cycle, and will occur in large numbers, even achieving close to 100% parasitism of the next generation (Bedding 1979).

In all male siricids, juvenile nematodes migrate to the testes. Males are not sterilized due to the transfer of spermatozoa from the testes to the vesiculae seminales before the migration of juvenile nematodes to the testes. Infected males mate and transfer effective spermatozoa and nematodes cannot be transferred during mating. A male siricid is considered a “dead-end” host, with the nematodes unable to leave (Bedding 1972, Bedding 2009).

Deladenus have been found to develop not only in siricids but also *Rhyssa* parasitoids, *Ibalia leucospoides* and a siricid associated beetle, *Serropalpus barbatus* (Schall.) (Bedding 1967, Bedding 1968). All seven nematode species have been found to enter parasitoid larvae but have failed to fully develop, except *B. wilsoni* which is able to develop in rhyssines causing a reduction in fecundity and longevity of the host (Hocking 1967, Bedding and Akhurst 1978).

Deladenus were quickly considered for use in biological control of *S. noctilio* due to their dual life-cycle and ability to sterilize female siricids. All seven species of *Deladenus* were considered for biological control and all but two were eliminated due to their specificity with *Amylostereum chailletii* instead of *A. areolatum*. *Deladenus siricidicola* was chosen for its ability to sterilize *S. noctilio* without affecting parasitoids.

Deladenus have been found to cause a reduction in the body size of the host. In Japan, nematode-infected female *Xeris spectrum* had a smaller body weight than uninfected females (Fukuda and Hijii 1997). Not only are smaller females not able to fly as far, they also are only capable of pulsating flight patterns as opposed to the longer sustained flight of large females (Bruzzone et al. 2009). The ability of nematode-infected females to effectively disperse is important. The effect on *S. noctilio* size by different strains of *D. siricidicola* was therefore evaluated and the strain chosen for biological control did not have a significant effect on *S. noctilio* size (Bedding and Akhurst 1978, Bedding 1979, Bedding 2009).

The free-living mycetophagous life cycle allowed *D. siricidicola* to be cultured easily and quickly. Using a culture of *A. areolatum* grown on a plate of potato dextrose agar (PDA), a few thousand nematodes can be produced. However, to culture enough to use for field inoculations the plates are used to inoculate flasks containing autoclaved wheat, which can yield between 3 million to 10 million juveniles after 4 to 6 weeks (Bedding and Akhurst 1974, Bedding 1979, Bedding 2009). Experience is needed to assess when to subculture the fungus and the ratio of fungus to nematodes. Too many nematodes and the fungus will stop growing, not enough nematodes and the fungus will grow rapidly and smother the nematodes (Bedding 2009).

For inoculation in the field, easily accessible trees infested with *S. noctilio* are felled. A specially made tool is used to create holes for inoculums. The use of a specially made tool to create the holes is necessary, because other methods, such as drilling, create twisted tracheids which impede the ability of the nematode to enter and spread. The tool consists of a wad-punch with one side cut away to release wood cores and a heavy head welded on top. The tool is self sharpening and creates clean holes allowing nematodes to easily enter the wood and disperse (Bedding and Akhurst 1974). A one mL aerated mixture of gelatin and nematodes is placed in

holes at every meter along the tree bole. Most female *S. noctilio*, emerging from trees inoculated using this method, are parasitized with parasitism levels nearing 99% (Bedding and Akhurst 1974, Bedding 1979, Bedding 2009). Inoculating more nematodes causes competition among nematodes and developing *S. noctilio* for the fungal resource and fewer nematodes means lower parasitism (Bedding and Akhurst 1974, Bedding 1979, Bedding 2009). Although, *S. noctilio* will naturally spread the nematodes, human intervention is necessary for continual control (Bedding 2009). Because parasitized *S. noctilio* are smaller and do not fly as far, unparasitized *S. noctilio* usually initiate new infestations (Villacide and Corley 2008). To be maximally effective, nematodes must be reintroduced once *S. noctilio* are discovered in a new area.

Deladenus siricidicola has been a successful biological control agent throughout the southern hemisphere. In 1972, a massive inoculation program was started in a forest in Northern Tasmania where several thousand trees were being killed annually by *S. noctilio*. By the second year, over 90% parasitism was found in the area. In 1974, only 200 trees were killed and the next year only 5 trees were killed. The nematodes also naturally spread to other nearby forests up to 13 kilometers away (Bedding 1979). From 1990 to 1993, nematodes were inoculated into a 12,000 ha plantation in Brazil where 30% of trees were infested by *S. noctilio*. In 1991 45% of *S. noctilio* were parasitized, 75% in 1992 and 90% in 1994. In 1995, *S. noctilio* infested trees were difficult to find (Bedding 2009).

Despite numerous successes, unexpected problems have occurred. A problem with nematode control was detected in Australia when inoculated trees produced 25% parasitism instead of close to 100%. This drop in parasitism was not the result of incorrect inoculation procedures but instead a result of genetic changes in the nematodes themselves. These changes were attributed to the continual subculture of the nematodes for 20 years without an intervention

of the parasitic cycle and a new colony was cultured and used with success (Haugen 1990, Bedding 1991).

Despite the success of *Deladenus siricidicola* in the southern hemisphere, multiple problems arise when discussing introductions of the nematode into the United States. Ecological differences between release areas in the southern hemisphere and the United States are important when considering success of *D. siricidicola*. The southern hemisphere lacks indigenous softwoods and *Pinus radiata* was introduced for timber production, restricting pines to multi-acre timber plantations. Although introductions of *P. radiata* gave these countries a fast growing timber alternative, it also created a monoculture system devoid of any native pine specialists. This not only allowed *S. noctilio* to quickly reach outbreak levels in these areas, but also provided *D. siricidicola* with a competitor free system when introduced as control (Williams et al. 2012).

The eastern United States has 13 native *Pinus* species, which are well-adapted to the environment and dominant components of forests throughout the region. In contrast to the southern hemisphere, U.S. pine forests not only have a complex of specialists competing with *Sirex* and its associated fungus, but also other *Deladenus* competing for the resource (Williams et al. 2012). In addition, the strain of *Amylostereum areolatum* found in the U.S. is different from those found in other countries and grows at one-third the rate of the Australian strain used for *D. siricidicola* cultures, which could affect establishment (Williams and Mastro 2008).

Deladenus siricidicola have been found parasitizing *S. noctilio* in New York and southern Canada and most likely were introduced with *S. noctilio*. However, the strain of *D. siricidicola* found is different than the one traditionally used for biological control programs. This strain invades the ovaries of *S. noctilio* and not the eggs. This means these nematodes do

not sterilize female *S. noctilio* and will therefore provide little control. Additionally, the strain of *D. siricidicola* found in New York could interbreed with the introduced strain and decrease the strain's virulence (Bedding 2009).

Native *Sirex*

In existing literature, two species of *Sirex* have been recorded from the southeastern U.S., *S. edwardsii* and *S. nigricornis* F., with *Pinus* being the main hosts for both species. However, current research suggests that *S. edwardsii* is only a color morph of *S. nigricornis* (Goulet 2012). Therefore, only one species of *Sirex*, *S. nigricornis*, is recorded from the southeast. In addition to *Sirex*, other siricids are documented from the southeast. *Urocerus cressoni* Norton which also attacks conifers, particularly firs and pines, *Urocerus taxodii* (Ashmead) a specialist on bald cypress and *Tremex columba* and *Eriotremex formosanus* (Matsumura), which are hardwood specialists (Schiff et al. 2006).

Beyond a broad understanding of host preference and range, very little is known about the siricids native to the southeast. Southeastern pine forests have a complex of associated organisms, many of which are interacting with native *Sirex*, likely affecting host colonization and survivorship. *Sirex* rely on the successful establishment of the symbiotic wood-rotting fungus *Amylostereum chailletii* (Madden and Coutts 1979, Kukor and Martin 1983). Pines within the southeast are also host to multiple wood-rotting fungi, some of which are associated with other wood-borers, e.g. *Ips-Ophiostoma* complex (Yearian et al. 1972, Kim et al. 2003). These fungi could be competing with *A. chailletii* and affecting siricid colonization. Other wood-boring insects, such as bark beetles and cerambycids, could be additional competitors during initial host colonization and also compete for resources within the tree. Clerids, trogossitids and

cerambycids, such as *Monochamus* and *Xylotrechus*, are potentially preying on *Sirex* larvae within the trees. Various parasitoids, such as members of *Rhyssa*, *Megarhyssa*, and *Ibalia*, are targeting native siricids, along with parasites such as native nematodes (Kirk 1974, Taylor 1976, Bedding and Akhurst 1978). *Deladenus proximus* Bedding has been found in the testes of male *S. nigricornis* from South Carolina and *D. wilsoni* has been found in areas of the southeastern U.S. (Bedding 1968, Bedding 1974, Bedding and Akhurst 1978), but effects on *Sirex* populations are undocumented. Understanding how native siricids interact with these other organisms and their behavior within southeastern forests is not only important from a basic natural history standpoint, but also when accessing potential impact and control of related invasive species, such as *S. noctilio*.

Research Objectives

The first objective of this research was to gain an understanding of species composition of Siricidae and flight periods of *Sirex* within Arkansas pine forests. The research focused on pine forests in three distinct forested regions of Arkansas: Ozark National Forest, Ouachita National Forest and Gulf Coastal Plain. Panel traps with semiochemical lures were used to determine species composition and dates of adult *Sirex* flight activity within the sites.

The second objective was to investigate whether native parasitic nematodes, *Deladenus*, were present within *Sirex* collected in the different regions of Arkansas pine forests. Following that confirmation, the third objective was to compare parasitism rates between the regions. *Deladenus* have been found to reduce population numbers in related *Sirex* and are expected to have an effect on native *Sirex* populations. *Sirex* females trapped in the three Arkansas regions were dissected and the presence of native nematodes and percent parasitism determined.

Percentage of eggs containing juvenile nematodes was also investigated along with the relationship between body size and nematode parasitism. The last objective was to use molecular techniques to determine the number of *Deladenus* species found and possible *Deladenus* population differences between the regions.

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CHAPTER TWO
SPECIES COMPOSITION AND FLIGHT PERIOD OF NATIVE *SIREX* WITHIN
ARKANSAS PINE FORESTS

ABSTRACT

Sirex noctilio F., an invasive European woodwasp, has caused extensive damage in pine stands planted in the southern hemisphere. Discovered in the northeastern United States in 2004, *S. noctilio* has caused mortality primarily to suppressed pines. However, fears are high that it will become both economically and ecologically damaging. Dispersal into other areas of the United States, particularly the southeast, is a concern. The southeast houses 42.5 million acres of pine forest with ten native pine species, all of which could be susceptible to *S. noctilio*. However, these pine forests harbor a native complex of insects, parasites and fungi which could prevent successful establishment. Included in this complex, is a native siricid, *Sirex nigricornis* F., the biology of which is poorly understood. The objective of this study was to determine species composition and flight period of native *Sirex* within Arkansas pine forests, which are a small representation of the competition *S. noctilio* could encounter. Panel traps baited with Contech Inc. *Sirex* lures were erected at sites in three distinct Arkansas forest regions: Ozark National Forest, Ouachita National Forest, and Gulf Coastal Plain. Traps were monitored in fall of 2009 and 2010. *Sirex nigricornis* F. was found in all three Arkansas regions. Trapping results show *Sirex* flight occurs from late September to early December.

Keywords: invasive monitoring, Siricidae, *Sirex*, flight period

INTRODUCTION

Invasive species constitute a growing problem in North American forests and can be serious threats to forest sustainability (Orwig 2002, Haack 2006). Despite efforts to combat introductions, the rate of establishment for exotic arthropods remains constant (Aukema et al. 2010, Gandhi & Herms 2010). Regardless of ongoing introductions, little is known about the impact these pests are having on the forest ecosystem and risk to these systems can be difficult to assess (Orwig 2002, Kenis et al. 2009, Dodds et al. 2010). While many introduced forest insects pose little notable risk to forest stability, others, such as emerald ash borer and hemlock woolly adelgid, are eliminating tree species from North American forests (Cappaert et al. 2005, Poland and McCullough 2006). Exotic insect herbivores and even the programs used to control them can start a cascade of direct and indirect effects on the ecosystem that can change the community dynamics of North American forests (Lovett et al. 2006, Gandhi and Herms 2010).

An invasive woodwasp, *Sirex noctilio* F., recently was added to the list of forest pests in North America (Hoebeke et al. 2005). Found in New York in 2004, *S. noctilio* has now spread to surrounding areas in the northeastern United States and parts of Canada (Hoebeke et al. 2005, de Groot et al. 2006, Dodds et al. 2010). Although a secondary pest in its native European range, *S. noctilio* has caused extensive damage in pine forests throughout the southern hemisphere (Rawlings 1948, Madden 1975, Haugen 1990). In North America, *S. noctilio* is reported to attack stressed or dead trees and is not causing excessive damage (Dodds et al. 2010). However, with its high reproductive potential and strong dispersal capability, there is considerable risk of *S. noctilio* spreading naturally throughout the United States (Neumann and Minko 1981, Borchert et al. 2007, Bruzzone et al. 2009). In addition, human mediated dispersal through movement of

firewood, logging material and shipments from overseas could greatly accelerate spread of this invasive species (Haack et al. 2010).

Sirex noctilio is in a family of wood-boring wasps (Siricidae), characterized by an ovipositor modified for boring into wood and a horn (cornus) at the apex of the abdomen that is retained throughout development (Schiff et al. 2006). Larvae develop within wood, giving them the common name woodwasps. The family is widespread in deciduous and coniferous forests of the northern hemisphere; whereas none are native to Australia or South America (Smith and Schiff 2002). Siricidae are divided into two subfamilies, Tremicinae and Siricinae. Distinguished by host preference, Tremicinae are associated with hardwoods and Siricinae are associated with conifers (Benson 1943, Talbot 1977, Schiff et al. 2006).

All siricids, including *S. noctilio* in its native range, colonize stressed or dying trees and are thus considered to be pests of secondary importance. Despite their close association with trees, woodwasp larvae are unable to digest cellulose (Gilmour 1965). In order to overcome this hurdle, siricids rely on an obligate symbiosis with *Amylostereum* (Amylostereaceae), a genus of wood-rotting fungi. Fungal spores are carried by siricids in specialized internal sacs called mycangia near the ovipositor and are oviposited into the tree with the egg (Buchner 1928, Coutts and Dolezal 1969, Smith and Schiff 2002). When ingested, the fungal symbiont supplies the larvae with essential enzymes needed to digest cellulose, while the siricid supplies the fungus with a method of dispersal (Kukor and Martin 1983). This relationship is thought to be species-specific, with each species of siricid only carrying one species of *Amylostereum*. For example, native North American *Sirex* species are considered to be associated with *A. chailletii*, while *Sirex noctilio* F. is associated with *A. areolatum* (Gaut 1969, Smith and Schiff 2002). However, since *S. noctilio* was introduced to the United States, native *Sirex* have been found carrying *A.*

areolatum, suggesting the associations are not as species specific as originally believed (Nielsen et al 2009).

Not only do siricids oviposit a symbiotic fungus into the tree, but paired glands in the female secrete colorless “mucus” which is oviposited with *Amylostereum* (Coutts 1969b). This substance is a protein-mucopolysaccharide complex and is found in all siricids (Boros 1968, Wong and Crowden 1976, Bordeaux and Dean 2012). However, the mucus found in *S. noctilio* is the only siricid mucus found to cause pathological symptoms within the tree (Spradbery 1973).

Neither *Amylostereum* nor the mucus provided by *S. noctilio* is able to overcome tree defenses on its own (Vaartaja and King 1964, Coutts 1969b, Titze and Stahl 1970). Early signs of suppressed tree defenses can be attributed to the mucus, lessening the tree’s initial resistance to the fungus (Coutts 1969b, Fong and Crowden 1973). The fungus subsequently restricts the sap supply to the crown by drying out the sapwood and eventually killing the tree (Coutts 1969a, Coutts 1970, Kile and Turnbull 1974).

Although currently found only in the northeastern United States, *S. noctilio* is a threat to pine forests throughout the southeast. Softwood timber production is a highly valued industry and the southeast produces 60% of the nation’s timber valued at \$11 billion dollars annually (Borchert et al. 2007). When introduced in the southern hemisphere, *S. noctilio* outbreaks drastically affected pine stands, killing up to 30% of the pines in areas of New Zealand (Rawlings 1948) and causing a loss of over 1.8 million pines in the Green Triangle of Australia (Haugen and Underdown 1990). Outbreaks of *S. noctilio* tend to occur in conditions that are stressful to pines, such as in overstocked stands or during prolonged drought and can be triggered by weather related damage (Carnegie et al. 2005). Overstocked, weather stressed stands are

common throughout the southeast and could provide ideal conditions for *S. noctilio* population growth.

Concern exists that *S. noctilio* could spread into southern pine forests. However, a complex of pine inhabiting insect predators, competitors, and parasites already exists in these forests and it is possible that they could affect *S. noctilio* establishment. Among these potential competitors are native siricids. Like *S. noctilio*, *Sirex* native to the southeastern U.S. utilize pines as a preferred host, but they only colonize damaged or dying trees. Little is known about the natural history of native *Sirex* and their interactions within the forests.

A first step in investigating native *Sirex* is to gain an understanding of species composition and flight periods in southern pine forests that are likely to be colonized by *S. noctilio*. Forest insects' emergence and flight periods can be influenced by weather and factors such as temperature can cause a change in phenology between populations of the same species (Pozo et al. 2008, Dukes et al. 2009, Jepsen et al. 2011). Warmer temperatures can have an indirect effect by stimulating growth in pines and a direct effect by increasing insect metabolism (Thomson et al. 2010, Tryjanowski et al. 2010). The objective of this study was to determine the species composition and flight period of native *Sirex* within Arkansas pine forests. Knowing flight periods for native *Sirex* can eventually lead to an understanding of dispersal, inter-species interactions and oviposition behavior.

Two species of *Sirex* have been documented within Arkansas, *Sirex nigricornis* F. and *Sirex edwardsii* Brullé (Schiff et al. 2006). However, Goulet (2012) groups these as distinct color morphs of the same species, *Sirex nigricornis*. While this claim is based on morphological and genetic proof, the research has not yet been published (Goulet 2012). For the purposes of this

paper, we will refer to the original *S. nigricornis* as color morph A and the former *S. edwardsii* as color morph B.

MATERIALS AND METHODS

Sites

Weather disturbances occurred in three regions of Arkansas in 2009 causing extensive damage in pine forests. These disturbances included a severe ice storm occurring from 26-27 January 2009 that was widely distributed across the Ozark National Forest (Barjenbruch 2009) and tornados occurring on 9 April 2009 in both the Ouachita National Forest and the Gulf Coastal Plain of southern Arkansas (National Weather Service 2009). The ice storm caused severe breakage of crowns and boles and resulted in downed trees due to excess weight. The tornados were concentrated in a smaller area but caused severe local damage, causing stem breakage and uprooting of trees. Both events generated a timber salvage effort throughout all three regions. A drastic increase in fresh pine volatiles within active logging sites causes an increase in siricid activity (Johnson et al. 2009). These three regions of the state were selected as research sites due to these disturbances and to the topographic and ecological differences between the areas.

Three sites were chosen in pine forests within each region: Ozark National Forest (OzNF), Ouachita National Forest (OuNF), and Gulf Coastal Plain (SAR) (See Figure 1 & Table 1 for specific site information). Logging occurred within each site in the summer or fall of 2009 and much of the slash was left in the sites through the 2010 siricid collecting season. Tree stands in the Ozark and Ouachita sites were mostly pine with a component of hardwoods, mainly in the

understory. These stands had a mixture of tree age and diameter. Pines within the stands were loblolly pine (*Pinus taeda* L.) or shortleaf pine (*P. echinata* Mill.). These sites ranged from moderately to heavily managed stands. A fourth site was selected in the OuNF region, because the site was poorly managed and stressed due to frequent flooding. In the Gulf Coastal Plain, the stands were younger, intensively managed, and pure loblolly pine.

Stand measurements were taken at each site including, basal area of pine, basal area of all other trees, average DBH of pine, average DBH of all other trees and approximate stand age. Basal area measurements were taken using point sampling. Three points were randomly selected in each site and basal area was measured using a JIM-GEM[®] Cruz-All with a BAF of 10 ft²/acre (Avery and Burkhart 2002). Basal area was later converted to m²/hectare. Two fixed area plots with a radius of 10m were randomly selected at each site and DBH of each tree within the plot was measured. The fixed plots were used to determine average DBH at each site. A core was taken using an increment borer from three randomly selected pines in each site. Tree cores were taken back to the lab and the rings were counted to determine age. These ages were used to determine an approximate age range for the pines in each site based on the majority of trees within the stand being close to the same age. We assumed the majority of trees were close to the same age based on tree size and because our sites were within planted pine stands.

Trapping

Siricids were collected at each site with three APTIV Intercept[™] black panel traps baited with Contech Inc. *Sirex* lure (α - β pinene) plus 95% high-release ethanol (Figure 2). Panel traps were used because it is suggested that they outperform Lindgren[®] funnel traps in capturing large bodied insects, such as siricids (Johnson et al. 2009). The traps were 80cm long and 30cm wide

and hung from a 2cm electrical conduit ca. 1m above the ground (Figure 2). Trapped specimens were captured in propylene glycol in a collection container at the bottom of the trap. Traps were placed approximately 15m apart at each site.

Trapping was initiated from late October to early November in 2009 and early to mid August in 2010. Establishment of sites was delayed in 2009 due to difficulty in obtaining permits for trapping in actively logged areas. Trap catches were collected every two weeks until early December in both years. Lures were changed according to manufacturer recommendations, approximately once every 4 weeks.

All trap catches were transferred into Whirl-Pak[®] bags in the field, brought back to the lab and stored at 1°C. Specimens from each trap were categorized as siricids, parasitoids, and large and small unidentified arthropods. Each group was stored in separate vials in 95% ethanol. Siricids were further identified to species using the manual of Schiff et al. (2006). Each specimen was placed in a separate vial and labeled with an identification number, site, trap, and collection date and entered into a database. All siricids were stored at -20°C to avoid DNA degradation (Rubink et al. 2003).

Data Analysis

Number of *Sirex nigricornis* caught was analyzed using a split plot design with region by color morph factorial as the whole plot and year as the split plot. Site was the whole plot replication. A normal ANOVA could not be performed because the counts were too small to justify the assumption of normality. Therefore, the data were transformed to log (count + 1) scale for purposes of performing an ANOVA and normality was assumed. The factors region, color morph and year were fixed effects, while sites within region were a random effect. For

appropriate effects in ANOVA, LSD procedures were used to compare means. The responses in log were back transformed for purposes of presenting the data.

RESULTS

Site measurements

Site and stand measurements were taken from all ten research sites. In the Ouachita sites, basal area of pines ranged from 12 to 17 m²/hectare, while all other trees ranged from 0 to 9 m²/hectare. In the Ozark sites, basal area varied from 11 to 25 m²/hectare, while all other trees only had a basal area between 1.5 and 3 m²/hectare. Basal area of pine in the Gulf Coastal Plain ranged from 14 to 21.5 m²/hectare. Basal area of other trees within the sites was too small to be measured. However, other trees were present in these sites (Table 1). Pine DBH ranged from 8 to 48cm in the Ouachitas, 9 to 46cm in the Ozarks and 4 to 33cm in the Gulf Coastal Plain. Diameter at breast height of all other trees within the sites ranged from 2 to 84cm in the Ouachitas, 2 to 28cm in the Ozarks and 2 to 7cm in the Gulf Coastal Plain (Table 1).

Siricid Trapping

In 2009, 174 *S. nigricornis*, 3 *Tremex columba*, and 3 *Eriotremex formosanus* were caught in traps at 10 sites in Arkansas (Table 2). In 2010, 105 *S. nigricornis*, 2 *Tremex columba* and 1 *Eriotremex formosanus* were caught in traps at 10 sites in Arkansas (Table 2). *Sirex nigricornis* color morph A was approximately twice as abundant as color morph B in both years. All siricids trapped were female.

Analysis of the number of *Sirex nigricornis* caught indicated a significant region by year interaction and a significant color morph effect (Table 3). Therefore, there are regional differences that are not the same in all years and color morph differences are not dependent on year or region. Comparison of means for the region by year interaction show the least square means for each region are statistically different between years (Table 4). Comparison of means for the species effect show the least square means for color morph A and B are significantly different (Table 5). When looking at the REML variance component estimates, 31.6% of the total variability can be attributed to the variability of sites within regions interacting with species and the other 68.4% is attributed to experimental error.

In 2009, the flight period for both color morphs of *S. nigricornis* peaked in late October and early November in the Ouachita and Gulf Coastal Plain sites and numbers of adults caught dropped in late November and early December (Figs. 3, 4 & 5). In the Ozarks, adults were only caught in mid November; albeit only 6 adults were caught. In 2010, flight period peaked in mid October for both color morphs of *S. nigricornis* in the Ozarks and began decreasing from early to late November (Figs. 6, 7 & 8). Flight period in the Ouachitas and Gulf Coastal Plain was from early to late November in 2010.

DISCUSSION

Although pine was found to be the major component in stands throughout the study, differences were seen among sites. Basal area was similar among sites in the Ozarks and Ouachitas, with the bulk of basal area comprised of pines interspersed with small diameter hardwoods. Basal area of pine was similar in the Gulf Coastal Plain stands to the other regions. However, presence of hardwoods in the Gulf Coastal Plain stands was so minimal the basal area

of hardwoods was calculated as zero. On average, the DBH for pines was similar between all stands, with stands in the Gulf Coastal Plain having slightly smaller pines than in the Ozarks and Ouachitas. The differences seen can be explained by the management tactics used within the stands. Stands in the Gulf Coastal Plain were intensively managed, even-aged loblolly pine, while the stands in the Ozarks and Ouachitas are mixed age pine stands within the National Forests.

Five siricids are reported from these regions: *Sirex nigricornis*, *Tremex columba*, *Eriotremex formosanus*, *Urocerus cressoni*, and *Urocerus taxodii*. We collected all except both species of *Urocerus*. We did not expect to collect *U. taxodii*, because it utilizes bald cypress as a host, a habitat we did not investigate (Schiff et al. 2006). The only anticipated siricid we did not trap was *Urocerus cressoni*. This species is found throughout the southeastern United States utilizing fir, spruce, and pines; therefore, it is likely attracted to our lures (Schiff et al. 2006). However, none were found.

Of the three siricids trapped, only *S. nigricornis* colonize pines and thus responded to the *Sirex* lures. *Tremex columba* is a commonly collected and well-known siricid throughout the eastern United States. It is a hardwood specialist and thus would not be attracted to the *Sirex* lure. However, each site had a hardwood component and the addition of an ethanol lure could have attracted this species or they were passively trapped.

Eriotremex formosanus is an exotic horntail native to Japan, Taiwan, and Vietnam and was discovered in Florida and Georgia in 1974 (Togashi and Hirashima 1982, Smith 1996). In 2006, a dead female was found in Arkansas, extending its range 230 miles inland (Warriner 2008). Although *E. formosanus* is considered a hardwood specialist, individuals have been found ovipositing in pine (Smith 1996). While there is evidence this horntail can develop in various

hardwoods, there is no evidence they can complete development in pine (Ulyshen and Hanula 2010). Low catch numbers suggest they may not yet be a dominant species in Arkansas pine forests.

All siricids trapped in both years were female. In natural populations, siricids have been shown to have a male-biased sex ratio (Morgan and Stewart 1966, Morgan 1968). However, it is possible males are not cueing into pine volatiles and therefore are not attracted to the traps. In addition, males have been shown to fly up to the canopy upon emergence and may not come into contact with panel traps (Morgan and Stewart 1966, Smith and Schiff 2002).

Examining the numbers of *Sirex nigricornis* caught, reveals twice as many color morph A as color morph B. This is confirmed by the LSD, which showed the means of color morph A and B to be statistically different. This difference was not associated with other factors such as region or year. Without more data and a better understanding of the relationship of the two color morphs, a reason for such a consistent difference in numbers is unknown.

Differences in numbers of *Sirex nigricornis* trapped among regions were not the same in both years. In fact, the number of *S. nigricornis* caught in each region in 2009 was significantly different than the number caught in 2010 (Table 4). This may suggest a peak in emergence in one year and then a decrease the next. However, to truly understand these differences trapping over multiple years in the regions would be necessary.

Data collected in 2009 may be slightly skewed due to a delay in receiving funding coupled with difficulty in obtaining permission to establish sites. Sites were not established until late in the season of 2009 and traps were not erected until mid to late October. Adults flying earlier in the fall would have been missed. In 2010, more complete trapping allowed us to delineate flight periods for *S. nigricornis* as occurring from mid September to late November

with peaks in mid October. Traps were erected in mid August and thus the full flight period was able to be documented.

High variation in trap catch was seen in the Ozarks with six *S. nigricornis* collected in 2009 and 87 in 2010. In January 2009, an ice storm caused significant damage throughout the Ozarks with an estimated 5% overall loss in pine timber. This triggered a timber salvage effort throughout the region. Previously, logging and weather disturbance were minimal in the sites and *S. nigricornis* populations were likely low. In the summer of 2009, logging throughout the sites created piles of coarse woody debris and flooded the areas with pine volatiles. Attracted by these volatiles, siricids were likely drawn into the sites looking for fresh oviposition sites. While they may have been drawn into sites, traps were erected late in 2009. Not only would the traps have missed peak flight of *S. nigricornis* within the sites but also peak migration to the sites as well. This combined with potentially low populations of *S. nigricornis* in 2008 could explain low trap abundance in 2009. However, attracted to the increase in dead and dying pines, *S. nigricornis* oviposition likely increased in 2009, subsequently increasing emergence in 2010.

The opposite was seen in the Ouachitas with a decrease in trap abundance in 2010 (123 *S. nigricornis* in 2009 and 14 in 2010). Reason for a drop in catches in the Ouachitas is still unknown. Weather conditions or natural population fluctuations could be responsible for the decrease. However, data collection over multiple years would be needed to fully understand the differences seen.

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Figure 1. Map showing the location of regions and placement of study sites throughout Arkansas. Red dots represent sites.

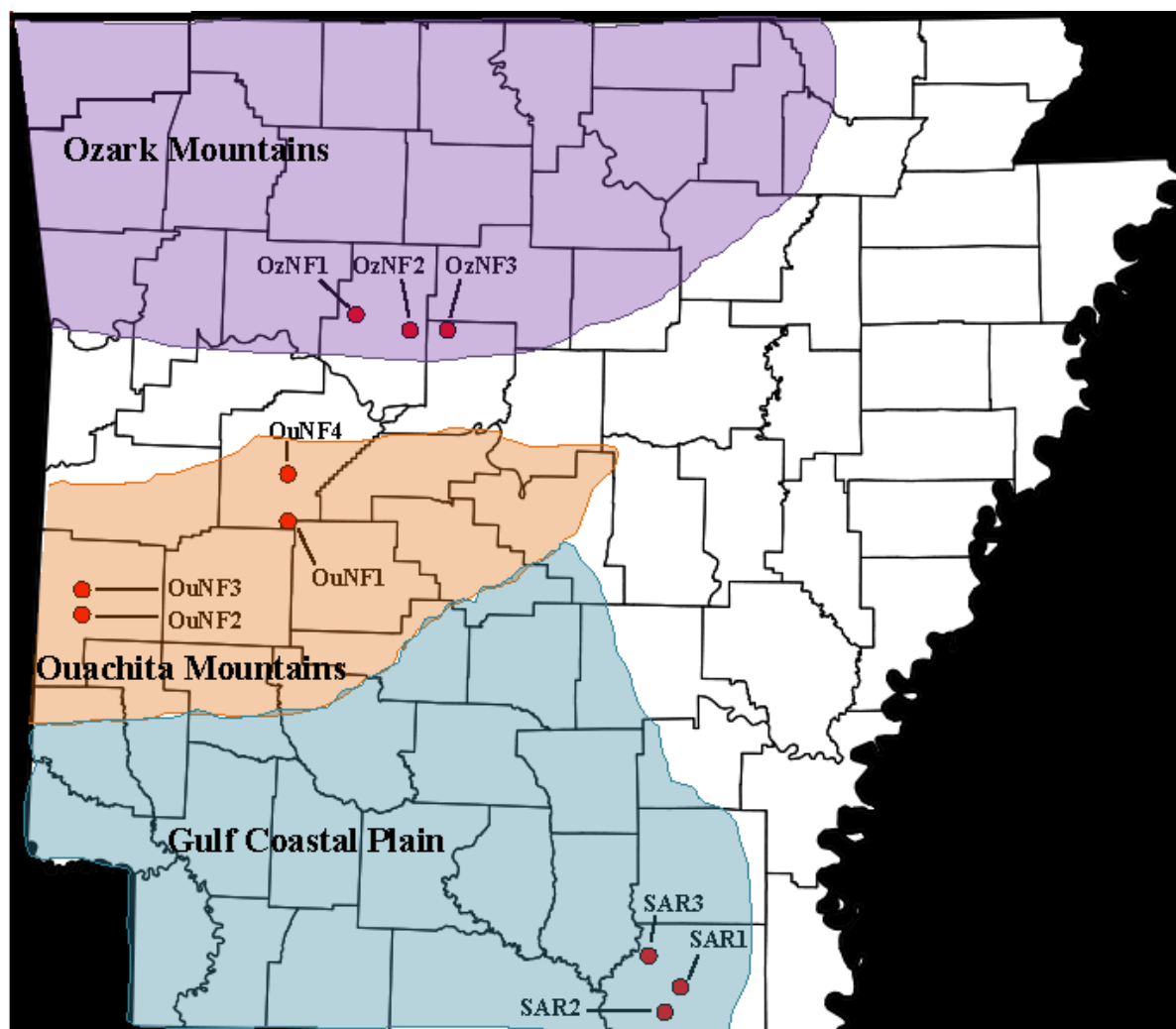


Figure 2. APTIV Intercept™ black panel trap set up at OuNF4.



Figure 3. Flight period for *S. nigricornis* color morph A in 2009 by region.

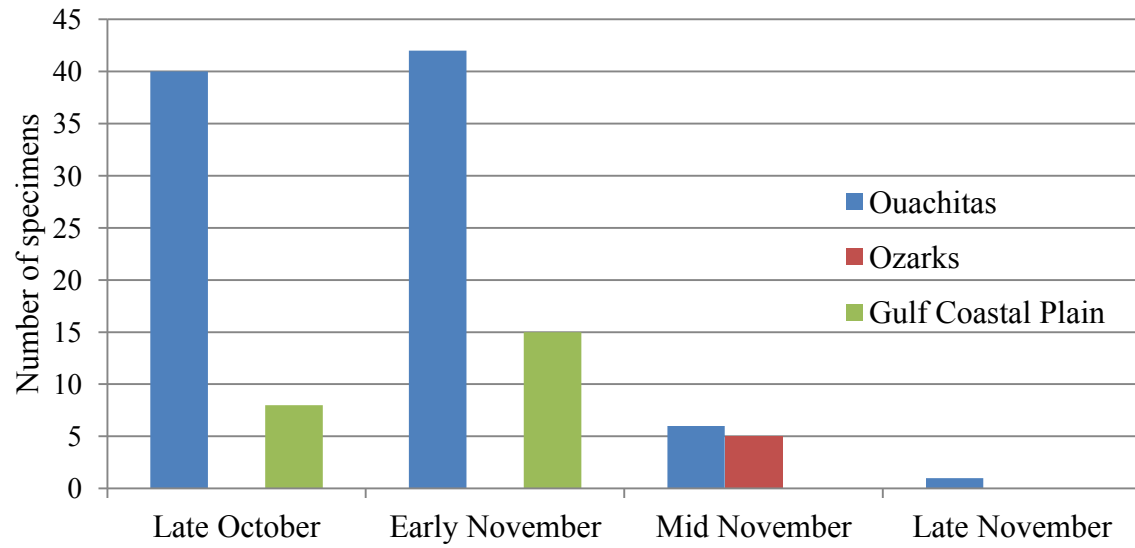


Figure 4. Flight period for *S. nigricornis* color morph B in 2009 by region.

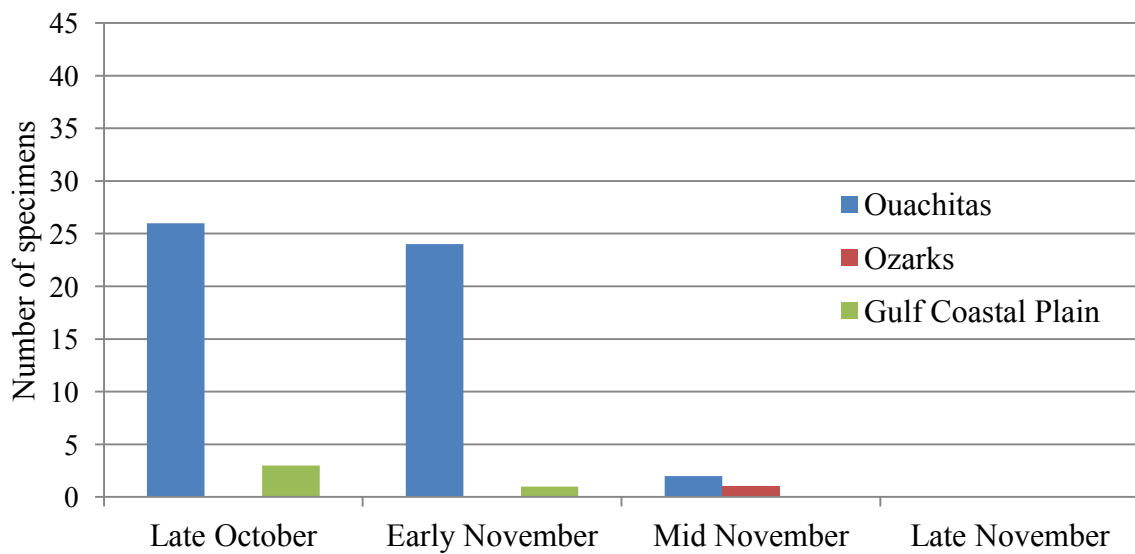


Figure 5. Flight period for *S. nigricornis* in 2009 by region.

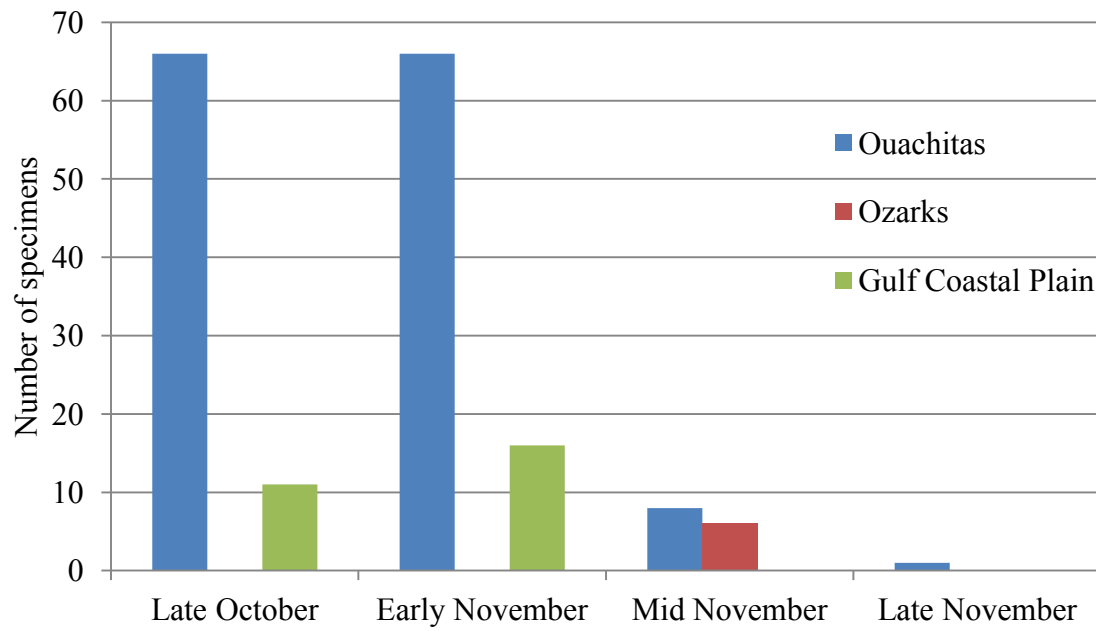


Figure 6. Flight period for *S. nigricornis* color morph A in 2010 by region.

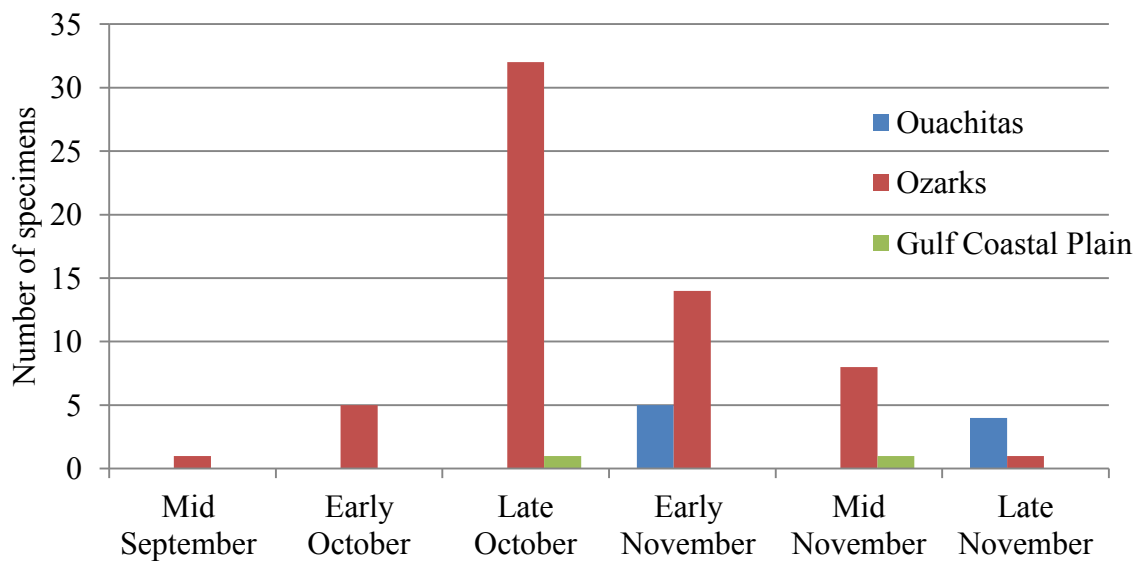


Figure 7. Flight period for *S. nigricornis* color morph B in 2010 by region.

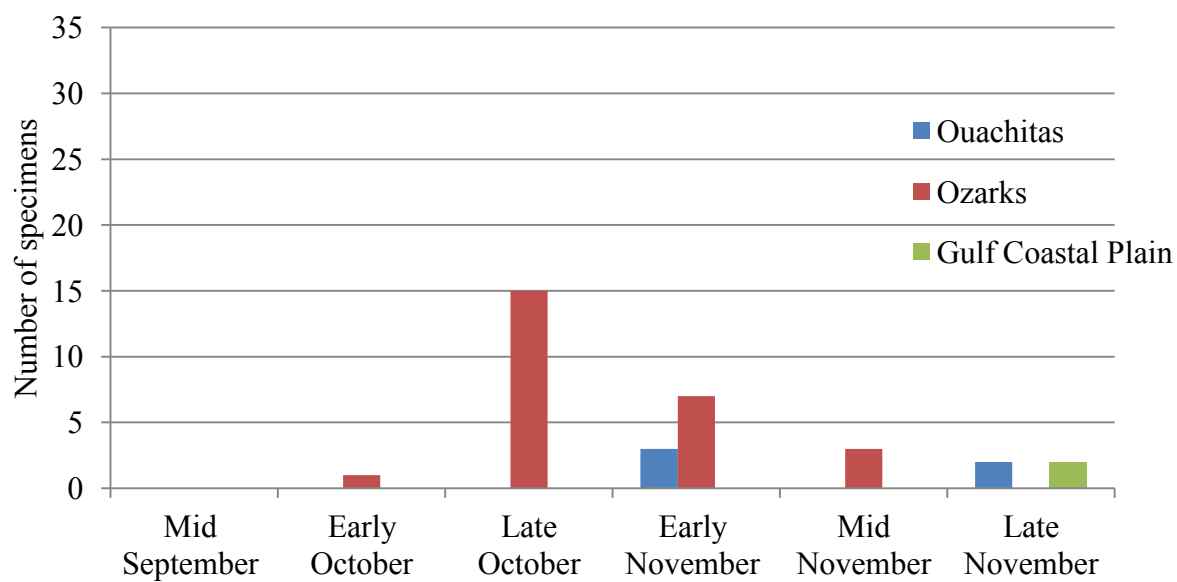


Figure 8. Flight period for *S. nigricornis* in 2010 by region.

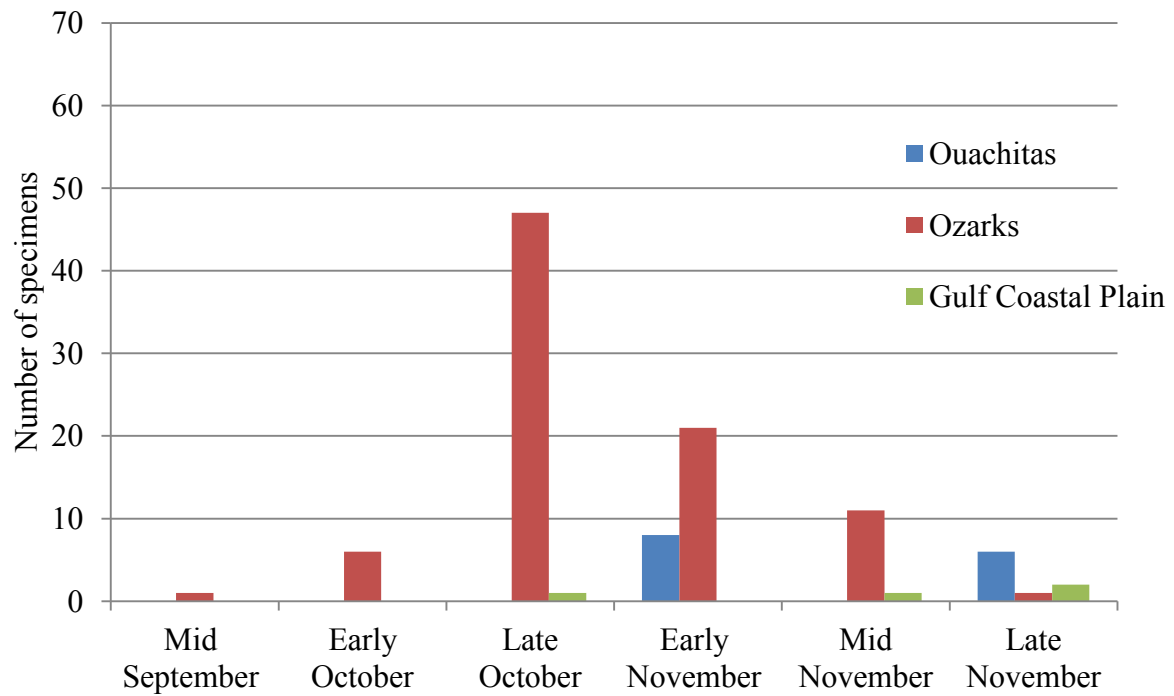


Table 1. Site and stand characteristics.

Sites	UTM 15N	Dominant pine species	Pine basal area (m ² /ha)	Hardwood basal area (m ² /ha)	Mean (Range) Pine DBH	Mean (Range) Hardwood DBH	Stand age (years)
Ouachita National Forest							
OuNF1	0461513 3846260	loblolly	16.8	0.8	24 (8-35)	8(2-20)	80-90
OuNF2	0383226 3807198	shortleaf	12.2	0	22 (10-28)	6 (4-11)	20-30
OuNF3	0383226 3808738	shortleaf	13.0	3.8	31 (23-40)	10 (3-25)	20-30
OuNF4	0459573 3864039	loblolly	12.2	8.4	24 (8-48)	15 (5-84)	80-90
Ozark National Forest							
OzNF1	0491867 3928173	loblolly	25.3	1.5	33 (9-45)	6 (2-11)	50-80
OzNF2	0518906 3923735	shortleaf	11.5	3.1	32 (10-45)	15 (5-27)	30-40
OzNF3	0511810 3923514	shortleaf	8.4	2.3	40 (33-46)	16 (6-28)	30-40
Gulf Coastal Plain							
SAR1	0611789 3663854	loblolly	19.1	0	21 (6-28)	4 (3-7)	15
SAR2	0605491 3658027	loblolly	13.8	0	17 (4-24)	3 (2-4)	15
SAR3	0598202 3682044	loblolly	21.4	0	26 (20-33)	3 (3-5)	15

Table 2. Number of specimens collected at each site in 2009 and 2010.

Site	<i>S. nigricornis</i> Color Morph A		<i>S. nigricornis</i> Color Morph B		<i>T. columba</i>		<i>E. formosanus</i>	
Ouachita National Forest								
	2009	2010	2009	2010	2009	2010	2009	2010
OuNF1	22	3	13	3	0	0	0	0
OuNF2	28	2	17	2	1	0	2	0
OuNF3	22	1	18	0	1	0	0	0
OuNF4	3	0	0	3	0	0	0	0
Ozarks National Forest								
OzNF1	2	39	1	8	0	0	0	0
OzNF2	1	7	0	4	0	0	0	0
OzNF3	2	15	0	14	0	1	0	0
Gulf Coastal Plain								
SAR1	2	0	1	0	0	0	1	0
SAR2	13	2	1	0	1	0	0	1
SAR3	8	0	2	2	0	1	0	0

Table 3. ANOVA table for number of *Sirex nigricornis* trapped.

Effect	DF	DFDen	F ratio	P-value
Region x Year x Color Morph	2	14	0.8318	0.4557
Region x Year	2	14	30.3261	<0.0001*
Region x Color Morph	2	14	0.0395	0.9614
Year x Color Morph	1	14	1.1112	0.3097
Region	2	14	3.1484	0.0743
Year	1	14	1.1793	0.2958
Color Morph	1	14	5.3506	0.0364*

Table 4. LSD comparison of means for region by year interaction. Means with different letters are significantly different.

Region (Year)	Least Square Mean
Ozarks (2010)	12.788 A
Ouachitas (2009)	11.442 A
Gulf Coastal Plain (2009)	4.069 B
Ouachitas (2010)	2.414 BC
Ozarks (2009)	1.817 BC
Gulf Coastal Plain (2010)	1.442 C

Table 5. LSD comparison of means for color morph effect. Means with different letters are significantly different.

Color Morph	Least Square Mean
A	5.411 A
B	2.875 B

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CHAPTER THREE
PARASITISM OF ARKANSAS *SIREX* BY *DELADENUS* NEMATODES

ABSTRACT

Discovery of an invasive woodwasp, *Sirex noctilio*, in pine forests throughout the eastern U. S. has led to extensive research to monitor and manage this destructive pest. A parasitic nematode, *Deladenus* (= *Beddingia*) *siricidicola*, has been found to be a successful biological control for *S. noctilio* in other areas of accidental introduction, such as Australia and New Zealand. However questions exist about use of *D. siricidicola* for biological control in North America, including differences in climate and forest structure, its potential colonization of native *Sirex* and competition with native *Deladenus*. Not only are *D. siricidicola*'s effects on native *Sirex* poorly understood, but we also know very little about the distribution of native *Deladenus* and their relationship to native *Sirex*. The objectives of this research were to confirm the presence of *Deladenus* within native *Sirex* in Arkansas pine forests, to compare parasitism rates for three regions of Arkansas, and to verify, using molecular techniques, the number of *Deladenus* species found. APTIV Intercept™ black panel traps baited with Contech Inc. *Sirex* lures were erected at sites in three regions of Arkansas: Ozark Mountains, Ouachita Mountains and Gulf Coastal Plain. *Sirex nigricornis* caught were dissected and examined for presence of parasitic *Deladenus*. *Deladenus* sequences were obtained from a portion of the cytochrome oxidase I (COI) gene and compared. Presence of *Deladenus* was confirmed within *S. nigricornis* collected from all three regions. *Sirex nigricornis* collected in the Ouachitas had a parasitism rate of 18% in 2009 and 24% in 2010 and in the Ozarks, *S. nigricornis* exhibited a rate of 44% in 2010. Sequences suggest we only collected one species of *Deladenus*.

Keywords: Siricidae, *Sirex*, *Deladenus*

INTRODUCTION

Sirex noctilio F., an invasive woodwasp, was discovered in the United States in 2004. Native to Europe, *S. noctilio* has been introduced into pine plantations throughout the southern hemisphere and caused widespread damage. In North America, this invasive species was first found in New York and has since been trapped in northern Pennsylvania, eastern Michigan, northern Vermont, southern Ontario and western Quebec (Hoebeker et al. 2005, de Groot et al. 2006, Dodds et al. 2010). Unlike other siricids, *S. noctilio* can attack and kill healthy pines when the wasp reaches high population levels (Coutts 1969a, Coutts 1969b). The ability to become a tree killer is not attributed to the woodwasp alone but is bolstered by injection of a toxic mucus and a symbiosis between the wasp and a wood-rotting fungus, *Amylostereum areolatum* (Fr.) Boid. When ovipositing an egg into the tree, *S. noctilio* also inserts fungal spores and toxic mucus (Rawlings 1948, Coutts and Dolezal 1969). The mucus immediately causes tissue desiccation and collapse of cells in the phloem, lessening the tree's resistance to the fungus (Coutts 1969b, Fong and Crowden 1973). The fungus itself restricts the sap supply to the crown by drying out the sapwood (Coutts 1969a, Kile and Turnbull 1974). Through this symbiosis, the fungus gains a method of spread through the forest, and *S. noctilio* gains a nutritional resource within the tree through digestive enzymes provided by the fungus (Gilmour 1965, Kukor and Martin 1983).

Outbreaks of *S. noctilio* have been documented throughout areas of the southern hemisphere, including Australia, New Zealand, South America, and South Africa. These outbreaks are initiated by conditions that are adverse to tree health, including overstocked pine stands and weather-related events such as prolonged drought (Carnegie et al. 2005). In New Zealand, overstocked stands, a severe drought throughout the region and a widely spread forest

fire led to a massive *S. noctilio* outbreak in 1946 (Elliot 1976). By 1949, *S. noctilio* had killed 30% of pines in 600,000 acres of forest (Rawlings 1948). Introduced into Victoria, Australia in 1979, *S. noctilio* killed 1.8 million trees by 1987 and another 3 million the following year (Bedding and Iedes 2005). In response to losses of marketable timber, a successful monitoring and control program was developed (Madden 1988). The program included silvicultural techniques and biological control using parasitoids and parasitic nematodes. The most effective control technique was the use of the parasitic nematode, *Deladenus* (= *Beddingia*) *siricidicola* Bedding. If correctly introduced into a forest, *D. siricidicola* achieves close to 100% parasitism and sterilization of female *S. noctilio* (Bedding 1979).

Deladenus siricidicola have two life stages, a mycetophagous or fungal feeding stage and a parasitic stage. During the mycetophagous stage, the juvenile and adult nematodes feed on *A. areolatum* and move throughout the tree's tracheids and resin canals. This life cycle can continue indefinitely without intervention from a siricid host (Bedding 1972). If eggs or juvenile nematodes are exposed to high CO₂ and low pH, as would be encountered near a siricid larva, they will develop into infective adults and begin a parasitic stage. In this stage, females are bright green and have a tubular stylet which they use to penetrate and enter the cuticle of the siricid larva (Bedding 1979). On average, 5 to 20 nematodes will enter a single siricid host (Bedding 1972). Shortly after host pupation, the nematode rapidly produces juveniles, which are released into the host's haemocoel. When the host is reaching the end of pupation, the female nematode is so full of juveniles that they rupture the oviduct and burst from her body. Once released, the juveniles migrate from the haemocoel to the host's reproductive organs (Bedding 1972).

Each species of *Deladenus* affects their host siricid differently. For example, the strain of *D. siricidicola* used for biological control of *S. noctilio* enters the ovaries well before the end of

host pupation. Not only do all the host's eggs become filled with juvenile nematodes, sterilizing the host, but the ovaries become suppressed and egg size is reduced (Bedding 1972). Other *Deladenus* species enter the ovaries after host pupation and may infect only a portion of the eggs. Others only surround the ovaries and never enter any of the eggs (Bedding 1972). Despite complete or partial sterilization of female siricids, mating and oviposition behavior is minimally affected. Parasitized females oviposit eggs packed with juvenile nematodes along with *Amylostereum* (Bedding 2009). Male siricids are considered a "dead end" host for *Deladenus*. Despite entering the testes, *Deladenus* cannot be spread from male to female and have no way of leaving the host (Bedding 1972).

A well established method for maintaining cultures and high parasitism rates have made *D. siricidicola* an effective biological control agent against *S. noctilio* within Australia and New Zealand (Bedding and Iedes 2005, Hurley et al. 2007, Slippers et al. 2012). Using the nematode's mycetophagous stage, cultures containing up to 10 million nematodes can be produced (Bedding and Akhurst 1974). Trap trees are created in the field and inoculated with an aerated mixture of gelatin and nematodes. Newly felled trees are ideal for *S. noctilio* oviposition and females are immediately attracted to the trap trees. By the time the next generation emerges, parasitism rates within the tree can approach 100% (Bedding and Akhurst 1974, Bedding 1979).

Despite the success of *D. siricidicola* in Australia, various factors could impede introductions into the southeastern United States. Pine diversity and forest composition are starkly different in Australia than in the eastern United States. Within Australia, all *Pinus* are introduced and are normally restricted to intensively managed multi-acre timber plantations. In contrast, the eastern United States has 13 species of *Pinus* which occur in both intensively managed plantations and more diverse forest ecosystems. Introduced pines give Australia a fast-

growing timber alternative. However, they also are devoid of native wood borers, fungi and the *Deladenus* species that are native to and prevalent in southeastern U.S. pine forests. While the exclusion of these pine-feeding species means that Australian pine plantations are somewhat pest-free, it also provides *D. siricidicola* with a competitor-free system (Williams et al. 2012). Not only may *D. siricidicola* experience direct competition from native *Deladenus* within the southeast, but its food resource, *Amylostereum areolatum*, may have to compete for space with native fungi, such as *A. chailletii* (Pers.:Fr.) Boid. and blue stain fungi, such as *Ophiostoma* (Williams et al. 2012). Climatic differences between Australia and the southeastern U.S. may affect the timing of *D. siricidicola* introduction and nematode reproduction. Mild Australian winters allow nematodes to produce generations year round, while temperature extremes in the southeast, particularly in the areas furthest north, may prevent reproduction during winter months or may even reduce survival (Williams et al. 2012).

Environmental factors alone may hinder *D. siricidicola*'s ability to colonize the southeast, but host potential must also be considered. Southeastern pine forests are home to native *Sirex* and *Urocerus*, which could come into contact with *D. siricidicola* should it be introduced into the southeast. While *D. siricidicola* is likely able to parasitize native siricids, an additional factor to be considered is the fungal symbiont of both *Deladenus* and the siricids (Williams et al. 2012). All southeastern *Sirex* and *Urocerus* are associated with *A. chailletii*, while *D. siricidicola* will only feed and reproduce on *A. areolatum* (Bedding and Akhurst 1978). As long as native siricids are feeding upon *A. chailletii* within the tree, they will remain isolated from *D. siricidicola*. However, the association between siricid and fungal symbiont may not be species-specific. Nielson et al. (2009) found *Sirex edwardsii* Brullé (now recognized as *S. nigricornis* F. (Goulet 2012)) carrying *A. areolatum*. If native siricids are able to carry both

species of fungus, they could be at risk should *D. siricidicola* be introduced into the United States. Further investigation into these relationships is needed to fully understand the threat introductions of *D. siricidicola* will pose on native siricids.

Not only are the potential effects of *D. siricidicola* on native *Sirex* poorly understood, but very little is known about the distribution or abundance of native *Deladenus* and their effects on native *Sirex* populations. Two native nematodes, *D. wilsoni* Bedding and *D. proximus* Bedding have been found in the eastern United States, but their distribution and impact on native siricid populations has not been investigated. Also, native *Deladenus* have not been documented within Arkansas forests.

Two species of *Sirex* have been reported from Arkansas, *Sirex nigricornis* and *S. edwardsii* (Schiff et al. 2006). However, Goulet (2012) groups these as distinct color morphs of the same species, *Sirex nigricornis*. While this claim is based on morphological and genetic proof, the research has not yet been published (Goulet 2012). For the purposes of this paper, we will refer to the original *S. nigricornis* as color morph A and the former *S. edwardsii* as color morph B.

The objectives of our research were to 1) establish whether native *Deladenus* occur in *Sirex* collected from three pine forested regions (Ozark Mountains, Ouachita Mountains and Gulf Coastal Plain) in Arkansas; 2) evaluate nematode parasitism rates within these regions; and 3) determine, using molecular techniques, whether *Deladenus* found in the three regions are one or multiple species.

MATERIAL AND METHODS

Sites

Sites were established within three Arkansas regions: Ozark Mountains, Ouachita Mountains and Gulf Coastal Plain. In 2009, large scale disturbance events in all three regions caused widespread damage within pine forests (Chapter 1). An ice storm within the Ozark Mountains (26-27 January 2009) caused breakage of crowns and downed trees throughout that national forest (Barjenbruch 2009). Localized, but severe tornados in the Ouachita Mountains and Gulf Coastal Plain (9 April 2009) uprooted trees and caused stem breakage (National Weather Service 2009). Release of pine volatiles caused by newly felled trees is highly attractive to emerging siricids making the regions ideal for our research (Johnson et al. 2009).

Fourteen sites were established in the three Arkansas regions; six sites within the Ozark Mountains (OzNF), five sites within the Ouachita Mountains (OuNF) and three in the Gulf Coastal Plain in southern Arkansas (SAR) (Figure 1 & Table 1; see Chapter 2 for more complete site data). Sites are predominately pine, interspersed with small diameter hardwoods. Pines within the sites are shortleaf (*P. echinata* Mill.), loblolly (*P. taeda* L.) or a combination of both. Three sites in the Ozarks were only used in 2010 and were relatively unmanaged, overstocked pine stands in which little to no logging had occurred in recent years (Wed1, Wed2, Wed3). The remaining three sites were in actively managed areas, which were thinned in 2009 (OzNF1, OzNF2, OzNF3). Three sites in the Ouachita National Forest were actively managed and commercially thinned in 2009 (OuNF1, OuNF2, OuNF3). One site in the Ouachitas was chosen due to stress caused by frequent flooding and salvage logging occurred in 2009 (OuNF4). The last site in the Ouachita Mountains was added in 2010 (OuNF5) and was burned in early October

2010. The three sites in the Gulf Coastal Plain (SAR1, SAR2, SAR3) were intensively managed even aged pine stands owned by Plum Creek Timber and were also commercially thinned in 2009.

Trapping

Trapping was conducted at each site using three APTIV Intercept™ black panel traps baited with Contech Inc. *Sirex* lure (α - β pinene) plus 95% high-release ethanol. The traps are 80cm long and 30cm wide and hung from a 2cm electrical conduit ca. 1m above the ground. Trapped specimens were captured in propylene glycol in a collection container at the bottom of the trap.

In most sites, trapping was initiated in late October in 2009 and mid August in 2010 and traps were removed both years in early December (OuNF1-4, OzNF 1-3, SAR 1-3). The OuNF5 and Wed 1-3 sites were not established until 2010 and trapping occurred from September to early December. Trap catches were collected every two weeks and stored in 95% ethanol at -20°C to avoid DNA degradation (Rubink et al 2003).

Dissections

Sirex nigricornis trapped in 2009 and 2010 were dissected to determine nematode presence. Each specimen was submerged in a dissecting dish with 95% ethanol. A dorso-longitudinal incision was made in the abdomen to expose the ovaries. A LEICA MZ75 dissecting microscope was used for visual inspection of the haemocoel for adult nematode presence as well as the presence of juveniles, which could occasionally be seen just outside the ovaries. This approach was justified by the observation of Bedding (1974) that juvenile *Deladenus* are

released from their mother into the host's haemocoel and then migrate to the ovaries.

Unfortunately, no adult *Deladenus* were found. To determine presence and number of juveniles, 25 to 30 eggs from each female were mounted on a slide in lacto-phenol and examined with a LEICA DM 2500 compound microscope using Phase microscopy, which was necessary to detect juveniles within the eggs. Number of eggs containing nematodes was recorded. After examination, each siricid was preserved in 95% ethanol and stored at -20°C.

Each siricid specimen was measured in a dissecting dish dorsum-up using a digital caliper. Body length was determined from the tip of the ovipositor to front of the head. Head width was measured as the distance between lateral margins of the eye.

Molecular Protocol

Species-level identification of *Deladenus* relies on adult morphology. Unfortunately, we were only able to obtain juveniles during this study; therefore, we attempted to use molecular tools for identification. Approximately 1 to 10 nematode infested eggs were taken from 30 *Sirex nigricornis* specimens (Table 2) and used for extraction. Genomic DNA was extracted from the nematodes using Qiagen® DNeasy Blood and Tissue kits and recommended protocol was followed.

A portion of the gene cytochrome oxidase I (COI) was amplified using primers from Kanzaki and Futai (2002). Polymerase chain reaction (PCR) was carried out using the following protocol: initial denaturation at 95°C for 5min; followed by 35 cycles of denaturing at 95°C for 30sec, annealing at 51°C for 45sec, and extension at 72°C for 2min; with a final extension at 72°C for 10min. Each reaction contained a 23µl mixture of 2.5µl PCR reaction buffer, 1.75µl

MgCl₂, 1.75µl dNTP, 1µl of each primer, 0.2µl Platinum Taq, 12.8µl H₂O, and 2µl DNA template.

The product was separated on a 1% agarose gel using gel electrophoresis and DNA stained with gel red (Biotium) was visualized under UV light. Successful amplifications were purified using Qiagen® QIAquick PCR purification kit and recommended protocol was followed. Purified sequences were sent to Macrogen Corp. in Rockville, Md. for sequencing. Forward and reverse sequences were reconciled using Lasergene SeqMan Pro (DNASTAR, Madison, Wis., USA). Sequences were aligned using ClustalX (Thompson et al. 1997). All molecular methods and analysis were conducted in consultation with Dr. Ashley Dowling, Department of Entomology, University of Arkansas.

Data Analysis

Body length of *Sirex nigricornis* with nematodes and *S. nigricornis* without nematodes was compared using a two sample *t*-test. Head width was also tested using the same method. Regression equations for *S. nigricornis* with nematodes and *S. nigricornis* without nematodes were determined using an analysis of covariance with body length as the dependent variable and head width as the independent variable. The analysis of covariance was used to identify differences in the two equations.

The percentage of eggs infected with nematodes within a female was compared between forest regions using a two sample *t*-test. The same method was used to compare infected eggs between years and between color morphs (A and B). A linear regression was fit to the scatterplot of body length by percentage of eggs within a *S. nigricornis* infected with nematodes and the

coefficients were found to be significant. The same methods were used for head width by percentage of eggs infected with nematodes.

RESULTS

Dissections

The haemocoel of each female was examined, but no adult nematodes were detected. However, juvenile nematodes were found within the reproductive system of *Sirex nigricornis* specimens. According to Bedding (1974), juvenile *Deladenus* migrate from the haemocoel to the ovaries and enter the eggs of the host. Our observations agree with his findings. Juveniles were found inside the eggs of the host (Figure 2) and also surrounding the ovaries (Figure 3). However, hosts were also found with juveniles surrounding the ovaries and with the eggs free of nematode infection. Unfortunately, only female *S. nigricornis* were collected in the panel traps; therefore, presence of *Deladenus* within male *S. nigricornis* was not observed in this study.

In 2009, 18% of the 134 *S. nigricornis* dissected from the Ouachita National Forest were nematode positive (Table 3). Percent infection for *S. nigricornis* trapped in the other regions is probably not relevant because the sample size was insufficient (6 dissected from the Ozark National Forest and 24 dissected from the Gulf Coastal Plain). However, one *S. nigricornis* female from each of these regions was nematode positive. In 2010, 24% of the 37 *S. nigricornis* dissected from the Ouachita National Forest and 44% of the 142 *S. nigricornis* dissected from the Ozark National Forest were nematode positive (Table 3). No percentage in 2010 can be given for the Gulf Coastal Plain because the sample size was not large enough (4 dissected). None of the *S. nigricornis* dissected from the Gulf Coastal Plain were nematode positive in 2010.

The percentage of eggs within a *S. nigricornis* female infected by nematodes had a range from 10 to 100% in 2009 and 0 to 100% in 2010 (Figure 4). Individuals recorded as having nematodes but with no eggs infected had juvenile nematodes surrounding the ovaries but none inside the eggs. In both years, the highest numbers of *S. nigricornis* were in the category 90 to 100% egg parasitism (32% in 2009 and 24% in 2010) with 43% of *S. nigricornis* having 80 to 100% of eggs being parasitized in 2010.

No difference was found in the percentage of eggs infected with nematodes within female *S. nigricornis* between the three forest regions ($t=-0.021$, $P=0.983$, $DF=96$) or the two years ($t=0.650$, $P=0.517$, $DF=97$). There was also no difference in percentage of eggs infected between the two color morphs of *S. nigricornis* ($t=-0.863$, $P=0.391$, $DF=97$).

The regression for body length on percentage of eggs infected with nematodes showed a significant linear relationship ($P<.0001$, $R^2=0.210$) (Figure 5). The regression for head width on percentage of eggs infected with nematodes also showed a significant linear fit ($P<.0001$, $R^2=0.217$) (Figure 6).

The regression equation for body length on head width of *S. nigricornis* with nematodes was not significantly different from the equation for *S. nigricornis* without nematodes (Figure 7). The difference in both the slopes ($P=0.658$) and the intercepts ($P=0.363$) were not statistically significant.

However, mean body length of *S. nigricornis* with nematodes ($M=21.59$, $SE=0.41$) was significantly different from mean body length of *S. nigricornis* without nematodes ($M=23.64$, $SE=0.26$) with a t -statistic of -4.165 and a p -value of $<.0001$ based on 342 DF. Mean head width was also significantly different between *S. nigricornis* with nematodes ($M=2.80$, $SE=0.06$) and *S.*

nigricornis without nematodes ($M=3.05$, $SE=0.04$) with a t -statistic of -3.563 and a p -value of 0.0004 based on 342 DF.

Molecular Identification

We amplified 644 base pairs of cytochrome oxidase I (COI) from 27 of the 30 sequences (Table 2). Twenty-five of these sequences were identical. The remaining two were identical to each other, but different from the other 25 by two base pairs (Table 2). The latter two sequences were both from nematodes infecting *Sirex nigricornis* color morph B. However, nematodes from color morph B specimens were also in the identical 25. The sequences were also found to be 11% different than *Deladenus siricidicola* sequences found on GenBank. Both sequences can be found in Appendix 1.

DISCUSSION

Our study confirmed the presence of *Deladenus* within *Sirex nigricornis* collected from Arkansas pine forests. Although no adult *Deladenus* were found, juveniles were located within the ovaries and the eggs. Absence of adult nematodes is seemingly impossible. However, once the female nematode stops producing juveniles, instead of continuing to release them, they burst through her body (Bedding 1974). This burst of juveniles may make the adult nematode unrecognizable within the haemocoel of the host and explain the absence of adult *Deladenus* within our specimens.

Although sample sizes were too small to obtain a percentage of *S. nigricornis* infected with *Deladenus* for each investigated region, nematodes were found within specimens from all three regions (Table 3). In the Ouachitas, percentage of infected *Sirex* was similar between the two years (18% in 2009 and 24% in 2010). However, comparisons cannot be made between the two years in the Ozarks and the Gulf Coastal Plain because of small sample size in the Ozarks in 2009 and in the Gulf Coastal Plain in both 2009 and 2010. No significant differences were seen in percentage of infection between sites. *Deladenus* was found within *S. nigricornis* collected from all sites in the Ozarks and the Ouachitas (Table 5). Only one site in the Gulf Coastal Plain had *Deladenus*, but when considering the sample size in the region (27 specimens) we could have easily missed *Deladenus* populations in the other sites. More extensive data collection would be necessary to make an accurate assessment of infection rates for all three regions.

Although we estimated a percentage of parasitism for the Ozarks and Ouachitas (Table 3), we still do not know how parasitism varies in natural populations of our native *Sirex*. Parasitism by *Deladenus siricidicola*, the nematode used for biological control of *S. noctilio*, has reached over 90% in some programs (Bedding 1979, Bedding 2009). In our study, our native *Deladenus* achieved much lower parasitism rates, between 18 and 44%. However, they may still be making an impact on *S. nigricornis* populations and may ultimately affect population dynamics.

There are many potential effects that *Deladenus* may have on their hosts, including an impact on female *Sirex* development from larval stage to adulthood. To determine whether nematodes affect *S. nigricornis* size, the body length and head width of each specimen was measured. These measurements were used to represent *S. nigricornis* size because neither measurement would be affected by the intake of fluids during specimen preservation.

Female *S. nigricornis* parasitized by *Deladenus* were found to be significantly smaller than those without nematodes. Unparasitized *S. nigricornis* were approximately 10% larger than parasitized *S. nigricornis*. Similar patterns have been shown in *S. noctilio* infected by *Deladenus siricidicola* (Villacide and Corley 2008) and *Xeris spectrum* infected by *Deladenus* (Fukuda and Hijii 1997). Smaller body size is thought to be a result of a loss of fat reserves and a modification of metabolic function in *Sirex* caused by nematodes feeding in the haemocoel (Bedding and Iedes 2005). Although feeding by *Deladenus* is minimal, *Sirex* do not feed as adults and any mass lost during development cannot be regained once the individual reaches adulthood.

While a loss in mass will not affect *Sirex* survival, larger *Sirex* have been shown to fly farther than smaller individuals. Smaller *Sirex* are incapable of flight patterns needed for sustained flight and are therefore less likely to disperse (Bruzzone et al. 2009). The ability of female *Sirex* to effectively disperse and deposit her eggs is important to nematode success. To ensure the spread of nematodes into new areas, healthy *Sirex* need to be ovipositing into the same trees as parasitized *Sirex*. However, if parasitized *Sirex* are unable to effectively disperse to new areas, then neither is *Deladenus*. Body size has also been positively correlated with fecundity (Madden 1974, Fukuda et al. 1993). Thus, *Deladenus* may indirectly reduce *S. nigricornis* fecundity by reducing its size. The decreased body size observed in parasitized *S. nigricornis* may negatively affect populations of both *Deladenus* and *S. nigricornis*.

While *Deladenus* indirectly affects fecundity by causing a reduction in *S. nigricornis* size, more importantly they directly reduce fecundity by invading the reproductive system. Once *S. nigricornis* reaches pupation, adult *Deladenus* rapidly produce juvenile nematodes which enter the haemocoel of the host. These juveniles migrate into the reproductive organs and, in the case of a female *Sirex*, usually enter some fraction of the eggs making them inviable (Bedding 1972).

Although all known *Deladenus* parasitize siricids, apparently a few species only enter a portion of the eggs, while others colonize every egg, completely sterilizing the *Sirex* (Bedding 1972).

Our results show nematodes colonizing a range from all of the available eggs to none. No consistent colonization pattern was detected (Figure 3) and region of the state, year or color morph of *S. nigricornis* had no effect on percentage of eggs infected. The variability in percentage of eggs parasitized was not anticipated. Bedding (1972) found that some strains of *Deladenus siricidicola* parasitized 100% of the eggs, while others were only found surrounding the eggs and not inside them at all. However, prior research has not shown such variability within *Deladenus* of the same species and, what appears to be, the same strain. This variability could be an advantage for *Deladenus*. While those *Sirex* with close to 100% of their eggs parasitized ensures juvenile nematodes are spread, those with a lower percentage of infected eggs ensure the next generation of *Deladenus* have resources, such as *Sirex* larva, available. *Deladenus* species which parasitize 100% of the eggs may be controlling the siricid population too effectively and therefore it may be an advantage to have variability in the number of eggs parasitized.

Sirex nigricornis size was negatively correlated with percentage of eggs infected (Figures 4 & 5). This relationship suggests that those *S. nigricornis* that have a higher portion of eggs infected may be losing more resources as larvae to the adult *Deladenus* feeding within the haemocoel than do *S. nigricornis* with a low percentage of infected eggs. Timing of parasitism by the adult nematodes could explain the relationship seen between size and percentage of eggs infected. *Sirex nigricornis* with no or very few infected eggs may have been parasitized at a later larval stage than those with most of their eggs infected. Since the reproductive system of the female *Deladenus* develops while she is inside the host, those nematodes which enter a larva late

in its development may lag in releasing juveniles and in turn be exploiting less of the larval insect's fat reserve. Bedding (1972) emphasizes the importance of release timing of the juvenile *Deladenus*. If the juveniles are released too late, the eggs may be too far along in development for the nematodes to successfully enter. If this is the case, the *S. nigricornis* would be larger upon emergence and the juveniles may be released too late to enter the eggs or were only able to enter a portion of the eggs. This may also explain why we observed a few *S. nigricornis* with juvenile nematodes inside the ovaries but none within the eggs.

Sequences obtained for *Deladenus* showed minimal differences in COI. The differences can be attributed to differences within a population and do not indicate different species. Therefore, our data suggests *Deladenus* found in the Ozarks, Ouachitas and Gulf Coastal Plain are the same species. Sequences were compared to published *Deladenus siricidicola* sequences on GenBank and were found to be 11% different. *Deladenus siricidicola* only feeds on *Amylostereum areolatum*, which was only recently introduced into the United States with *Sirex noctilio*. Since *S. noctilio* has yet to be introduced into the southern United States, *Amylostereum areolatum* should not be found in Arkansas. Furthermore, our nematodes were found in *S. nigricornis*, which carries a different species of fungus, *Amylostereum chailletii*. These geographical and biological restraints along with an 11% difference suggest the nematodes found within our specimens are not *D. siricidicola* (Hebert et al. 2004). However, further identification of the species was not possible, because no identified specimens were available for comparison and juvenile *Deladenus* cannot be identified morphologically.

The results observed in this study confirmed the presence of *Deladenus* colonizing native *S. nigricornis* females within Arkansas pine forests. The nematodes found were a single, as yet undetermined species and infected the eggs of both color morphs of *S. nigricornis*. The presence

of *Deladenus* was correlated with reduced *S. nigricornis* body size. Further research is needed to identify the species of *Deladenus* found in *S. nigricornis* populations in the southeastern United States.

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Figure 1. Map showing the location of regions and placement of study sites throughout Arkansas. Red dots represent sites.

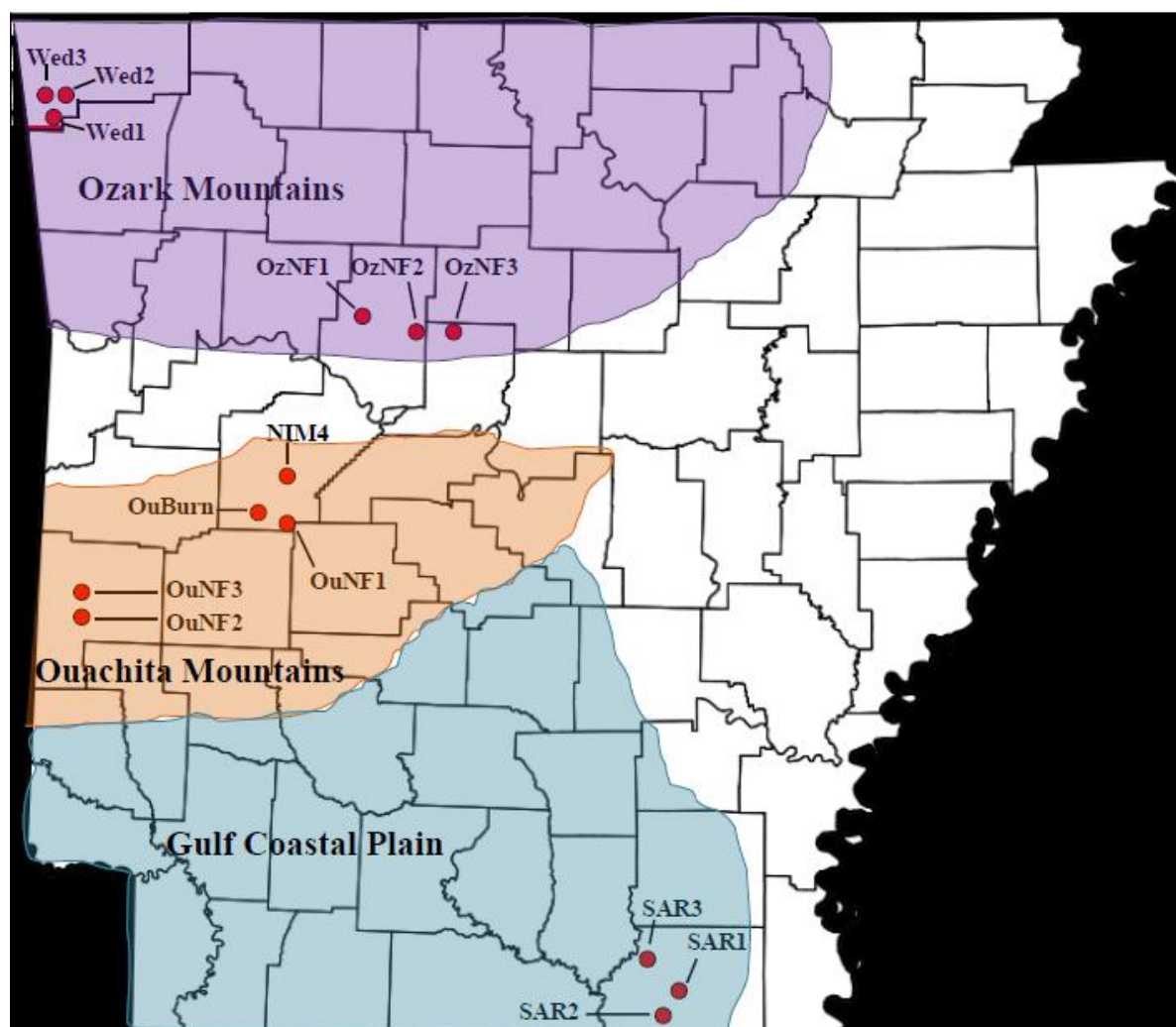


Figure 2. Compound micrographs of *Sirex nigricornis* eggs. **a)** Uninfected; **b)** Infected with juvenile *Deladenus*; **c)** *Deladenus* removed from the egg. Images by D. C. Steinkraus.



Figure 3. *Sirex nigricornis* eggs surrounded by juvenile *Deladenus* within the ovaries.

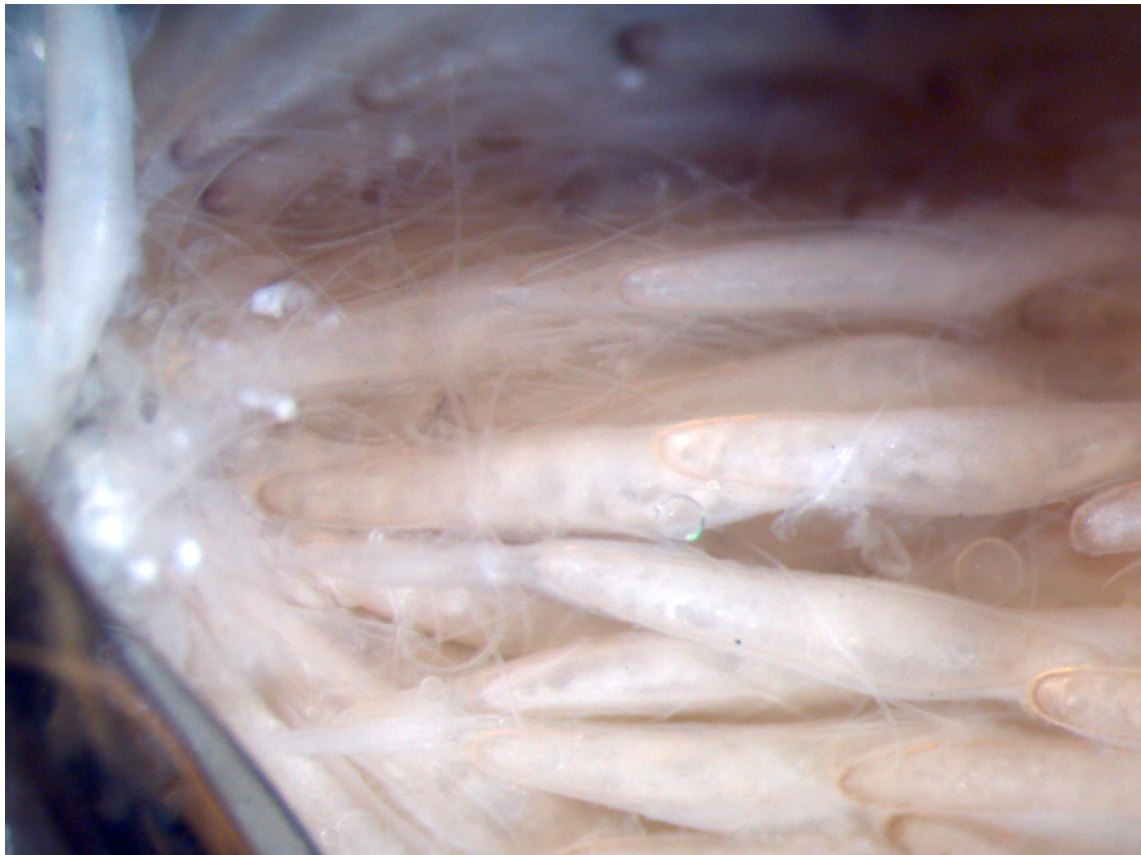


Figure 4. Percentage of eggs infected with nematodes within the ovaries of *Sirex nigricornis* caught in panel traps.

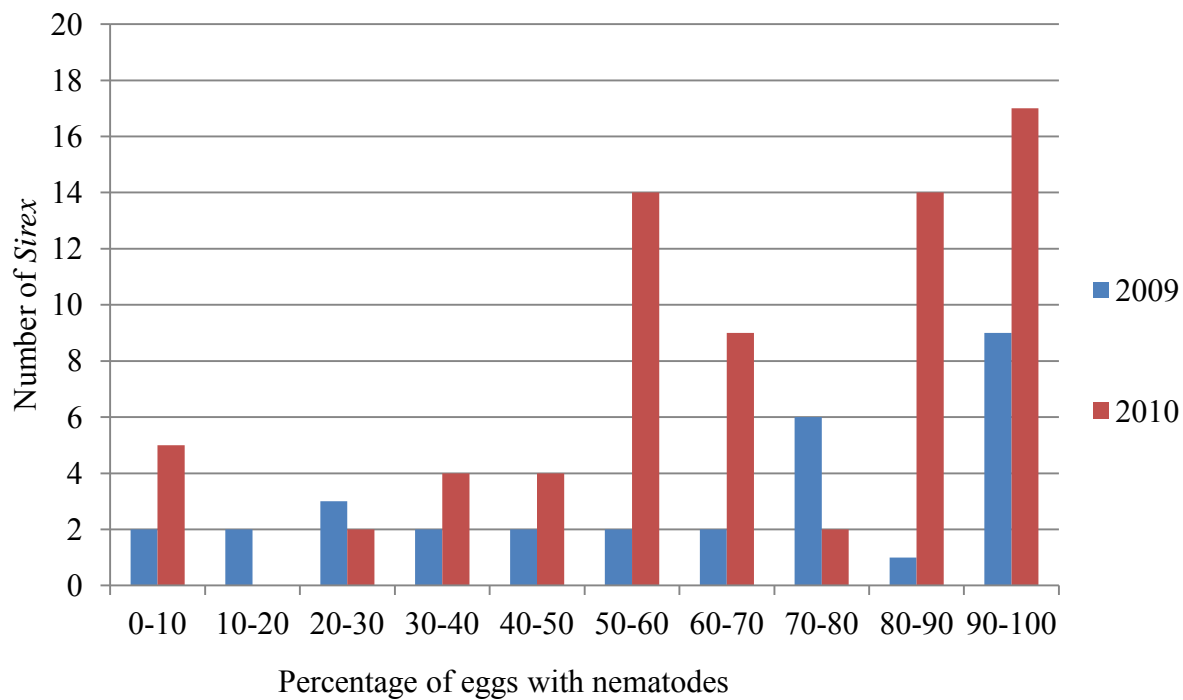


Figure 5. Regression for body length on percentage of eggs infected within a female *Sirex nigricornis*, including the line of best fit and R-squared value.

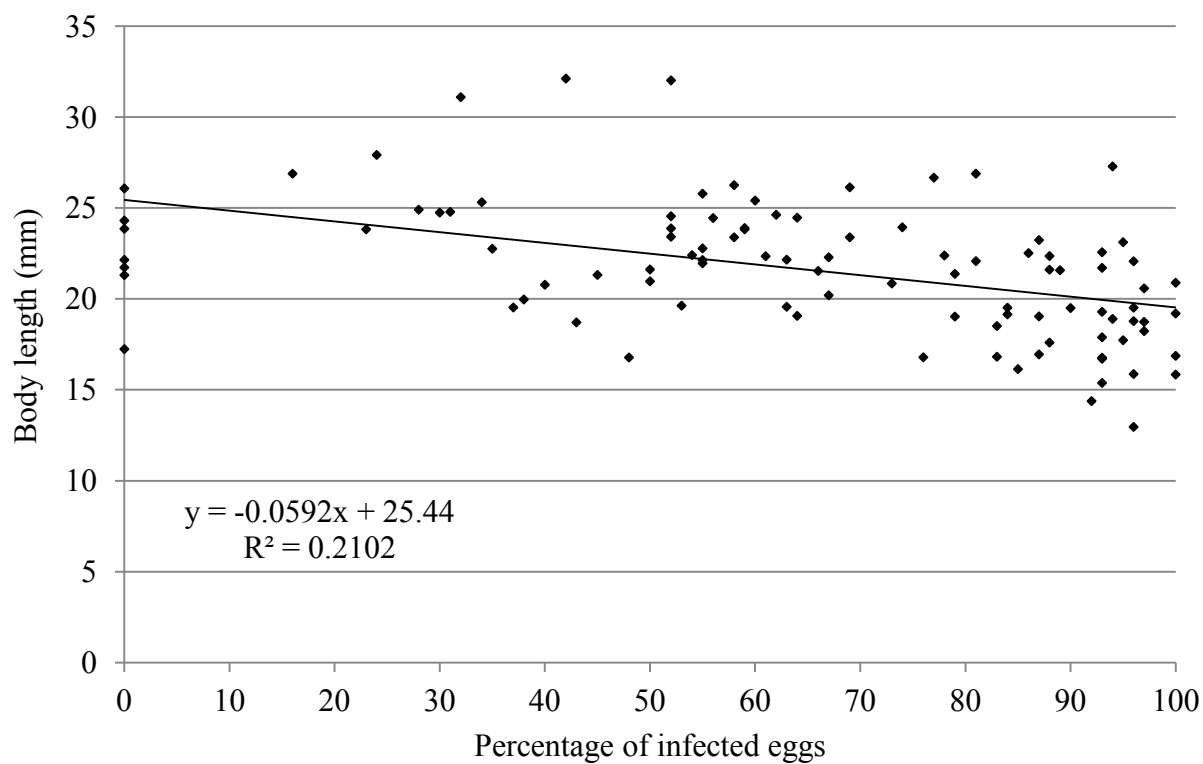


Figure 6. Regression for head width on percentage of eggs infected within a female *Sirex nigricornis*, including the line of best fit and R-squared value.

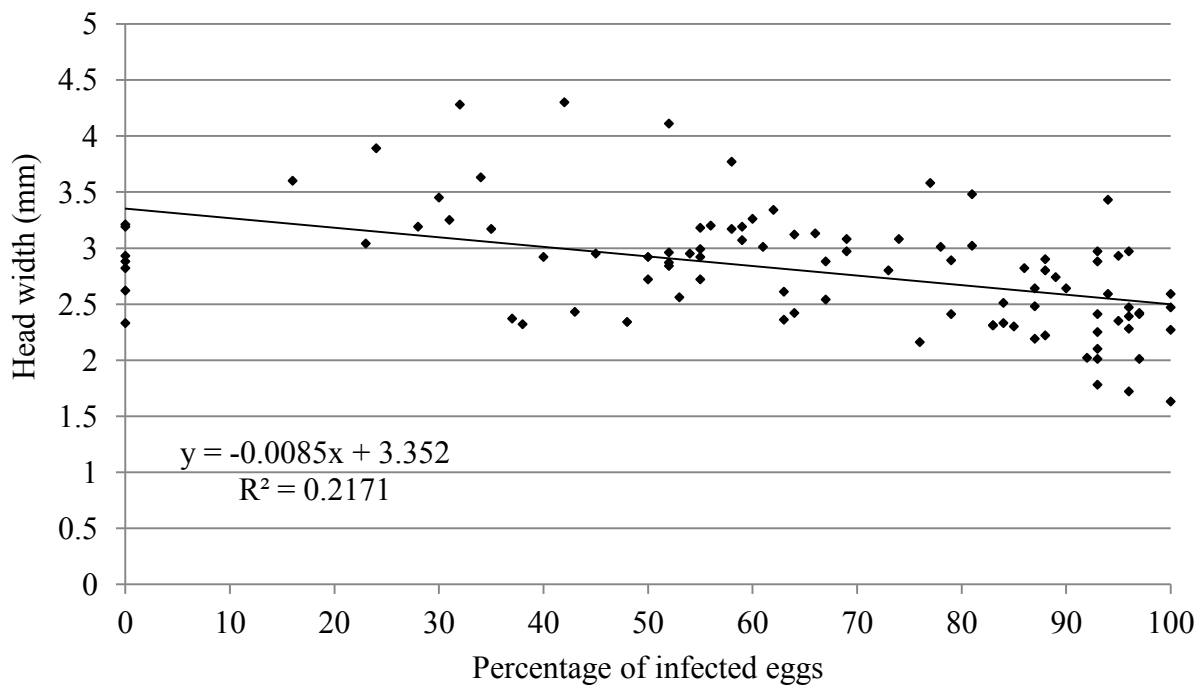


Figure 7. Regression for body length on head width showing the lines for *Sirex nigricornis* with nematodes and those without nematodes.

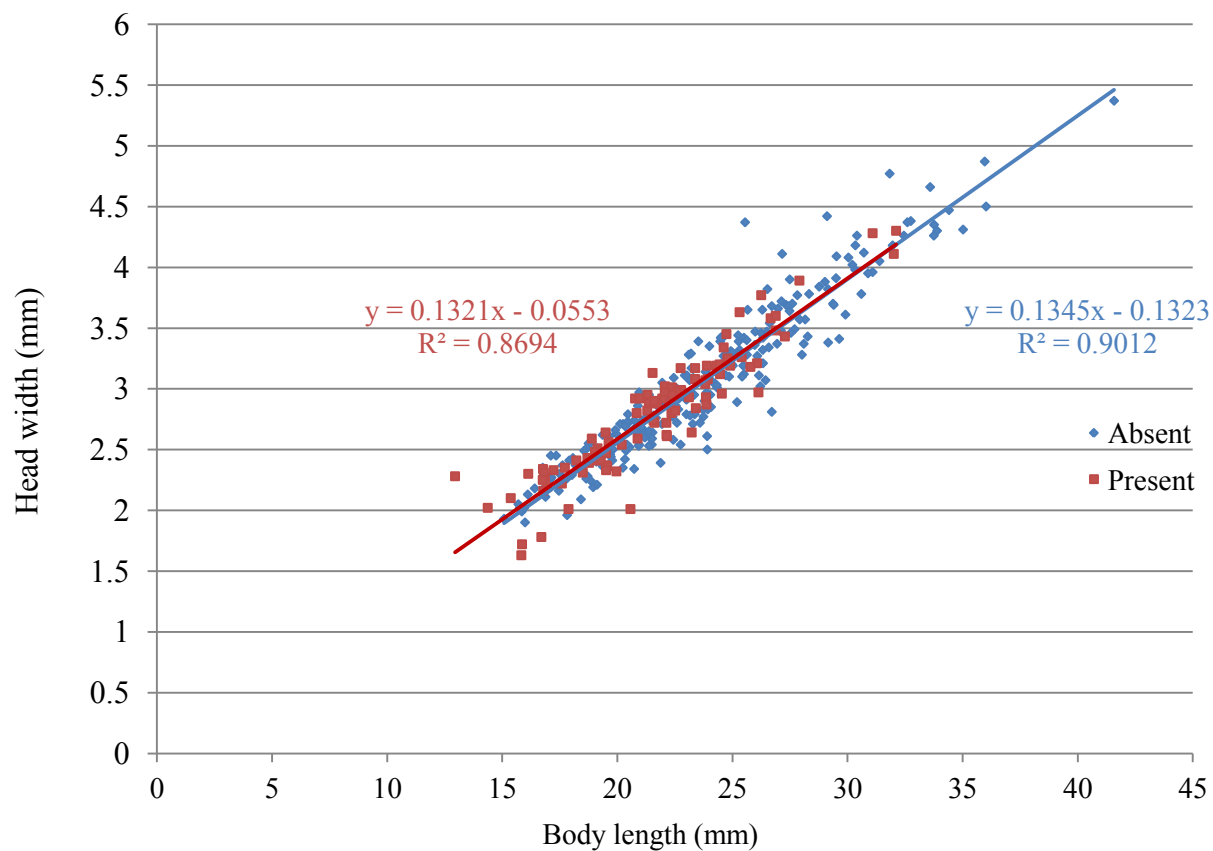


Table 1. UTM coordinates for sites.

Sites	UTM 15N
Ouachita National Forest	
OuNF1	0461513 3846260
OuNF2	0383226 3807198
OuNF3	0383226 3808738
OuNF4	0459573 3864039
OuNF5	0455844 3845070
Ozark National Forest	
OzNF1	0491867 3928173
OzNF2	0518906 3923735
OzNF3	0511810 3923514
Wed1	0373952 3998821
Wed2	0373156 3999732
Wed3	0370165 4000441
Gulf Coastal Plain	
SAR1	0611789 3663854
SAR2	0605491 3658027
SAR3	0598202 3682044

Table 2. *Sirex nigricornis* specimens used for polymerase chain reaction (PCR).

Specimen number	Site	Date collected	Color morph	Sequence obtained	Sequence identity
1	OuNF1	11/4/2009	A		X
2	OuNF1	11/13/2009	A	+	X
3	OuNF1	11/25/2009	A	+	X
4	OuNF2	11/30/2010	A	+	X
5	OuNF2	11/6/2009	B	+	X
6	OuNF2	11/6/2009	A	+	X
7	OuNF2	11/6/2009	A	+	X
8	OuNF3	11/18/2009	B	+	Y
9	OuNF3	11/6/2009	A	+	X
10	OuNF3	11/6/2009	B	+	X
11	NIM4	11/13/2009	A	+	X
12	NIM4	11/11/2010	A	+	X
13	NIM4	11/13/2009	A	+	X
14	OuBurn	11/11/2010	A	+	X
15	OuBurn	11/30/2010	A	+	X
16	OuBurn	11/30/2010	A	+	X
17	OzNF1	11/24/2009	B	+	Y
18	OzNF1	10/22/2010	B	+	X
19	OzNF1	11/6/2010	A	+	X
20	OzNF2	10/22/2010	B	+	X
21	OzNF2	11/6/2010	A	+	X
22	OzNF2	10/22/2010	B	+	X
23	OzNF3	10/22/2010	A		X
24	OzNF3	10/22/2010	B	+	X
25	Wed	10/15/2010	A	+	X
26	Wed	11/15/2010	B	+	X
27	Wed	10/28/2010	B	+	X
28	Wed	10/28/2010	A	+	X
29	Wed	11/15/2010	A	+	X
30	SAR2	11/17/2009	A		X

Table 3. Number and percent of trapped *Sirex nigricornis* infected with nematodes in 2009 and 2010 by region.

	Ouachita National Forest	Ozark National Forest	Gulf Coastal Plain
2009			
No. dissected/No. with nematodes Color Morph A	85/17	5/0	22/1
No. dissected/No. with nematodes Color Morph B	49/8	1/1	2/0
No. dissected/No. with nematodes total	134/26	6/1	24/1
Percent infected (total)	18%		
2010			
No. dissected/No. with nematodes Color Morph A	21/7	99/41	2/0
No. dissected/No. with nematodes Color Morph B	16/2	43/22	2/0
No. dissected/No. with nematodes total	37/9	142/63	4/0
Percent infected (total)	24%	44%	

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APPENDIX 1

Sequences obtained from native *Deladenus* found within *Sirex nigricornis*.

X:TCAAGAGTACGTGAAATCGGTAAGGTGGAAACGGATAGAGTTGACGTATCCGGCACGTATTCAGCTGCCGCTTGC
GTTTGTGCGAGGGTCGGTCGTCGCCGATGGTGGTTCGCCGCCCGTTGGTGTGTTGCTGATTGTCGTACGAATGCGAG
TGGTGGTGCAATTTACGGGCTAATGTGCGCTGGGAGGTGCGCGCTGGCTGTTTGCGGTTTAGGTAAGAGGATGCACGC
TTCGGCATGTAAACCAGACCTTTACTCGTGGGCAGTTCGGCGTGCGCGTTGATTTGCGGCGAGGCCTACGGGCGCA
ATGGCTTATCGTTTTCTGTGGGGCATGGCGGTTCGCATGCGACACGTGTGCTTTGTGGATTCCGGTGGTTGTTGTGTTCA
CTCGTTCATTCCCGGTGTAAGAGTCAATCATCTATCCGACCCGTCTTGAAACACGGACCAAGGAGTTTAAAGTATGC
GCGAGTTATTGGGTGTTGAAAACCTATTAGCGTAATGAAAGTGAATGCGTCCTCGAGATGCTTATATGCGATCCAAT
GCACTTCGATGTGTGTAGGCGCAGCATAGCCCCGTTCTCGTCGCTTGCGATGGAGCGGAGGAAGAGCGTATGCGTTG
AGACCCGAAAGATGGTGAAGTATGCTTGAGCAGGACGAAGTCAGAGGAAACTCTGATGGAAGTCCGAATCGGTTCTG
ACGTGCAAATCGATCGTCTGACTTGAGTATAGGGGCGAAAGACTAATCGAACTATCTAGTAGCTGGTTCTCTCC

Y:TCAAGAGTACGTGAAATCGGTAAGGTGGAAACGGATAGAGTTGACGTATCCGGCACGTATTCAGCTGCCGCTTGC
GTTTGTGCGAGGGTCGGTCGTCGCCGATGGTGGTTCGCCGCCCGTTGGTGTGTTGCTGATTGTCGTACGAATGCGAG
TGGTGGTGCAATTTACGGGCTAATGTGCGCTGGGAGGTGCGCGCTGGCTGTTTGCGGTTTAGGTAAGAGGATGCACGC
TTCGGCATGTAAACCAGACCTTTACTCGTGGGCAGTTCGGCGTGCGCGTTGATTTGCGGCGAGGCCTACGGGCGCA
ATGGCTTATCGTTTTCTGTGGGGCATGGCGGTTCGCATGCGACACGTGTGCTTTGTGGATTCCGGTGGTTGTTGTGTTCA
CTCGTTCATTCCCGGTGTAAGAGTCAATCATCTATCCGACCCGTCTTGAAACACGGACCAAGGAGTTTAAAGTATGC
GCGAGTTATTGGGTGTTGAAAACCTATTAGCGTAATGAAAGTGAATGCGTCCTCGAGATGCTTATATGCGATCCAAT
GCACTTCGATGTGTGTAGGCGCAGCATAGCCCCGTTCTCGTCGCTTGCGATGGAGCGGAGGAAGAGCGTATGCGTTG
AGACCCGAAAGATGGTGAAGTATGCTTGAGCAGGACGAAGTCAGAGGAAACTCTGATGGAAGTCCGAATCGGTTCTG
ACGTGCAAATCGATCGTCTGACTTGAGTATAGGGGCGAAAGACTAATCGAACTATCTAGTAGCTGGTTCTCTCC

FUTURE DIRECTIONS

While this research filled in gaps in our understanding of *Sirex nigricornis*, *Deladenus* and their interactions, it also left many unanswered questions. Trapping results in Chapter 2 show a drastic increase in trap catches in the Ozarks from 2009 to 2010 and a decrease in the Ouachitas between the two years. Although possible reasons for the change in trap catches were discussed, trapping should be carried out over several years to determine if patterns seen are relatively consistent and *Sirex* emergence increases in an area every other year or it is merely coincidental. Although we were able to estimate parasitism rates for *Deladenus*, these were rough estimates because of small sample sizes and only two years of sampling. Further study is needed to understand the impact *Deladenus* has on *Sirex* populations. Nematodes were found parasitizing only a portion of the eggs and whether or not the unparasitized eggs are viable is still unknown. While *Deladenus* were found within *Sirex nigricornis* specimens, we have not recovered the free-living mycetophagous form of the nematodes from pine wood samples. This should be pursued to give more insight into the life history of the nematode, and also provide specimens of adult nematodes. During our research, only juvenile nematodes were found making morphological identification impossible. Lack of previous molecular work on *Deladenus* meant we were unable to find identified sequences to compare to the sequences we obtained from our nematodes. Either identified specimens need to be sequenced for comparison or adult nematodes need to be found within pine samples or larval *S. nigricornis*. Being able to identify the species of *Deladenus* found within Arkansas is an important step towards understanding these organisms.