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Emergence phenology and nematode associates of the woodwasp, *Sirex nigricornis* F.
(Hymenoptera: Siricidae), in Arkansas and Mississippi forests

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Entomology

By

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Abstract

Sirex nigricornis F. (Hymenoptera: Siricidae) is a native woodwasp in eastern North America that inhabits dead and dying pine trees during its univoltine development. *Sirex noctilio* is native to Eurasia and North Africa and was discovered in the northeastern United States in 2004 after a century of accidental introductions across the Southern Hemisphere. Since then, it has spread to seven states and southern Ontario. There is concern about its potential to negatively affect the multi-billion dollar pine timber industry of the southeastern United States. Effective monitoring tools are necessary to track the spread and establishment of *S. noctilio*. *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae) is a parasitic nematode associated with *S. noctilio* in its native range and has been accidentally introduced along with the woodwasp. While these nematodes have been successfully used in biological control in much of the Southern Hemisphere, the strain introduced into North America appears to be non-sterilizing and is not providing natural control. *Deladenus proximus* Bedding is a native nematode that infects *S. nigricornis* and has added *S. noctilio* to its host range in the northeastern U.S., implicating it as a potential control agent for *S. noctilio* in North America, circumventing potential issues associated with classical biological control using generalists. My objectives were to 1) examine the effect of temperature on emergence patterns of *S. nigricornis* and *S. noctilio*, 2) use molecular techniques to identify the native nematode parasitizing southeastern *S. nigricornis*, and 3) examine the effects of nematode infection on southeastern *S. nigricornis* female body size and fecundity. Trapping data from Arkansas, Mississippi, Louisiana, and Ontario were examined to determine the effect of temperature on emergence of adult female siricids. Over 1,200 native female woodwasps were collected, measured, and dissected in the fall of 2011-2015 from Arkansas and Mississippi. Nematode prevalence and virulence were higher in Arkansas than Mississippi. A 750 base pair

region of the cytochrome c oxidase subunit I gene extracted from southeastern nematodes was sequenced. Nematode sequences from New York and Illinois were obtained from GenBank to examine genetic diversity across the eastern United States. Genetic distance among all nematode sequences collected was less than 0.5% indicating a single species, and continuous genetic flow, of nematodes across the eastern United States. Nematodes significantly negatively impacted woodwasp size and fecundity; infested females were smaller and produced fewer eggs than uninfested females. We believe these impacts demonstrated on *S. nigricornis* will translate to *S. noctilio*. Future directions should include inoculating *S. noctilio*-infested trees using virulent populations of Arkansas-collected *D. proximus* to test these predictions in the field.

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Special thanks to my committee members for their help throughout this process. They were, and will continue to be, essential to my professional and personal growth throughout my career. Sincere appreciation to my co-authors who helped from afar. Thank you to my friends and family for their continued support through the trials and tribulations of graduate school.

Dedication

I dedicate this dissertation to my husband, Jeremy Rathje, who brought me light when there was none to see.

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List of Published Papers

1. Hartshorn, J. A., L. J. Haavik, J. D. Allison, J. R. Meeker, W. Johnson, L. D. Galligan, K. D. Chase, J. J. Riggins, and F. M. Stephen. Emergence of adult female *Sirex nigricornis* F. and *S. noctilio* F. (Hymenoptera: Siricidae) coincides with a decrease in daily minimum and maximum temperature. Agricultural and Forest Entomology (in press).

Literature Review

Introduction

Siricid woodwasps (Hymenoptera) colonize and develop in both hard- and soft-wood trees of the Palearctic and Nearctic regions and, within their respective native ranges, do not kill healthy trees. While woodwasps are routinely intercepted at ports, only one (*Sirex noctilio* F.) of the 122 extant species has caused significant economic damage. In the late 1800s, this European woodwasp was accidentally introduced to several countries in the Southern Hemisphere in which significant commercial pine industries had been initiated. Following these accidental introductions, *S. noctilio* often remained innocuous until abiotic factors (e.g. significant drought), combined with overstocked stands sufficiently stressed trees and led to outbreaks (Rawlings and Wilson 1949). Control measures during these outbreaks ranged from silvicultural treatments to biological control, with each method exhibiting benefits and disadvantages depending on the region affected and severity of infestation (Hurley et al. 2007).

In 2004, *S. noctilio* was detected in upstate New York and has since spread to seven states and Canada (southern Ontario and western Quebec), where it has yet to cause mortality to healthy, vigorous pine stands (Dodds et al. 2010, Hoebeke et al. 2005). It is difficult to translate past effects of *S. noctilio* on pine stands in the Southern Hemisphere to future effects in North America. *Pinus* L. (Pinales: Pinaceae) is not native to the Southern Hemisphere, and thus the full complex of pine-inhabiting natural control agents normally found along with *Sirex* are not present in those settings. Additionally, pines in the Southern Hemisphere are typically grown in large discrete plantations, whereas North America contains both pure pine stands and millions of hectares of mixed stands, which may be managed or unmanaged. Since the accidental introduction of *S. noctilio* into the United States, research on the European woodwasp, its native

counterpart *S. nigricornis*, and their associates has dramatically increased. Morphologically the two *Sirex* species are very similar but can be distinguished by the larger size of adult *S. noctilio* and by orange coloration on its legs (Figure 1a, b).

Native Ranges and *S. noctilio* Introduction History

Sirex noctilio F. is native to Eurasia and North Africa where it inhabits dead and dying pine trees (Hall 1968). *Sirex nigricornis* F. (Hymenoptera: Siricidae) is native to the eastern United States and Canada (Schiff et al. 2012) where it similarly inhabits dead and dying pine trees. *Sirex noctilio* was accidentally introduced into exotic commercial pine plantations of the Southern Hemisphere, beginning with New Zealand in the late 1800's and continuing to Australia (Chrystal 1928, Miller and Clark 1935, Nuttall 1989, Haugen 1990), South America (Iede et al. 1996), and South Africa (Tribe and Cillié 2004). Exact figures are not available to calculate the economic damage to pine plantations caused by *S. noctilio* in these countries, however, negative effects on timber in the Southern Hemisphere are regularly referred to as 'significant' (e.g. Carnegie et al. 2005). While woodwasps have been detected at ports in the northeastern United States several times, the first reported established population of *S. noctilio* in a novel environment of the Northern Hemisphere was in 2004 in New York (Hoebeke et al. 2005). Although populations have spread, pine mortality has been limited to stressed trees in natural stands and economically significant losses in North America have not yet been reported. Models, however, predict anywhere from \$86 to \$254 million in economic losses per year after 20 years, with the majority of losses most likely occurring in eastern Ontario (Yemshanov et al. 2009a, b).

Conversely, *Sirex nigricornis* has never been reported as an economically significant pest and also has never been reported outside its native range of eastern North America. Only

recently have studies begun to investigate the ecology of this native woodwasp in pine forests and these studies describe it as being found, along with several additional pine-inhabiting insects, in stressed or damaged stands (Ryan et al. 2012a, b, Chase et al. 2014).

Phenology

Adult *S. noctilio* emergence in its native range of Eurasia and North Africa is reported to begin in late summer or early autumn and continue for several weeks (Morgan 1968, Spradbery and Kirk 1978). This is similar to its flight patterns reported in Tasmania (Taylor 1981) and the northeastern United States (Zylstra et al. 2010, Haavik et al. 2013, Myers et al. 2014). However, its phenology in other areas of the Southern Hemisphere differs significantly, with adult emergence lasting longer and often beginning earlier, sometimes as early as mid-summer (December) (Corley and Villacide 2012, Nahrung et al. 2015). Cumulative degree-day (CDD) models have been used to predict emergence of *S. noctilio* and *S. nigricornis* with variable results and implications (Haavik et al. 2014, Myers et al. 2014). While CDD models may differ significantly in their results and predictions, it appears that both *S. noctilio* and *S. nigricornis* females respond to decreasing minimum and maximum temperatures and that this trend will continue upon the spread of *S. noctilio* into the southeastern United States (Hartshorn et al. in press).

In the southeastern United States, *S. nigricornis* emergence begins in early October and continues through December (Dinkins 2011, Keeler 2012, Lynn-Miller 2012, Chase 2013, Hartshorn et al. 2015). Further north, in New York and Ontario, Canada, *S. nigricornis* emergence begins in September and extends through November (Dodds 2007, Zylstra et al. 2010, Haavik et al. 2013). At a similar latitude, emergence of *S. nigricornis* in Minnesota began in late July (Coyle et al. 2012) and these differences in emergence in northern regions may be

related to interactions with *S. noctilio* or additional factors (e.g. relative humidity) not examined by those studies.

Oviposition and Development

Sirex noctilio and *S. nigricornis* share similar patterns of emergence, oviposition, and development. Life cycles lasting one to three years have been reported for various *Sirex* species, including *S. noctilio* (Gilbert and Miller 1952, Corley and Villacide 2012). Males tend to emerge prior to females and immediately fly towards light in the forest canopy, waiting there for females to emerge. Both sexes live a week to ten days. Potential fecundity of females varies considerably, with both *S. nigricornis* and *S. noctilio* containing anywhere from less than ten to several hundred eggs, and the underlying causes of this variation have been speculated but are still largely unknown (Keeler 2012, Yousuf et al. 2014). Female *Sirex* are parthenogenetic, meaning that unmated females can oviposit viable progeny without fertilization. Specifically, female *Sirex* are arrhenotokous, a mechanism prominent in Hymenoptera by which unfertilized eggs are male while fertilized eggs produce females (Morgan 1968, Madden 1974). Neither sex feed as adults; females are pro-ovigenic and, therefore, do not require additional adult nutrition to complete egg development (Madden 1974, 1981). A contact pheromone that induces copulation attempts by males has been described for *S. noctilio* (Böröczky et al. 2009) and this pheromone likely assists in mate recognition and lekking behavior of males. Male *S. noctilio* have also been shown to emit pheromones which attract both females and males, with preliminary tests showing less response by mated females (Cooperband et al. 2012). Pheromones of *S. nigricornis* have not been evaluated but lures mimicking host volatiles are effective for attracting both species (Barnes et al. 2014).

Adult females of both species are attracted to host volatiles that are released from stressed or damaged pines (i.e. pinenes and ethanol), and they use these chemicals to orient to suitable host trees (Böröczky et al. 2012). After landing at a host tree, they drill their flexible ovipositor approximately 3 cm into the xylem, presumably testing for host suitability. If a host is unsuitable, females may test another spot on the same tree or fly to a new host. Once a suitable host is detected, females deposit 1-2 eggs into the first drill hole. They partially remove their ovipositor from the initial drill hole, changing direction and inserting it one or more times to deposit additional eggs. The final drill hole, however, does not contain eggs but rather a symbiotic fungus, *Amylostereum* (Boidin) (Russulales: Amylostereaceae), and a phytotoxic venom called noctilisin, thought to condition the tree for successful larval development (Coutts 1969a, Coutts and Dolezal 1969, Spradbery 1977, Bordeaux et al. 2014) (Figure 2). Exact mechanisms behind the process by which females test for host suitability are unknown, but moisture content is thought to play a role (Coutts and Dolezal 1965, Hartshorn 2012).

After eggs eclose, larvae tunnel into the xylem, creating long, winding galleries (Madden 1981). Until very recently, it was believed that larvae obtained limiting nutrients, such as nitrogen (N), by directly ingesting *Amylostereum* (Talbot 1977). However, Thompson et al. (2014) demonstrated the presence of a N-fixing bacterial symbiont in the gut of *S. noctilio* larvae, suggesting larvae do not feed directly on the fungus. Instead, the gut symbiont fixes N using sugars ingested when larvae, using their specialized mandibles, squeeze ‘juice’ from fungus-degraded wood (Figure 3). Gut-inhabiting bacteria in wood boring insects have been identified several times (Vasanthakumar et al. 2006) and they help to explain how woodwasps develop successfully in such a nutrient poor environment. It is expected that these bacteria would be present in *S. nigricornis* guts as well, however, this has not been investigated.

Woodwasp larvae develop in pine xylem for one to three years, completing a variable number of instars (usually 6-12, Morgan and Stewart 1996), eventually turning towards the phloem before pupation and emergence.

Symbiotic Fungi

Amylostereum arthrospores, sometimes referred to as ‘oidia’, are held in specialized abdominal sacs (mycangia) of adult female *S. noctilio* and *S. nigricornis* and injected into the tree during probing and oviposition (Büchner 1928, Coutts and Dolezal 1965, Morris and Hajek 2014). These fungi stain the wood and can negatively affect the health of pines (Coutts 1969b, Shain and Hillis 1972), even without developing larvae or the addition of noctilisin. Complex associations among *Sirex* woodwasps and these fungi have been described several times (e.g. Boros 1968, Talbot 1977) and for several decades these relationships were thought to be species specific (Slippers et al. 2003). However, *A. areolatum*, believed to have been introduced along with *S. noctilio*, was identified from mycangia of *S. nigricornis* in the southeastern United States where *S. noctilio* is not yet present (Nielsen et al. 2009), indicating that multiple species of *Amylostereum* are currently present across the United States and that woodwasps can likely use more than one species for development. The ability of *S. noctilio* to utilize native fungi could allow for rapid establishment and spread of *S. noctilio* throughout the United States, and may have a significant impact on the future of biological control by promoting or inhibiting growth and development of both *S. noctilio* larvae and parasitic nematodes (described later) (Nielsen et al. 2009, Hajek et al. 2013).

The fusing of different fungal hyphal strains creates vegetative compatibility groups (VCG), which are important in evaluating and determining introduction pathways of non-native fungi and the insects with which they have an intimate relationship (Leslie 1993). Some possible

S. noctilio invasion routes have been proposed based on VCGs of some non-native populations of *A. areolatum*. These include introduction into the Northern Hemisphere from several native populations in Europe and from introduced populations in the Southern Hemisphere (Nielsen et al. 2009, Bergeron et al. 2011, Boissin et al. 2012). The most likely scenario is that woodwasps have been introduced several times across the world from multiple places, and that no single introduction is solely responsible for their establishment in any locale (Slippers et al. 2002).

Monitoring

Much of the recent research on woodwasps in North America has focused on detection, monitoring, and preventing the establishment of *S. noctilio* in the southeastern United States. Barnes et al. (2014) found no significant differences among native *S. nigricornis* capture rates for either funnel or panel traps in the southeastern United States. Similar studies have also found no significant differences among these two trap types and their ability to collect siricids in other parts of North America and in South Africa (Haavik et al. 2014, Hurley et al. 2015). Trap height has been shown to be important with respect to sex ratio captured, due to males and unmated females flying higher than mated females, so trap placement may be altered to reflect this behavior (Martinez et al. 2014).

While trap type does not significantly affect the number or species of woodwasps collected in the field, bait material has been shown to drastically affect numbers of native woodwasps collected. *Sirex* lures that are simply combinations of host volatiles (e.g. 70/30 α -/ β -pinene in combination with ethanol) are adequate in some situations (Coyle et al. 2012; Hartshorn et al. 2015) but actual host material is significantly more effective (Barnes et al. 2014; Chase et al. 2014) and volatiles may not work at all in large plantations due to the massive volatile release within the stand and the inability of adult *Sirex* to differentiate between a trap and

a tree (Bashford and Madden, 2012). *Sirex noctilio* pheromone combinations have been largely unsuccessful at capturing *S. noctilio* in the field even after showing promise in wind tunnel experiments (Hurley et al. 2015). These studies indicate that, while pheromones are being produced by woodwasps, their use for trapping and monitoring may be very limited. Visual cues also play an important role in attraction of both males and females to traps. While females are attracted to black panel traps without the addition of lures, males seem to avoid dark objects even when being attracted to them with semiochemicals (Sarvary et al. 2015). Elucidation of these confounding effects will enhance our ability to detect and monitor siricid presence and abundance in the field.

Abundance of *S. nigricornis* is positively correlated with conditions typical of stressed stands (e.g. smaller tree diameter at breast height, higher basal area) (Chase et al. 2014) which is consistent with the high degree of spatial aggregation found in populations of *S. noctilio* in Argentina (Corley et al. 2007). Tree resistance plays a significant role in keeping *Sirex* populations below damaging levels (Haavik et al., 2015a). Thinning to increase tree health and vigor by removing stressed and susceptible trees significantly reduces tree mortality caused by *S. noctilio* in North America, Europe, and South America (Neumann et al. 1987, Aparacio et al. 2013, Ayers et al. 2014). Dodds et al (2007) provide specific recommendations for modifying stand basal area to increase tree health in existing stands and, in New York, these silvicultural treatments were found to reduce the number of trees attacked by *S. noctilio* by 75% several years later (Dodds et al. 2014). In North America, insect and tree diversity, while a potential hindrance to monitoring efforts, will most likely be beneficial in slowing the spread and damage caused by *S. noctilio* (Dodds and de Groot 2012). Field and laboratory studies have been inconclusive in

determining flight ability of females and males and how environmental factors and parasitism affect flight capacity (Bruzzone et al. 2009, Corley and Villacide 2012, Haavik et al. 2016). Degree-day models have been proposed for both *S. noctilio* and *S. nigricornis* in the eastern United States (Haavik et al., 2013; Myers et al., 2014). However, when tested against native *Sirex* populations in Arkansas, no model was able to accurately predict emergence of females, regardless of model parameters. When *S. noctilio* and *S. nigricornis* emergence datasets ranging from southern Ontario to Louisiana were combined and examined, a clear inverse relationship between increasing emergence and decreasing temperature was found. The trend for female woodwasps to emerge as temperatures cool was consistent across species and across a wide landscape. This indicates that foresters and land managers wanting to monitor the spread of *S. noctilio* should do so based on local temperature patterns rather than the accumulation of degree days (Hartshorn et al., in press).

Biological Control

The most commonly used, and most successful, biological control agent of *S. noctilio* is the parasitic nematode *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae). *Deladenus* was erected by Thorne (1941) to differentiate it from other neotylenchid nematodes due to its lack of a valvular median oesophageal bulb. A bicyclic life cycle of seven species within the genus was later described by Bedding (1967). Within these seven species, females may either remain in their associated host-plant as free-living and mycetophagous, or they may detect a suitable insect larva living in the same host plant and become parasitic. Blinova and Korenchenko (1986) then used this biological characteristic to erect a new genus, *Beddingia*, within Phaenopsitylenchidae. *Deladenus* was moved to Allantonematidae by Fortuner and Raski (1987) and *Beddingia* was classified as a junior synonym of *Deladenus* later by Chitambar

(1991). *Deladenus* *apopkaetus* was erected in that same review of *Deladenus* (Chitambar, 1991), and since then, seven additional species have been described: *D. leptosoma* (Gagarin, 2001), *D. minimus* (Chizhov and Sturhan, 1998), *D. pakistanensis* (Shahina and Maqbool, 1992), *D. cocophilus* (Nasira et al., 2013), *D. valveus* (Yu et al., 2014), *D. albizicus*, and *D. processus* (Tomar 2015). Twenty-five species of *Deladenus* are currently recognized and are represented both by species parasitic on insects such as Buprestidae (Blinova and Korenchenko, 1986), Ichneumonidae (Hocking, 1967), and Siricidae (Bedding, 1972) and monocyclic plant-inhabiting mycetophagous species (Nasira et al., 2013).

The bicyclic life history of *Deladenus* involves both free-living and parasitic stages, which are morphologically unique. While free-living in pine hosts, nematodes feed on *Amylostereum*, which can continue in this stage indefinitely. When female nematodes detect a woodwasp larva (e.g. changes in wood pH, increased CO₂) they become sexually mature and mate, after which time they morph into a parasitic form and, with specialized stylets, penetrate the cuticle of the immature woodwasp. Once inside a woodwasp host, female nematodes produce eggs which soon hatch and the resulting juveniles, during host pupation, migrate towards woodwasp genitalia. Woodwasp eggs, potentially filled with juvenile nematodes, are then deposited into new pine trees during oviposition and nematodes again become free-living (Bedding 1968, 1972).

The effectiveness of *D. siricidicola* at controlling populations of *S. noctilio*, while often successful, is widely variable in certain regions of the Southern Hemisphere. For example, *S. noctilio* in Australia has experienced significant (up to 99%) parasitism by *D. siricidicola* (Bedding 1979, Carnegie et al. 2005) while inoculation success in South Africa has remained at, or below, 25%, regardless of nematode origin and inoculation effort (Bedding and Akhurst 1974,

Haugen 1990, Hurley et al. 2007). One hypothesis is that low pine wood moisture content inhibits nematode parasitism, however, success remained low even in wood with higher moisture (Hurley et al. 2008). The underlying factors of this variation are still being examined and there is no clear reason why these nematodes would be successful in one region and not another. In eastern North America, a native congener, *D. proximus*, infects the haemocoel, eggs, mycangia, and testes of woodwasps (Yu et al. 2011, Kroll et al. 2013). Much like previously described woodwasp-fungal associations (Hajek et al., 2013), recent research has revealed that relationships among these organisms are not entirely specialized. *Deladenus siricidicola* is associated with *S. noctilio* and *D. proximus* with *S. nigricornis* (Bedding and Akhurst 1978), however, both nematode species have been found parasitizing both woodwasp species in New York and Canada (Morris et al. 2013, Haavik et al. 2015b). Additionally, both nematode species are able to grow on multiple species of *Amylostereum* (Morris et al. 2014). Nematode infection results in smaller females and partial or complete sterilization of eggs (Kroll et al. 2013, Zieman et al. 2015, Haavik et al. 2016) and the ability of native nematodes to utilize *S. noctilio* as hosts implicates them as potential biological control agents of *S. noctilio* in eastern North America. Inoculation studies to evaluate the effects of native nematodes on *S. noctilio* are necessary to accurately assess their use in biological control.

North American *D. proximus* vary considerably in regional abundance and in the proportion of woodwasp eggs they parasitize (virulence) (Keeler 2012, Zieman 2013). This may be related to a number of biotic and abiotic factors such as nematode growth on different species of *Amylostereum*, climatic differences, genetic diversity of nematodes and woodwasps, and tree species. Increasing temperatures negatively affect woodwasp size and fecundity, potentially hindering nematode sterilization (Yousuf et al. 2014). Tree defenses have significant negative

effects on *Sirex* survival (Haavik et al. 2015a) and these defenses among different species may dictate nematode virulence. Genetic diversity of a 620-750 bp region of COI in *D. proximus* COI gene is low enough (<0.5% genetic distance) to suggest that a single breeding population is present across all of eastern North America (Chapter 3). Mlonyeni et al. (2011) suggest that extreme homozygosity of introduced *D. siricidicola* may result in lower virulence over time and the area of population genetics should be further explored for both woodwasps and nematodes. *Deladenus proximus* do negatively affect size and fecundity of *S. nigricornis* in the southeastern United States (Chapter 4) but predictions of underlying mechanisms behind variability in virulence cannot yet be made for these species.

Nematodes are not the only mortality-causing agents of woodwasps. Development of *S. noctilio* and *S. nigricornis* in North America overlaps with several other pine-inhabiting insect species, particularly longhorned beetles (Cerambycidae), and weevils and ambrosia beetles (Curculionidae), which significantly affect survival of *S. noctilio* in Ontario, and most likely also affect *S. nigricornis* (Ryan et al. 2012a). Several hymenopteran parasitoids (e.g. *Ibalia leucospoides* (Hochenwarth) (Ibaliidae), *Rhyssa persuasoria* L. and *R. lineolata* (Kirby) (Ichneumonidae)) are associated with native siricids in the southeastern United States (Kirk 1974) and are routinely collected from *Sirex*-infested material across eastern North America. However, their specific effects on *S. noctilio* mortality are debated (Ryan et al. 2012b, Zylstra and Mastro 2012, Haavik et al. 2015). Additionally, not all parasitoids are species specific so it is not known which wood-inhabiting insects they are parasitizing or the amount of mortality attributed to a particular species of parasitoid (Long et al. 2009). Molecular techniques have been developed to identify *Sirex* tissue within gut contents of hymenopteran parasitoids which may be beneficial to future studies of effectiveness of these natural control agents (Foelker et al.,

2015). After numerous release attempts in New Zealand, *I. leucospoides* finally became well established and successfully contributed to the control of *S. noctilio* (Rawlings 1951, Rawlings, 1953, Taylor 1976). Other North American hymenopteran parasitoids which, with varying levels of success, were introduced into Australasia for control of *S. noctilio* (*Megarhyssa nortoni* (Cresson), *R. persuasoria*, *R. lineolata*, (Ichneumonidae) and *Schlettererius cinctipes* (Cresson) (Stephanidae)) (Taylor 1976). Studies evaluating their impacts on native siricids may provide support for their use in future control efforts of *S. noctilio*.

Conclusions

Since its introduction into the northeastern United States in the early 2000s, *S. noctilio* primarily selects suppressed trees in which to oviposit and develop, and has not had a major impact on commercial pine stands (Dodds et al. 2010). Dinkins (2011) found that the European species, Scots pine (*P. sylvestris* L.), which is commonly grown in the northeastern United States but is not present further south, was the preferred host for *S. noctilio* females when presented with several pines grown in North America, although *P. resinosa* is reported as a preferred host in the field (Dodds et al. 2007). However, female woodwasps will oviposit, and their progeny can complete development, in eastern white pine (*P. strobus* L.) and Virginia pine (*P. virginiana* Mill.), two economically important species in the eastern United States. Other southeastern pines, namely shortleaf (*P. echinata* Mill.) and loblolly (*P. taeda* L.), were not attractive for *S. noctilio* oviposition, even in no-choice experiments. These results suggest a species specific approach to management and that the impact of *S. noctilio* on the southeastern United States will likely vary by forest stand composition. *Sirex noctilio* continues to be introduced, spread and become established throughout areas of introduction, regardless of prevention or control systems in place (Slippers et al. 2015). Therefore, additional research is needed to evaluate the

interactions among *S. noctilio* and other pine-associates so predictions can be made regarding its economic and ecological impact in the future.

Forestry and pine timber production in the southeastern United States are multi-billion dollar industries (Borchert et al. 2007). Additional research into the ecology of native *S. nigricornis* and its rich complex of natural enemies may help minimize damage by *S. noctilio* as it spreads across the United States. Proper silvicultural practices will remain important in the prevention, and treatment, of insect pests, including *S. noctilio* (Hurley et al. 2007, Dodds and de Groot 2012) while other options, like biological control, are evaluated.

Objectives

The main objective of my dissertation is to examine the effects of temperature and nematode infestation on woodwasp ecology in regions of the southeastern United States. Efficient monitoring of *S. noctilio* is essential as it continues to spread south and west throughout North America. Additionally, the use of a native parasitic nematode to control *S. noctilio* populations would alleviate the concerns associated with classical biological control using generalists and provide many potential sources of biological control agents. With this dissertation I intend to answer the following questions: 1) how does temperature affect female emergence of both *S. nigricornis* and *S. noctilio* in eastern North America, 2) what is the identity and genetic diversity of the parasitic nematode found in body tissues of adult female *S. nigricornis* in the southeastern United States, and 3) are these nematodes negatively affecting the fitness of native *S. nigricornis* in the southeastern United States? This information is essential to monitoring the spread and establishment of *S. noctilio* in eastern North America and evaluating the potential for the native nematode, *D. proximus*, to be used in biological control of *S. noctilio*.

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Figures

Figure 1. Adult *Sirex noctilio* (a) and *Sirex nigricornis* (b) [Photos from Schiff et al. (2012)].



Figure 2. Diagram depicting various types of *Sirex* tunnels for host suitability testing and oviposition [adapted from Coutts and Dolezal (1969)].

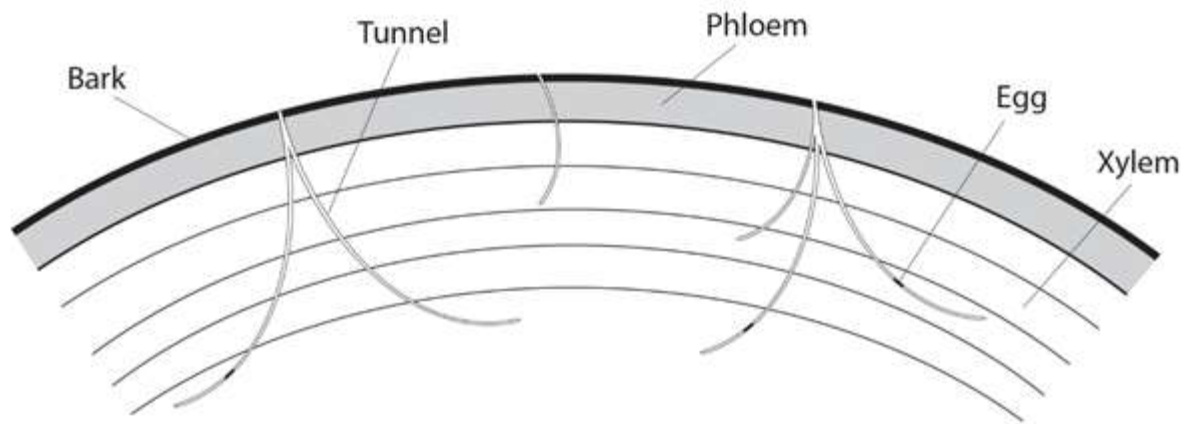
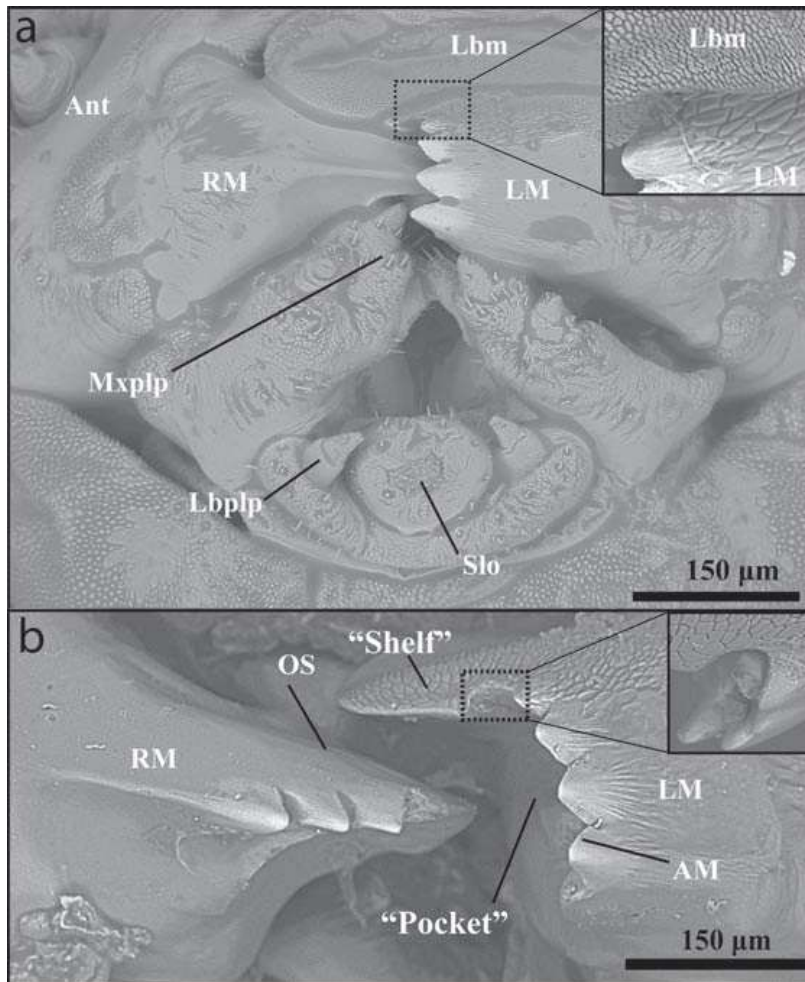


Figure 3. Scanning electron micrograph of the feeding appendages of larval *Sirex* with mandibles occluded (a) and open (b). Right and left mandible (RM and LM, respectively) are symmetrically aligned along the mesal margin. The mandibles are bordered by the maxillary (Mxplp) and labial (Lbplp) palps and the salivary orifice (Slo) from below and the highly textured labium (Lbm; inset a). The top of the left mandible extends as a “shelf” above the OS of the right mandible. The “pocket” created by the “shelf” of the left mandible holds wood cut by the AM and the “carpenter’s plane” (inset b), where it is pressed, releasing fluid fractions [taken from (Thompson et al. 2014) with permission from the Entomological Society of America].



Chapter 2 – Emergence of Adult Female *Sirex nigricornis* F. and *S. noctilio* F.

(Hymenoptera: Siricidae) Coincides With a Decrease in Daily Minimum and Maximum Temperature

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Abstract

The establishment and spread of *Sirex noctilio* F. (Hymenoptera: Siricidae), in northeastern North America necessitates reliable monitoring methods for this alien woodwasp pest of *Pinus*. The native congener, *S. nigricornis* F., is common across the eastern United States and has been studied as a proxy for *S. noctilio*. Predicting emergence of *S. noctilio* is important to guide monitoring efforts. Degree day models have been developed to predict flight phenology of *S. noctilio* and *S. nigricornis* across eastern North America. These model parameters were tested against three years of *S. nigricornis* collections from two locations in Arkansas. Neither of the proposed models accurately predicted emergence. Emergence of females for both *S. noctilio* and *S. nigricornis* was significantly negatively correlated with minimum and maximum temperature, indicating that emergence increases when minimum and maximum temperatures decrease. These results suggest that as *S. noctilio* spreads south, where higher mean temperatures are present, it will emerge later in the season as temperatures decline compared to the current northeastern population. We propose that monitoring for the spread of *S. noctilio* in the southeastern United States should begin slightly before daily temperatures are expected to decline (i.e. mid-September).

Keywords: *Sirex noctilio*, *Sirex nigricornis*, monitoring, phenology, Siricidae, invasive species, autumnal emergence

Introduction

There are 14 extant species in the genus *Sirex* L. in the New World that colonize various conifers. These include *S. noctilio* F., a European species recently discovered in northeastern North America (de Groot et al., 2006; Hoebeke et al., 2005), and a common native congener *S. nigricornis* F., both of which develop in *Pinus* L. (Schiff et al., 2012). In their respective native ranges, these woodwasps rarely, if ever, cause economic injury to their associated hosts (Hall, 1968), however, since the mid-twentieth century *S. noctilio* has been recorded as a significant pest in exotic commercial *Pinus* stands across much of the Southern Hemisphere, where it has been repeatedly introduced (Slippers et al., 2015). The introduction and spread of *S. noctilio* throughout the northeastern United States and southeastern Canada has necessitated new monitoring and detection technologies. While *S. noctilio* often inhabits *P. resinosa* and *P. banksiana* in the northeastern U.S. (Zylstra and Mastro, 2012), host preference testing has shown that *S. noctilio* is capable of ovipositing, and completing development, in economically important North American *Pinus* (e.g., *P. virginiana* Mill.) (Borchert et al., 2006; Dinkins, 2011). Most recent monitoring studies have focused on efficacy of traps and lures with less attention paid to the timing of adult emergence (Barnes et al., 2014; Cooperband et al., 2012; Haavik et al., 2014; Hurley et al., 2015).

Both species overwinter as larvae in the xylem of *Pinus* and require at least one year to complete development. Soon after emergence, females are attracted to host volatiles (e.g., ethanol and terpenes) released by damaged or dying *Pinus* and, after identifying suitable host material, insert their ovipositor into the xylem, depositing eggs, phytotoxic venom, symbiotic fungi (*Amylostereum* Boidin), and potentially parasitic nematodes (Coutts and Dolezal, 1969; Hartshorn, 2012; Lynn-Miller, 2012; Spradbery, 1977). The timing of emergence differs greatly

among geographic regions. For example, in Canada and the northeastern United States, both woodwasp species emerge in the late summer and fall with *S. noctilio* flying from July to early September, earlier than *S. nigricornis* emergence which occurs in late August to early October (Haavik et al., 2013; Ryan et al., 2012a). In the southeastern United States, *S. nigricornis* emerge from early-October through December (Haavik et al., 2013; Hartshorn et al., 2015; Johnson et al., 2013; Keeler, 2012; Lynn-Miller, 2012). In Patagonia, adult *S. noctilio* activity occurs during the hottest month of the year whereas peak flight in its native range of Europe occurs during fall and winter months (Corley and Villacide, 2012; Spradbery and Kirk, 1978). Thus, an accurate method to predict *S. noctilio* emergence across a variable landscape has yet to be defined.

Several model types have been developed to describe the emergence and phenology of insects, typically focusing on pest species, mainly in crop systems where insects are sensitive to ambient air temperatures (Briere et al., 1999; Lactin et al., 1995; Logan, 1988). Winter temperatures underneath the bark of ash trees can be more than 4°C warmer than air temperatures and differences between ambient and phloem temperature can differ between north and south-facing sides of the tree (Vermut et al., 2012). A phenology model for the phloem-feeding mountain pine beetle (*Dendroctonus ponderosae* Hopkins) based on phloem temperature was developed and it was noted as exceedingly difficult to model phenology of wood-borers using ambient temperatures (Bentz et al., 1991). Significant variation existed among emergence curves of *Monochamus alternatus* Hope populations in Korea and, even with 12 years of collection data, three separate Weibull models were needed to accurately predict population phenology (Park et al., 2014). The overall phenology of *M. saltuarius* Gebler was accurately predicted using temperature-based models, however, the start of emergence differed by more

than a week between some models (Jung et al., 2015). Degree-day accumulation prior to bark beetle flight in the southwestern United States differed significantly among collections within a species and maximum temperature was a much better predictor of beetle activity (Gaylord et al., 2008). Therefore, while accumulation of thermal heat units is important in the development of many insects (Ives, 1973; Tauber and Tauber, 1976; Wang et al., 2000), wood-borers are less sensitive to ambient open-air temperatures due to their cryptic larval feeding habits and buffering properties of the forest canopy and bark. Consequently, development rate models may predict overall phenological patterns but are often not accurate at predicting the start of woodborer emergence, or at identifying specific environmental cues which trigger continued emergence, and these factors can be important in monitoring the spread of non-native species, such as *S. noctilio*.

Cumulative degree-day (CDD) models that have been used to predict emergence of *S. nigricornis* and *S. noctilio* in eastern North America have used different data collection methods and different developmental thresholds and model parameters (Haavik et al., 2013; Myers et al., 2014). The start of *S. nigricornis* emergence was more difficult to predict using CDD models because emergence occurred over a shorter period of time compared to *S. noctilio* (Haavik et al., 2013). Additionally, other environmental factors (e.g., time of day) play important roles in the activity of females and may impact emergence and dispersal, resulting in varying emergence patterns among species (Madden, 1974; Morgan and Stewart, 1966). Myers et al. (2014) predicted *S. noctilio* emergence in the Southeast to occur as early as April and May, which often contain days with temperatures above the upper developmental threshold of the wasp. In one study, adult activity of *S. noctilio* began when temperatures reached 14°C and was unimpaired at 30°C, with the main emergence period occurring when mean air temperature ranged 14-21°C.

An increase in emergence was stimulated by a change from low to high atmospheric pressure. Adults were most active under stable high pressure systems and more emergence took place on bright, sunny days with moderate to low relative humidity (i.e., characteristic of conditions in the South following passage of a cold front in the fall) (Morgan, 1968). We predict that tracking local minimum and maximum temperatures may predict emergence of *S. noctilio* as well, or better than, CDD models, as it moves further south, and that this will improve decision-making about monitoring and control operations.

Our objectives were to 1) examine the validity of previously published degree-day models for predicting emergence of *S. nigricornis* in Arkansas and 2) investigate the relationships between declining daily minimum and maximum temperatures and emergence of female *S. noctilio* and *S. nigricornis* in Arkansas, Louisiana, Mississippi, and Ontario.

Materials and Methods

***Sirex* collections**

In Arkansas, eight sites, each containing two black APTIV™ intercept panel traps baited with a *Sirex* lure (75/25- α/β -pinene; release rate: 1.33 g/d) and 95% ultra-high release (UHR) ethanol (release rate: 0.3-0.8 g/d) (Synergy Semiochemicals, Burnaby, Canada), were established in the Ozark (four sites, 35.62°N, -93.55°W) and Ouachita National Forests (four sites, 34.90°N, -93.12°W). Traps were suspended with collection cups approximately 2.5 m above the ground. Collection cups contained propylene glycol which was replaced weekly. Adult female *S. nigricornis* were removed from each sample and stored in vials containing 95% ethanol. Traps were erected in late September of 2012, 2013, 2014, and 2015 based on observations from previous studies examining *S. nigricornis* emergence in Arkansas (Hartshorn et al., 2015; Keeler,

2012; Lynn-Miller, 2012) and collections continued until two consecutive collections contained no *S. nigricornis*, typically late December. Only female wasps were collected in baited traps. All Arkansas collections, regardless of sample size, were used in calculations of degree-day models. Years within regions were evaluated as independent collections and only those which exceeded n=30 wasps were included in analyses of temperature and emergence [2012: OU (n=141), OZ (n=184); 2013: OU (n=56), OZ (n=41); 2014: OU (n=53), OZ (n=10); 2015: OU (n=83), OZ (n=40)].

In central Louisiana during November of 2009, live uninfested *P. taeda* were felled and cut into 1.2-m length logs (down to an 8 cm top dia) at two sites on the Kisatchie National Forest, (Catahoula Tract: 31.59°N, -92.42°W; and Johnson Tract: 31.16°N, -92.67°W). These logs were cross-stacked in a “log cabin” style, and left adjacent to residual crown material to attract female woodwasps for oviposition. In September of the following year (2010), logs were transferred into rearing tents held in an open-sided pole-barn at the Catahoula Work Center (31.50°N, -92.46°W). Rearing tents were checked every 5-7 days until emergence was detected at which point collection intervals decreased to 2-3 days. Both sexes of *S. nigricornis* were reared from logs but only female emergence data were used for this study. In late September 2013, 2014, and 2015, 12 panel traps baited with freshly cut *Pinus* bolts and boughs of fresh foliage, which was replaced every 10-14 days, were erected around the Hunt Plywood Mill (31.59°N, -92.42°W). Wasps were collected from trap collection jars every 2-5 days. Only females were collected in panel traps (2010 Catahoula: n=299, 2010 Johnson: n=113; 2013: n=229; 2014: n=80; 2015: n= 125).

In northern Mississippi (33.89°N, -88.98°W) in September 2011, sixteen sites were established across four *P. taeda* forest types (unthinned plantation, thinned plantation, mixed

hardwood-softwood, old growth) (Chase et al., 2014). Each site contained three Lindgren® 12-funnel traps baited with small diameter (≤ 7.62 cm) *Pinus* slash which was partially stripped of bark and placed in propylene mesh bags. Traps (48 total) were hung at a height of ~1.7 m and each trap terminated in a collection cup filled with a 50/50 mix of antifreeze and water. The antifreeze mixture containing adult *S. nigricornis* was replaced weekly and wasps were stored in Whirl-Pak® bags in a -20°C freezer (n=594)

In southern Ontario, *P. sylvestris* L., *P. resinosa* Aiton, and *P. banksiana* Lamb. trees showing symptoms of *S. noctilio* infestation were identified and felled from May to early July in 2007, 2008, and 2011-2014 (Ryan et al. 2012, Haavik et al. 2013). Trees were cut into 1-m long sections and held at the Ontario Tree Seed Facility in Angus, Ontario (44.32°N, -79.87°W). Logs were cross-stacked in a “log-cabin” style inside a screen tent or placed in individual cardboard cylinders in a covered shed. Logs were checked every 5-7 days until emergence began, at which point they were checked every 2-3 days. The total number of both sexes of *S. noctilio* and *S. nigricornis* (which were collected incidentally) was counted, but only female emergence was included in this study. For each species, years were evaluated as independent collections and only those which resulted in more than n=30 wasps were included in analyses [*S. noctilio*: 2007 (n=539), 2008 (n=970), 2011 (n=256), 2012 (n=250), 2013 (n=344); *S. nigricornis*: 2012 (n=101), 2014 (n=262)].

Weather data

Daily maximum and minimum temperatures (°C) for Arkansas and Mississippi were obtained from the National Oceanic and Atmospheric Administration (NOAA, www.ncdc.noaa.gov/cdo-web/; accessed 31 January 2016). The majority of weather data for

Ozark sites were obtained from the Clarksville 2 weather station (ID: USC00031459). The Clarksville 6 station (ID: USC00031457) was used to supplement missing data. Weather data for Ouachita sites were obtained from the Jessieville weather station (ID: USC0000AJES). These data were also used to create CDD models for Arkansas collections. Weather data for Mississippi sites were collected from the Tupelo Regional Airport station (ID: USW00093862). Weather data for Louisiana collections were obtained from the Catahoula Remote Automatic Weather Station (RAWS, raws.wrh.noaa.gov; accessed 31 January 2016) (ID: BENL1). Missing Louisiana weather data were supplemented with the Alexandria International Airport station (ID: USW00093915), obtained from NOAA. Temperature for Ontario collections were obtained from Environment Canada, National Climate Data and Information Archive (www.climate.weatheroffice.gc.ca; accessed 4 December 2015) (Barrie-ORO station ID: 6117700).

Data analysis

Haavik et al. (2013) previously used Ontario and Louisiana emergence data to create CDD models thus only Arkansas-collected female *S. nigricornis* were used in the current evaluation of CDD models. We used the DEGDAY program to calculate CDD (Zalom et al., 1983) for each year of collection data from the Ozarks and Ouachitas (2012-2014) and chose the sine wave method for calculation of CDD (Allen, 1976). Several lower thresholds (LT) (i.e. 0°C, 5°C, 6.8°C, 10°C) and upper thresholds (UT) (i.e. 20°C, 25°C, 30°C, 35°C) along with several start dates (i.e. Oct 1 of previous year, Jan 1, Mar 1, May 1, July 1) were used to create CDD models and each provided similar results in terms of accuracy. Therefore, we provide analyses for a LT of 0°C and an UT of 25°C and a LT of 6.8°C and an UT of 35°C, both models

with a start date of May 1, based on previous published models (Haavik et al., 2013; Myers et al., 2014). We fit a Gompertz 3-parameter model to each regional CDD dataset, grouped by year, and tested for significant differences among years within each model using an F -test test for parallelism. An F -test for parallelism tests the null hypothesis that emergence rates are all within a single confidence interval. A significant F -test indicates that rates of woodwasp emergence among years are significantly different and, in this case, emergence data from one year of collections cannot be used to accurately predict emergence in other years. Conversely, a non-significant F -test would indicate that rates of emergence were similar enough to use in predictions. We created and compared CDD models using JMP Pro 11 (SAS Institute Inc., 2007).

We also examined the relationship among daily minimum and maximum temperatures during woodwasp emergence and female *Sirex* collections in Arkansas, Mississippi, Louisiana, and Ontario. Neither weather nor emergence data fit a normal distribution or had equal variances, so a Spearman's ranked correlation coefficient (ρ) was performed. We calculated ρ for the correlation between female woodwasp emergence and 1) daily maximum temperature, 2) daily minimum temperature, and 3) the difference between daily minimum and maximum temperature. Correlations were calculated separately for each species within its given region (i.e. Southeastern *S. nigricornis*, Ontario *S. nigricornis*, and Ontario *S. noctilio*) and for all collection data ($n=4760$ wasps). Data violated the assumptions for a linear model so we performed a generalized additive model (GAM) on the combined dataset using a Gaussian distribution in the R package 'mgcv' (Wood, 2011). We performed correlations and created the GAM using R 3.2.3 (R Core Team, 2015). This GAM was used to calculate the fitted line shown in Fig 2. All statistical tests were evaluated with a significance level of $\alpha = 0.05$.

Results

Arkansas CDD models

Both model types produced significantly different CDD curves in both the Ozarks and Ouachitas (Fig 1). In the Ozarks, an F -test of parallelism showed significant differences among years for the 0°C LT-25°C UT models (F -ratio = 142.53; d.f. = 2; $p < 0.0001$) and 6.8°C LT-35°C UT models (F -ratio = 163.39; d.f. = 2; $p < 0.0001$). In the Ouachitas, 0°C LT-25°C UT models (F -ratio = 13.82; d.f. = 2; $p < 0.0001$) and 6.8°C LT-35°C UT models (F -ratio = 14.05; d.f. = 2; $p < 0.0001$) were also significantly different among years. These significant differences indicate that one year of collection data could not accurately predict other years of emergence data.

Temperature data

In the southeastern United States, daily minimum ($\rho = -0.38$, $S = 125560000$, $p < 0.0001$), daily maximum ($\rho = -0.29$, $S = 117380000$, $p < 0.0001$), and the difference between minimum and maximum temperatures ($\rho = -0.08$, $S = 98189000$, $p = 0.0217$) were significantly negatively correlated to female *S. nigricornis* cumulative emergence. All variables were significant in the GAM for southeastern *S. nigricornis* as well (min: $F = 13.714$, d.f. = 4.95, $p < 0.0001$, max: $F = 15.441$, d.f. = 6.29, $p < 0.0001$, difference: $F = 3.049$, d.f. = 4.63, $p = 0.0036$). In Ontario, daily minimum ($\rho = -0.32$, $S = 6901700$, $p < 0.0001$) and daily maximum ($\rho = -0.49$, $S = 41004000$, $p < 0.0001$) were significantly negatively correlated with female *S. noctilio* cumulative emergence. The difference between minimum and maximum temperature was not significantly correlated with *S. noctilio* emergence in Ontario. Only minimum ($F = 66.09$, d.f. = 0.63, $p < 0.0001$) and maximum ($F = 45.297$, d.f. = 0.68, $p < 0.0001$) were significant in the

GAM for *S. noctilio*. Daily minimum ($\rho = -0.57$, $S = 65618$, $p < 0.0001$) and daily maximum ($\rho = -0.67$, $S = 69708$, $p < 0.0001$) were both significantly negatively correlated with *S. nigricornis* cumulative emergence in Ontario. The difference between maximum and minimum temperatures was nearly significant ($\rho = -0.24$, $S = 51774$, $p = 0.055$) for Ontario *S. nigricornis*. Again, only minimum ($F = 10.590$, d.f. = 1.92, $p = 0.0005$) and maximum ($F = 24.470$, d.f. = 0.57, $p = 0.0004$) were significant in the GAM for northeastern *S. nigricornis*. Each species in both regions responded similarly to decreasing temperatures (Fig 2) and were combined for additional analyses.

For the dataset containing emergence data for both *S. nigricornis* and *S. noctilio* across all regions, cumulative emergence was significantly negatively correlated with daily minimum temperature ($\rho = -0.37$, $S = 391220000$, $p < 0.0001$), daily maximum ($\rho = -0.16$, $S = 330860000$, $p < 0.0001$), and the difference between daily minimum and maximum temperature ($\rho = -0.07$, $S = 306260000$, $p = 0.0104$). Daily minimum ($F = 28.48$, d.f. = 4.149, $p < 0.0001$) and daily maximum ($F = 11.67$, d.f. = 5.74, $p < 0.0001$) were significant in the GAM ($R^2 = 0.23$) while the difference between daily minimum and maximum temperature ($F = 1.85$, d.f. = 7.39, $p = 0.0552$) was nearly significant. Daily minimum temperature being the strongest predictor in the GAM.

Discussion

The significant variation in *S. nigricornis* phenology predictions based on CDD models in Arkansas illustrates the need for a reliable and simple method to better forecast future *Sirex* emergence. While previous CDD models were created for Louisiana, predicted and observed emergence often differed by more than a week. Published models most accurately predicted the final 10% of emergence which is not necessarily beneficial for monitoring (Haavik et al., 2013).

Additionally, while Haavik et al (2013) did not use these models to predict future *S. noctilio* emergence in the southeastern United States, Myers et al. (2014) predicted significantly earlier emergence for *S. noctilio* (i.e. mid-April).

A lack of consistency among CDD models may be due to varying methodology to record *Sirex* emergence (i.e., logs vs. field-based traps) and frequency of collections (i.e., daily vs. weekly). Additionally, open air temperatures recorded by weather stations, which may be multiple km away from actual sites, are most likely different than what is experienced by flying insects under the canopy and wood-borers still in the phloem and xylem due to shading by trees (Karlsson, 2000). However, these open air temperatures are easier to measure than specific under-canopy conditions. The most likely scenario for future monitoring of *S. noctilio* spread is the use of baited field-based traps, which may be checked weekly or less often. This situation requires a simple method to predict and monitor the spread of *S. noctilio*.

While degree-day accumulation is important for insect development, wood-borers respond to buffered conditions within the tree which may affect their response threshold. Female *S. nigricornis* and *S. noctilio* cumulative emergence were significantly negatively correlated with minimum temperature, maximum temperature, and the difference between minimum and maximum temperature, indicating that woodwasp emergence for both species occurred when temperatures began to decline and when daily temperature changes became less extreme. Emergence of both species was negatively correlated with temperature even though *S. noctilio* began emerging earlier (July) compared to *S. nigricornis* (August) in Ontario.

Interactions between the two species should also be considered when monitoring. Most recent trapping efforts have focused solely on *S. noctilio* owing to its non-native status. Coyle et al. (2012) reported field collections of *S. nigricornis* in Minnesota beginning in late July, while

Ryan et al. (2012a) reported emergence of *S. nigricornis* occurring mostly in the month of September in Ontario at a similar latitude. However, the latter study involved *S. nigricornis* which were reared from *S. noctilio*-infested trees that were felled and stored in sheds. Additionally, relatively few *S. nigricornis* were captured during their Ontario study and they did not examine the possibility of within-tree interactions delaying *S. nigricornis* emergence. Ryan et al. (2012b) provided evidence that other wood-boring insects may affect the size of male *S. noctilio* due to competition and they speculated that these interactions may extend to population dynamics, including phenological patterns. The current study also provides a baseline with which to examine future changes in native *Sirex* phenology after the establishment of *S. noctilio*.

Sirex noctilio phenology is also different depending upon the region in which it is being studied. In Patagonia, *S. noctilio* begin emerging at the beginning of summer, in late December, and continue emerging through the start of snowfall in May (Corley and Villacide, 2012). In Australia *S. noctilio* emerges from summer to early autumn (November – March), as it does in North America (July – October), however, peak emergence is inconsistent (Haugen, 1990; Nahrung et al., 2015; Neumann et al., 1982). In South Africa, *S. noctilio* has two separate emergence patterns where emergence coincides with the rainy season (Tribe and Cillié, 2004). However, these affected regions consisted of exotic *Pinus* plantations that, at the time of *S. noctilio* introduction, did not have the complex of pine-inhabiting organisms that is native to the Northern Hemisphere. North America more closely resembles the European landscape with respect to forest composition (Niemelä and Mattson, 1996), compared to countries in the Southern Hemisphere, and we speculate that *S. noctilio* populations will behave in a manner similar to what is reported in its native range. Predictions of later *S. noctilio* emergence are supported by previous phenology studies that found *S. noctilio* emerging later in southern areas

(e.g., Morocco) compared to northern areas (e.g., Belgium) (Spradbery and Kirk, 1978). We assert that the significant negative correlation between *S. noctilio* emergence and temperature indicates that female *S. noctilio* will emerge at the same time as, or slightly earlier than, *S. nigricornis* in the southeastern U.S., despite the significant increase in CDD accumulation, and in contrast to predictions of significantly earlier emergence in warmer climates (Myers et al., 2014). Both species in Ontario emerge over a narrower window of temperatures compared to southeastern *S. nigricornis*, however, significant overlap of emergence is evident (Fig 2). The warmer climate of the Southeast may result in a wider window of *S. noctilio* emergence. While *S. noctilio* may emerge over a longer period of time as it moves further south, emergence as early as mid-April in the Southeast could result in woodwasps flying when maximum temperatures are known to exceed 38°C, well above the upper threshold for *Sirex* development and survival, and when minimum temperatures often remain above 15°C for long periods. Warmer temperatures could result in faster spread of the non-native through the Southeast by increasing metabolic activity (Lantschner et al., 2014), but will most likely not change the emergence and flight phenology of *S. noctilio* to occur before the late summer and fall.

We hypothesize that neither *Sirex* species relies solely on degree-day accumulation for emergence, and that monitoring decreasing daily temperatures, along with decreasing variation in daily temperatures, is the best predictor of *Sirex* emergence as *S. noctilio* moves further south. Previous CDD model parameters did not accurately predict *Sirex* emergence in Arkansas. We found significant differences among years of collection data within CDD models, suggesting that one year cannot be used to predict future emergence patterns. Conversely, emergence of both species was significantly negatively correlated with minimum and maximum temperature. Monitoring efforts to track the spread of *S. noctilio* should focus on placing traps prior to these

drops in temperature, based on local historical temperature data and forecasts. We suggest using regional daily temperatures to time the deployment of traps for *S. noctilio* monitoring in the southeastern United States which will reduce the amount of sampling prior to initiation of woodwasp emergence and capture.

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Figures

Figure 1. Fitted degree day models for Ouachita National Forest *S. nigricornis* collections from 2012-2014 with a LT of 0°C and UT of 25°C (a) and a LT of 6.8°C and a UT of 35°C (b); degree day models for Ozark National Forest *S. nigricornis* collections from 2012-2014 with a LT of 0°C and UT of 25°C (c) and a LT of 6.8°C and a UT of 35°C (d). All years were significantly different within regions.

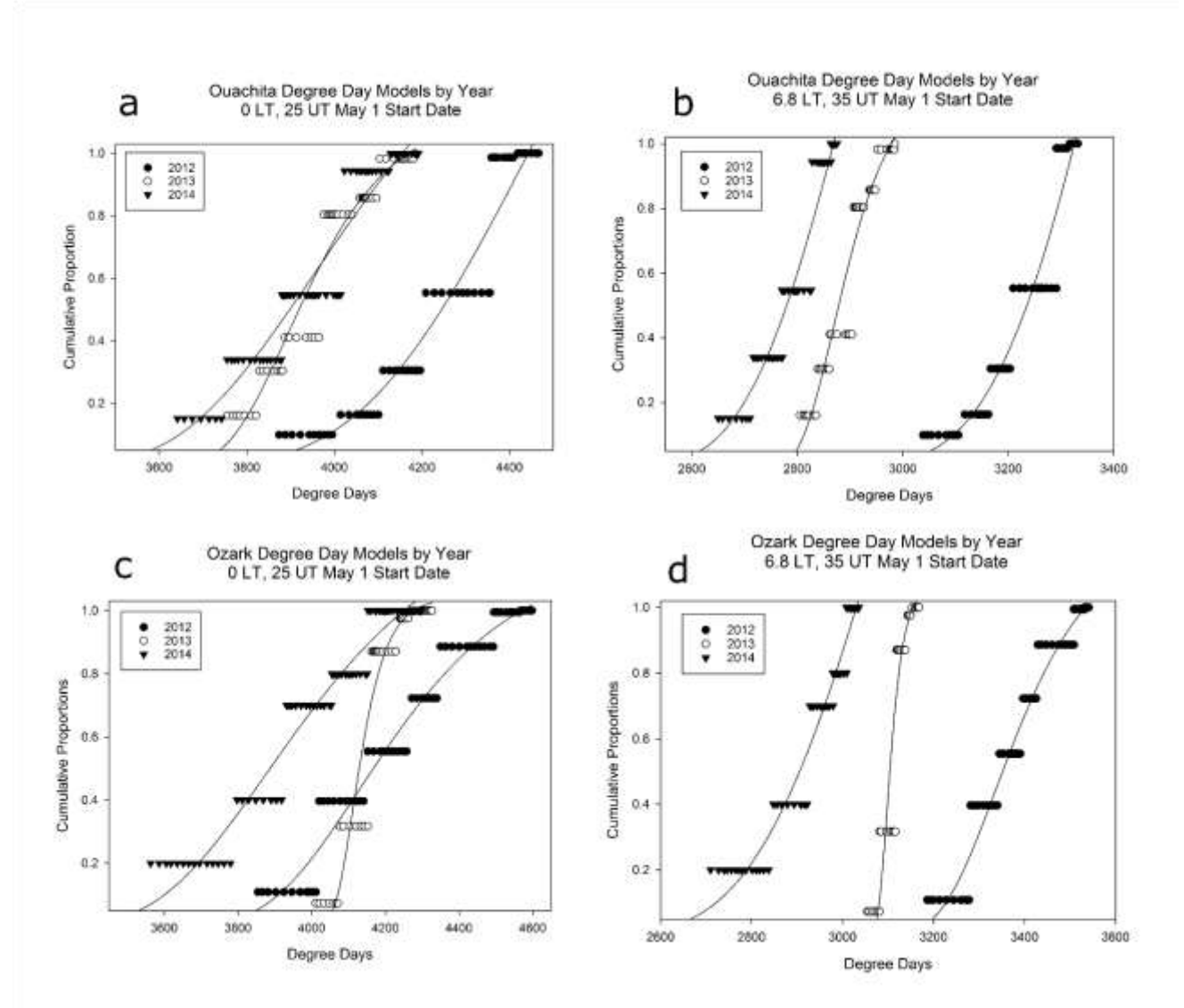
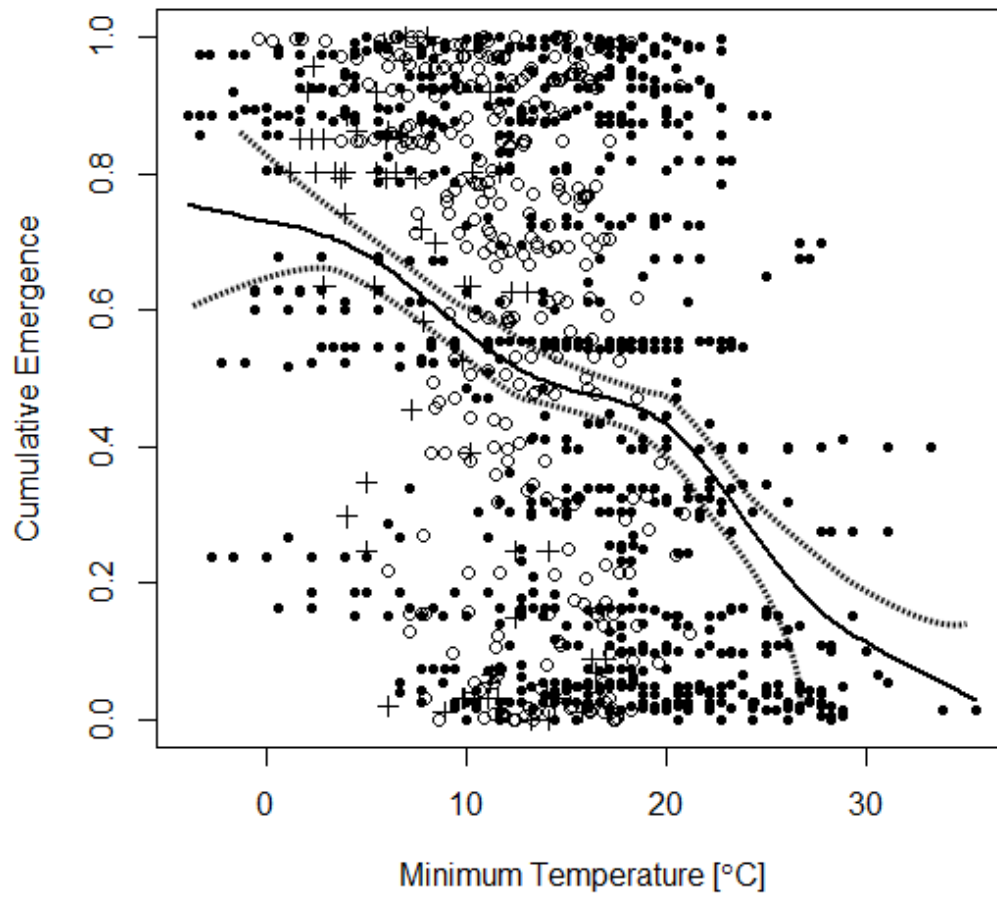


Figure 2. Cumulative emergence of *Sirex noctilio* (●), southeastern *S. nigricornis* (○), and northeastern *S. nigricornis* (+) vs. minimum temperature with minimum temperature GAM overlaid ($\rho = -0.37$, $S = 391220000$, $p < 0.0001$, $R^2 = 0.23$).



Chapter 3 – Molecular Identification of the Roundworm, *Deladenus proximus* Bedding, 1974 (Tylenchida: Neotylenchidae): a Parasite of *Sirex nigricornis* F., 1781 (Hymenoptera: Siricidae)

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Abstract

Parasitic nematodes were isolated from *Sirex nigricornis* F., 1781 (Hymenoptera: Siricidae) females collected in baited traps across pine forests of Arkansas and Mississippi, USA. We examined a 650-720 bp of cytochrome c oxidase subunit I and compared Arkansas and Mississippi sequences to sequences from nematodes collected in Illinois, Louisiana, and New York that were positively identified as *Deladenus proximus* Bedding, 1974 (Tylenchida: Neotylenchidae). We propose a single species, *D. proximus*, across Arkansas and Mississippi. Moreover, all sequences, which spanned a large geographic area (Louisiana to New York), exhibited only minor genetic variation (mean genetic distance of 0.004). This suggests that all examined specimens are a single species and suggests continuous gene flow across eastern North America.

Keywords: biological control, invasive species, forest entomology, *Sirex* woodwasps

Introduction

Deladenus Thorne, 1941 (Tylenchida: Neotylenchidae) are cosmopolitan roundworms that may remain free-living in a plant host, but can also act as parasites when a suitable host is encountered. There are 25 recognized species of *Deladenus* represented by both monocyclic plant-inhabiting mycetophagous species (Nasira et al., 2013) and species parasitic on insects such as Buprestidae (Blinova and Korenchenko, 1986), Ichneumonidae (Hocking, 1967), and Siricidae (Bedding, 1972). Recent attention has focused on *D. siricidicola* Bedding, 1968 due to its potential importance as a biological control agent for the invasive woodwasp, *Sirex noctilio* F., 1793 (Hymenoptera: Siricidae).

Thorne (1941) erected *Deladenus* to accommodate a new species, *D. obesus* Thorne, 1941, and a species previously named *Tylenchus durus* Cobb, 1922. These species were considered distinct from other neotylenchids in lacking a valvular median oesophageal bulb. Bedding (1967) then described the presence of a bicyclic life cycle in seven species, in which females feed on symbiotic fungi within the host plant, but can become parasitic after detecting a suitable insect larva living in the same host plant. This biological characteristic was then used by Blinova & Korenchenko (1986) to erect a new genus, *Beddingia*, within Phaenopsitylenchidae. *Deladenus* was moved to Allantonematidae by Fortuner & Raski (1987) and *Beddingia* was classified as a junior synonym of *Deladenus* by Chitambar (1991). Since the review of *Deladenus* (Chitambar, 1991), which considered 18 species, including one newly described (*D. apopkaetus* Chitambar, 1991), seven additional species have been described: *D. leptosome* Gargarin, 2001, *D. minimus* Chizhov & Sturhan, 1998, *D. pakistanensis* Shahina & Maqbool, 1992, *D. cocophilus* Nasira, 2013, *D. valveus* Yu, 2014, *D. albizicus*, and *D. processus* Tomar, 2015.

Thirteen *Deladenus* are able to parasitize insects. Of these, seven (*D. canii*, *D. imperialis*, *D. nevexii*, *D. proximus*, *D. rudyi*, *D. siricidicola*, and *D. wilsoni*) are known either from woodwasps (*Sirex* F., 1761, *Urocerus* Geoffroy, 1762, *Xeris* Costa, 1894), or their ichneumonid parasitoids (*Rhyssa* Gravenhorst, 1829, *Megarhyssa* Ashmead, 1858, and *Pseudorhyssa* Merrill, 1915) (Bedding and Akhurst, 1978). Five species (*D. durus*, *D. ipini*, *D. laricis*, *D. norimbergensis*, and *D. obesus*) are known only from various beetles (Buprestidae: Blinova and Korenchenko, 1986; Curculionidae: Massey, 1974; Cerambycidae: Sumenkova, 1989). One species (*D. aenea*) is known from two species of picture-winged flies (Ulidiidae: *Physiphora aenea* (F., 1794); *P. demandata* (F., 1794)) on maize, sorghum, and green gram in India (Rao and Reedy, 1982). Four species (*D. canii*, *D. proximus*, *D. siricidicola*, and *D. wilsoni*) are known from eastern North America, all associated with *Sirex* (Bedding, 1968; Morris et al., 2013; Zieman et al., 2015). Of these, three species are native (*D. canii*, *D. proximus*, and *D. wilsoni*) and one (*D. siricidicola*) was introduced with *S. noctilio* in the early 2000s (Hoebeke et al., 2005; Kroll et al., 2013). While the exact origin of *D. siricidicola* in North America is unknown, previous work has suggested Australia as a source (Yu et al., 2009). Despite previous assumptions that eastern North American *Deladenus* are specialists (Bedding and Akhurst, 1978), *D. siricidicola* and *D. proximus* will opportunistically parasitize *S. nigricornis* F., 1781 and *S. noctilio* (Morris et al., 2013).

There are 14 *Sirex* in North America, three from east of the Rocky Mountains: *S. nigricornis* and *S. cyaneus*, which are native; *S. noctilio*, which is invasive and currently restricted to the northeast (Schiff et al., 2012). All species utilize conifers as hosts, with *Pinus* L., 1753 (Pinales: Pinaceae) being the most commonly inhabited host for both *S. nigricornis* and *S. noctilio* and *S. cyaneus* most often inhabiting *Abies* Mill., 1754 and *Picea* A. Diet., 1824

(Pinales: Pinaceae). *Sirex* have a symbiotic relationship with fungi, *Amylostereum* Boidin, 1958 (Russulales: Amylostereaceae), of which there are two species in eastern North America (*A. areolatum* Boidin, 1958 and *A. chailettii* Boidin, 1958), each with several strains (Hajek et al., 2013; Talbot, 1977).

Sirex-parasitizing *Deladenus* feed on *Amylostereum* during their free-living stage. After detecting host larvae, mated nematode females use styletiform mouthparts to pierce host cuticle and enter the haemocoel. Once inside, female nematodes lay dozens of eggs, resulting in juvenile nematodes which may migrate to the host reproductive organs, likely during host pupation. After pupation, female woodwasps emerge and fly to a new host tree to oviposit (Bedding, 1972, 1967). If infested with *Deladenus*, eggs may be inviable and full of nematodes. Following oviposition, nematodes emerge from infected eggs and juvenile nematodes become free-living again. Mycangia, abdominal sacs that function in housing and transporting *Amylostereum*, may also be infested with nematodes and may serve to transport nematodes to new host trees during fungal inoculation. Alternatively, juvenile nematodes may be unsuccessful in finding the host eggs and instead remain in the host's haemocoel, where they will presumably die with the host. Male woodwasps are not able to vector nematodes. These latter scenarios are considered "dead ends" for *Deladenus* (Bedding, 1972, 1967).

Cytochrome c oxidase I is reliable in discrimination of species across the animal kingdom, excluding Cnidaria (Hebert et al., 2003). Past studies have shown clear patterns of intra- and interspecies variation within insects (Monaghan et al., 2005). Additionally, past studies evaluating *D. proximus* have morphologically identified nematodes isolated from native woodwasps (Zieman, 2013). Therefore, we are confident that sequence comparisons with those

that have been previously identified both molecularly and morphologically will result in a positive identification of nematodes parasitizing native woodwasps in Arkansas and Mississippi.

The continual spread and establishment of *S. noctilio* in the United States and Canada (Dodds et al., 2010), coupled with the uncertainty of nematode identification (Morris et al., 2013), requires a molecular evaluation of *Deladenus* across eastern North America. Herein we describe the 1) molecular identification of *Sirex*-parasitizing nematodes in Arkansas and Mississippi, and 2) genetic diversity among nematodes from several geographic locations in eastern North America (i.e. New York, Illinois, Louisiana, Arkansas, and Mississippi).

Materials and Methods

Collection and dissection In Mississippi in September 2011, sixteen sites were established across managed and unmanaged *Pinus taeda* L., 1753 forests as described by Chase et al. (2014). Each site contained three Lindgren® 12-funnel traps baited with small diameter (≤ 7.62 cm) *P. taeda* slash which was partially stripped of bark and placed in propylene mesh bags. Traps (48 total) were hung at a height of ~1.7 m and each trap terminated in a collection cup filled with a 50/50 mix of propylene glycol and water. The antifreeze mixture containing adult *S. nigricornis* was replaced weekly through December and wasps were stored in 95% ETOH in Whirl-Pak® bags in a -20°C freezer.

In Arkansas, four sites, two in each of two geographic regions (Ozark National Forest and Ouachita National Forest), were selected based on presence of suitable *S. nigricornis* host material. Within each site, two APTIV™ black panel traps were hung from bent steel conduit the last week of September. Traps (16 total) were hung so that the collection cup filled with propylene glycol was ~2.5 m above the ground. This collection cup containing female *S.*

nigricornis was collected weekly through December in 2012, 2013, and 2014. Arkansas and Mississippi site locations are shown below (Fig. 1).

All female wasps were dissected using a LEICA MZ75 (Leica Microsystems, Wetzlar, Germany) dissecting microscope following protocol described by Thomsen and Harding (2011). Mycangia were removed and placed in a separate Petri dish to be examined for the presence of nematodes. To determine nematode presence in eggs, a random subsample of 20 to 25 eggs from each female was mounted on a microscope slide in lacto-phenol and examined with a LEICA DM 2500 compound microscope using phase microscopy. Remaining eggs were counted and placed, along with the corresponding mycangia, in a labeled microcentrifuge tube containing 70% ethanol. Samples were stored at room temperature until molecular analyses.

Extraction and sequencing A total of 48 nematode isolates, representing both geographic locations and both management types, were sequenced using molecular methods to identify nematode species (Table 1). Nematode DNA was extracted from mycangia and eggs of infested *S. nigricornis* females using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. A 650-720 bp region of the nematode cytochrome oxidase subunit I gene was amplified using previously published primers (Morris et al., 2013) and PCR protocol (Ye et al., 2007). The presence of DNA within PCR products was confirmed using a 1.5% agarose gel electrophoresis which was visually examined using a BioDoc-It® Imaging system (UVP, LLC, Upland, CA). PCR products were purified for sequencing using a PureLink® PCR Purification Kit (ThermoFisher Scientific, Waltham, MA) according to manufacturer's instructions. Purified PCR products were sent to Macrogen USA (Rockville, MD) for Sanger sequencing. Raw sequence data were assembled and edited with DNA Baser

4.23.0.2 (Heracle BioSoft, Romania). For comparison, sequence data for New York, Louisiana, and Illinois-caught *Deladenus proximus*, and the out-group, were obtained from GenBank (Table 1). Pairwise and mean genetic distances were calculated using MEGA 6 (Tamura et al., 2013).

Results

From a total of 1,139 wasps collected, 244 were infested with nematodes (21%). In Mississippi, 72 of 594 (12%) had some level of nematode infection while 172 of 545 (32%) Arkansas wasps were infested. Mean genetic distance of all *D. proximus* evaluated was 0.004, indicating that nematodes from Arkansas, Mississippi, New York, Louisiana, and Illinois are all a single species. Isolates obtained in Illinois have been positively identified as *Deladenus proximus* (Zieman et al., 2015), therefore, we report that this species is also the species associated with *S. nigricornis* in the southeastern United States. Arkansas and Mississippi isolates had a mean genetic distance from the out-group of 0.206.

Discussion

Our results suggest that there is a single native nematode species associated with *S. nigricornis* in the eastern United States. Mean genetic distance of 0.004 not only indicates that these nematodes are the same species, but that there could be continuous gene flow among these nematodes across the geographic range examined in this paper. Minor genetic distances of the COI gene indicative of gene flow have been found in other organisms (e.g. Van Syoc, 1994). Previous studies have evaluated additional markers (i.e. ITS, 18S) for species delineation and genetic diversity of *Deladenus*, and additional analyses utilizing these markers would be necessary to conclusively discuss gene flow across eastern North America (Morris et al., 2013,

Zieman et al., 2015). This minor genetic distance is evident for nematodes collected from Louisiana to New York, across various management situations, from varying host tree species, and different symbiotic fungi. Additionally, *D. proximus* that were isolated from *S. noctilio* in New York and *S. nigricornis* in the Southeast show very little genetic distance from each other. Such minor differences among sequences covering a wide area also suggests that the geographic range, and potentially host range, of *D. proximus* may be larger than originally thought. Such small genetic distance over a large geographic range implies that the range of the species must be larger relative to current estimations. *Sirex nigricornis* and *S. noctilio* do not occur in North America outside of eastern region, however, *D. proximus* may be parasitizing other woodwasp species west of the Rocky Mountains (Schiff et al., 2012). Further investigation into nematode identity and diversity across other areas of North America and other *Sirex* is warranted.

Deladenus proximus occurs as a single nematode species, in the eastern United States. They have negative effects on body size and fecundity of female woodwasps (Haavik et al., 2016; Kroll et al., 2013), and can utilize *S. noctilio* as a novel host. These factors suggest that as *S. noctilio* spreads throughout North America, *D. proximus* should be considered as a biological control agent. However, sterilization of female woodwasps by *D. proximus* is highly variable across even small regions of the United States (Chapter 4). The strain of *D. siricidicola* that was introduced along with *S. noctilio* has the ability to infect *S. nigricornis*, but is not currently sterilizing native woodwasps. The introduction of a highly virulent strain of *D. siricidicola* could have significant non-target effects, whereas the use of a native nematode in biological control of *S. noctilio* avoids many of these concerns. Prior to any biological control efforts however, possible confounding factors such as multiple host tree species and woodwasp genetic diversity should be evaluated.

Acknowledgments

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Figures and Tables

Figure 1. Map showing all Arkansas and Mississippi sites; gold circles indicate unmanaged sites, blue squares indicate managed sites, green outlines indicate National Forest boundaries.

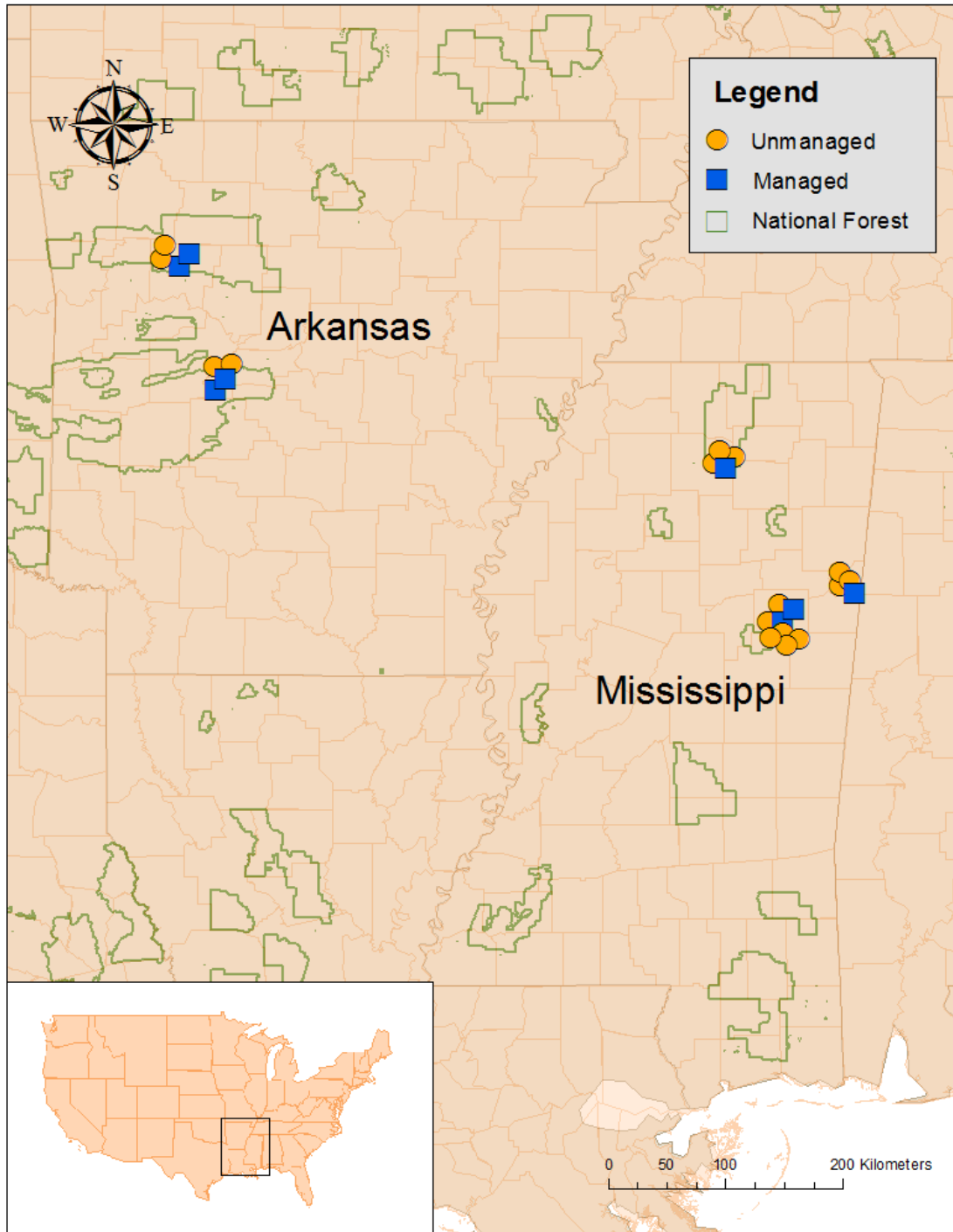


Table 1. *Sirex* nematodes and outgroup accession numbers used in genetic analyses.

Nematode ID	Collection		Treatment	Accession
	Date	Site		Number
MS632	12-Nov-11	Holly Springs	Unmanaged	KU705663
MS871	29-Oct-11	Holly Springs	Unmanaged	KU705664
MS1022	29-Oct-11	Holly Springs	Unmanaged	KU705665
MS1054	12-Nov-11	Holly Springs	Unmanaged	KU705666
MS1069	17-Nov-11	St. Mark Road	Managed	KU705667
MS1087	12-Nov-11	Holly Springs	Managed	KU705668
OZ157	10-Dec-12	Ozark NF	Unmanaged	KU705669
OZ158	10-Dec-12	Ozark NF	Unmanaged	KU705670
OZ165	29-Nov-12	Ozark NF	Unmanaged	KU705671
OU177	19-Nov-12	Ouachita NF	Managed	KU705672
OZ187	29-Nov-12	Ozark NF	Unmanaged	KU705673
OZ190	29-Nov-12	Ozark NF	Unmanaged	KU705674
OU269	30-Nov-12	Ouachita NF	Managed	KU705675
OZ329	18-Oct-12	Ozark NF	Managed	KU705676
OZ348	18-Oct-12	Ozark NF	Unmanaged	KU705677
OU386	21-Nov-13	Ouachita NF	Managed	KU705678
OU390	21-Nov-13	Ouachita NF	Unmanaged	KU705679
OU395	31-Oct-13	Ouachita NF	Unmanaged	KU705680
OU408	14-Nov-13	Ouachita NF	Managed	KU705681
OZ475	10-Dec-12	Ozark NF	Managed	KU705682

MS623	25-Nov-11	Holly Springs	Unmanaged	KU705683
MS765	5-Nov-11	Holly Springs	Managed	KU705684
MS882	9-Nov-11	Storage Units	Unmanaged	KU705685
MS885	12-Nov-11	Holly Springs	Managed	KU705686
MS895	29-Oct-11	Holly Springs	Unmanaged	KU705687
OU94	30-Oct-12	Ouachita NF	Managed	KU705688
MS1036	7-Nov-11	Ennis Road	Unmanaged	KU705689
OU160	11-Dec-12	Ouachita NF	Unmanaged	KU705690
OU226	11-Dec-12	Ouachita NF	Managed	KU705691
OU250	11-Dec-12	Ouachita NF	Unmanaged	KU705692
OU268	30-Nov-12	Ouachita NF	Managed	KU705693
OZ338	8-Nov-12	Ozark NF	Managed	KU705694
OU392	24-Oct-13	Ouachita NF	Unmanaged	KU705695
OZ466	21-Nov-13	Ozark NF	Unmanaged	KU705696
OU523	13-Nov-14	Ouachita NF	Managed	KU705697
OU270	19-Nov-12	Ouachita NF	Managed	KU705698
OU522	30-Oct-14	Ouachita NF	Managed	KU705699
OZ479	8-Nov-12	Ozark NF	Managed	KU705700
OU459	21-Nov-13	Ouachita NF	Managed	KU705701
OZ516	13-Nov-14	Ozark NF	Managed	KU705702
MS911	25-Nov-11	Holly Springs	Managed	KU705703
MS970	29-Oct-11	Holly Springs	Unmanaged	KU705704
OZ196	20-Nov-12	Ozark NF	Unmanaged	KU705705

OU230	11-Dec-12	Ouachita NF	Managed	KU705706
OU130	11-Dec-12	Ouachita NF	Managed	KU705707
MS856	12-Nov-11	Holly Springs	Unmanaged	KU705708
OZ412	14-Nov-13	Ozark NF	Managed	KU705709
OZ260	8-Nov-12	Ozark NF	Unmanaged	KU705710
nig12	15-Oct-08	Mt. Morris, PA	-	JX104240
nig162	5-Oct-10	Warrensburg, NY	-	JX104271
nig163	1-Nov-10	Grant Parrish, LA	-	JX104272
nig174	2008	Fabius, NY	-	JX104277
noc156	27-Aug-10	Warrensburg, NY	-	JX104267
EDIL008	-	-	-	KJ193986
NEIL034	-	-	-	KJ194018
EDIL079	-	-	-	KJ194001
NEIL125	-	-	-	KJ194026
ED2547	-	-	-	KJ193985
<i>Fergusobia</i> 311	-	South Australia	Shoot bud gall	AY589450

Chapter 4 – Interactions Among Female *Sirex nigricornis* F. (Hymenoptera: Siricidae) Egg Load, Body Size, Collection Location, and Nematode Parasitization

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Abstract

Sirex nigricornis F. (Hymenoptera: Siricidae) is an innocuous pine-inhabiting woodwasp native to eastern North America, utilizing dead or dying pine trees as hosts. Although *S. nigricornis* does not cause economic damage, a closely related species, *S. noctilio*, was discovered in New York in 2004 and has continually spread throughout the northeastern United States and southern Canada, potentially threatening the multi-billion dollar pine timber industry of the southeastern United States and raising interest about potential interactions with native woodwasps and associated mortality agents. A non-sterilizing strain of the biological control agent, *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae), was introduced along with *S. noctilio* but is not inhibiting the spread or establishment of *S. noctilio*. A North American congener, *D. proximus*, has recently been isolated from *S. noctilio* and shows promise as a biological control agent. To better understand the potential of *D. proximus* as a biological control agent for *S. noctilio* in the eastern United States, we measured and dissected nearly 1,200 *S. nigricornis* females from Arkansas and Mississippi and evaluated differences among collection location with regard to the nematode's ability to sterilize female woodwasps, woodwasp body size, and egg load. Body size and egg load were related to collection location, and nematode infection resulted in significantly smaller females who produced significantly fewer eggs compared to their uninfested counterparts. Female woodwasps, especially those collected in Arkansas, were often fully sterilized by nematodes and a higher percent sterilization was inversely related to body size and fewer eggs. We propose field studies to test the nematode's ability to sterilize *S. noctilio* in the northeastern United States.

Keywords: biological control, *Deladenus*, forest entomology, invasive species, *Sirex noctilio*

Introduction

Sirex noctilio F. (Hymenoptera: Siricidae) is a pine-inhabiting woodwasp native to Eurasia and North Africa where it causes little, if any, damage to natural and commercial pine stands. However, in the Southern Hemisphere, where the woodwasp has been repeatedly introduced, it has caused significant economic damage to exotic pine plantations (Carnegie et al., 2005; Slippers et al., 2015). *Sirex nigricornis* F. is native to eastern North America and, much like *S. noctilio* and other siricids in their respective native ranges, is not a primary cause of mortality to pines. The first non-native population of *S. noctilio* in North America was discovered in 2004. It has since been found in seven states and much of southern Ontario, while not yet causing substantial mortality (Dodds et al., 2010, 2007).

The southeastern United States houses a multi-billion dollar pine timber industry, and establishment of *S. noctilio* in the Southeast may have significant negative effects on this industry and natural forests, especially in stressed or overstocked stands (Borchert et al., 2006; Chase et al., 2014) and during periods of drought (Morgan, 1989; Neumann and Minko, 1981). Effective management techniques are necessary to ensure these forests are not decimated by establishment of *S. noctilio*.

Deladenus siricidicola Bedding (Tylenchida: Neotylenchidae) are parasitic nematodes commonly used in biological control of *S. noctilio* in the Southern Hemisphere (Bedding, 1979; Bedding and Akhurst, 1974; Slippers et al., 2012). These nematodes can be free-living within a pine host, feeding on the *Sirex* symbiont, *Amylostereum* Boidin (Russulales: Amylostereaceae) and are not pathogenic to the tree. When in proximity to *Sirex* larvae, female nematodes mature, mate, become parasitic, and pierce the cuticles of *Sirex* with their stylets. After moving into the hosts' haemocoel, females lay eggs which hatch and juvenile nematodes migrate to *Sirex*

reproductive organs during host pupation. *Sirex* adults emerge and infested females oviposit into new pine hosts, potentially laying eggs filled with nematodes. Sperm production has ceased by the time of juvenile nematode migration and, therefore, males are not able to transfer nematodes during copulation (Bedding, 1972, 1967).

For the purposes of this study, virulence equates to the percent of *S. nigricornis* eggs sterilized by *D. proximus*, that is, the percentage of total eggs within a female that contain nematodes and are presumably not viable (e.g. Jaenike, 1996). *Deladenus* have had variable success controlling *S. noctilio* populations. Over 99% parasitism of *S. noctilio* was reported from inoculated logs in Australia (Bedding and Akhurst, 1974), however, repeated inoculations of pines in South Africa resulted in an average parasitism rate of 25% or lower (Slippers et al., 2012). A strain of *D. siricidicola* which does not sterilize female woodwasps was introduced with *S. noctilio* into the northeastern United States, but have not been effective at reducing populations or slowing the spread of this invasive pest (Kroll et al., 2013). Although northeastern U.S. populations of infested *S. noctilio* females are smaller and produce fewer eggs, the eggs that are produced are not infected with nematodes and are presumably viable (Kroll et al., 2013). *Deladenus proximus* Bedding, are commonly found within body tissues of *S. nigricornis* across eastern North America (Keeler, 2012). While in various host tissues, *Deladenus* are thought to feed on *Sirex* fat reserves, resulting in smaller adult woodwasps (Bedding and Iede, 2005).

Eastern North American nematodes were assumed to be specialists (Bedding and Akhurst, 1978), however recent studies have documented both nematode species infecting both *S. noctilio* and the North American native, *S. nigricornis* (Haavik et al., 2016; Morris et al., 2013). The nematodes' ability to parasitize novel hosts raises questions about potential non-

target effects of biological control using a more virulent strain of *D. siricidicola*. While the accidentally introduced strain of *D. siricidicola* remains non-sterilizing in eastern North American *Sirex* (Haavik et al., 2016; Kroll et al., 2013), inoculation of pines with an introduced, highly virulent strain, could have significant negative effects on native woodwasp populations. Also, potential genetic bottlenecks, resulting in extreme homozygosity of cultured nematodes, and a loss of virulence over time, implies the need for repeated introduction of more virulent nematode strains (Mlonyeni et al., 2011). Conversely, *D. proximus* is found in native female *S. nigricornis*, but its virulence is inconsistent across the U.S. (Zieman et al., 2015). These conflicting potential outcomes indicate potential concern for biological control of *S. noctilio*. However, a virulent native strain of *D. proximus* could potentially sterilize *S. noctilio* females while not causing additional harm to native woodwasp populations.

Our objectives in this study were to determine if nematode virulence varied by collection location (i.e. Ozark NF, Ouachita NF, Mississippi) and if *S. nigricornis* female body size and egg load were related to collection location and nematode infection of *S. nigricornis*.

Materials and Methods

Specimen collections

In Arkansas during four consecutive years, late September 2012 – 2015, two APTIV Intercept™ black panel traps were erected at four sites in the Ozark (35.62°N, -93.55°W) and four sites in the Ouachita (34.90°N, -93.12°W) National Forests (NF), totaling eight traps per location (16 traps). Sites were split among unmanaged and recently thinned stands. Each trap was attached to bent steel conduit and raised so that a collection cup at the bottom of the trap, filled with propylene glycol, was approximately 2 m above the ground. Traps were baited with a

Sirex lure (75/25- α/β -pinene; release rate: 1.33 g/d) and 95% ultra-high release (UHR) ethanol (release rate: 0.3-0.8 g/d) (Synergy Semiochemicals, Burnaby, Canada) which only attracted *S. nigricornis* females (Böröczky et al., 2012). The propylene glycol containing woodwasps was collected every 7-10 days and stored at ~4°C until processed. Sampling continued until two consecutive collections contained no siricids, usually late December or early January. Female *Sirex* were placed in glass vials containing 70% ethanol, denoting trap location and collection date. During the four years of trapping, 601 woodwasps were collected in Arkansas, 275 in the Ozark NF and 326 in the Ouachita NF.

In northeastern Mississippi (33.89°N, -88.98°W) in 2011, three Lindgren multiple funnel traps were deployed at each of 16 sites (48 traps). As in Arkansas, sites represented both unmanaged and recently thinned stands. Traps were baited with pine slash and bark that was held in a propylene mesh bag hung parallel to the trap and replaced every 14 days. Traps were hung so that a collection cup filled with 50/50 mix of propylene glycol and water was 1.7 m above the ground. Traps were erected in late September and insects were collected weekly until late December. A total of 594 woodwasps was collected, measured, and dissected. All trapping locations are presented in Figure 1.

Specimen dissection

Female woodwasps were placed in a paraffin-filled Petri dish, covered with a layer of 70% ethanol, and examined using a Leica MZ75 (Leica Microsystems, Wetzlar, Germany) dissecting microscope. The body was secured in paraffin by placing one insect pin through the dorsal side of the pronotum and an additional insect pin through the dorsal side of the last abdominal segment. Wings were removed to allow better visibility and an ocular micrometer was used to measure (to the nearest 0.001 mm) the widest part of the pronotum and body length

from tip of the head to tip of the abdomen (not including ovipositor sheath). Dissecting scissors were used to cut through the entire right abdominal pleuron. Fine forceps were used to peel back the dorsal abdominal sclerites which were then secured to the paraffin with insect pins. This exposed abdominal contents of the woodwasp and allowed examination of the haemocoel for the presence of nematodes. Eggs and mycangia were removed and placed in a separate Petri dish, also filled with 70% ethanol. Mycangia were examined for nematodes and then stored in a labeled microcentrifuge tube containing 70% ethanol. Eggs within ovaries were separated using fine forceps and a random subsample of 20-25 eggs per female was mounted in lacto-phenol on a microscope slide. Remaining eggs were counted and stored in the same microcentrifuge tube containing corresponding mycangia. The slide-mounted subsample of eggs was examined for nematode infection with a Leica DM 2500 compound microscope using phase microscopy. The number of eggs infected with nematodes was divided by the total number of eggs in the subsample to obtain percent sterilization.

Data analyses

R 3.2 (R Core Team 2015) was used for all statistical analyses. Significant differences were judged at $\alpha = 0.05$. Pronotum width and body length were significantly correlated ($\text{cor} = 0.94$, $t = 18.829$, $\text{d.f.} = 1194$, $p < 0.0001$) so one variable was chosen for analyses. A Shapiro-Wilk test was used to test assumptions of normality for dependent variables. Body length was used in all analyses because those data could be natural-log transformed to fit a normal distribution while pronotum width could not. Egg load also fit a normal distribution after natural log transformation. A t-test was used to examine the effects of nematode infection on transformed body length and egg load, using measurements from both infested and uninfested wasps. A one-way Analysis of Variance (ANOVA) was used to test significance among

locations for log-transformed body length and egg load. ANOVA significance warranted separation of means using a Tukey's HSD test. Nematode infestation was examined among locations using a generalized linear model (GLM) with a binomial family distribution (0 = uninfested, 1 = infested).

Within infested females, effect of location on natural log-transformed body length and egg load was examined using a one-way ANOVA and Tukey's HSD test to separate means when necessary. Percent sterilization was examined among locations using a Kruskal-Wallis rank sum test because these data could not be transformed to fit normality assumptions. Comparison of means on Kruskal-Wallis rank sum results was performed using the 'pgirmess' package in R (Giraudoux, 2014). Effects of percent sterilization on natural log-transformed body length and egg load were examined using a linear model (lm).

Results

Female Sirex nigricornis body size and egg load in relation to location

Body length ($F = 20.46$, d.f. = 2, $p < 0.0001$) and egg load ($F = 8.522$, d.f. = 2, $p = 0.0002$) were significantly different among locations. Female body length increased in more southern locations, with females collected in the Ozark NF being smaller (10.13 – 32.91 mm; mean = 18.3 mm \pm 0.24) than those from the Ouachita NF (10.96 – 31.72 mm; mean = 19.15 mm \pm 0.23; $p = 0.0152$) and those from Mississippi (7.26 – 32.20 mm; mean = 19.90 mm \pm 0.14; $p < 0.0001$). The difference in egg load between females from the Ouachita NF and Mississippi was also significant ($p = 0.0026$). While egg load was significantly different among locations, the relationship was not the same. Females collected in the Ozarks contained significantly fewer eggs (122 \pm 5.20) than those collected in the Ouachita NF (144 \pm 5.45; $p = 0.0229$) but not fewer

than those collected in Mississippi (118 ± 3.19 ; $p = 0.6370$). The difference in egg load between females collected in the Ouachita NF and those collected in Mississippi was also significant ($p = 0.0001$) (Fig. 2a).

Nematode parasitism of S. nigricornis females in relation to location

Prevalence of *D. proximus* was measured as the number of infested females divided by the total number of females collected. Prevalence was significantly different among locations with the Ozark NF (31%) and Ouachita NF (24%) having similar rates of infection ($z = -1.749$, $p = 0.0803$), both of which were significantly higher than prevalence of nematodes in Mississippi (12%; $z = -6.541$, $p < 0.0001$). Body length was significantly different ($t = 6.9983$, d.f. = 359.12, $p < 0.0001$) with uninfested females being larger ($19.70 \text{ mm} \pm 0.12$) than infested females ($17.84 \text{ mm} \pm 0.24$). Egg load was also significantly different ($t = 6.9249$, d.f. = 379.76, $p < 0.0001$) with uninfested females (133 ± 2.84) having more eggs than infested females (97 ± 4.81).

Body length of infested females was significantly different among locations ($F = 7.698$, d.f. = 2, $p = 0.0006$). Females collected in the Ozark NF (10.62 – 31.78 mm; mean = $17.28 \text{ mm} \pm 0.41$) were not significantly different from those collected in the Ouachita NF (11.51 – 30.48 mm; mean = $17.37 \text{ mm} \pm 0.46$; $p = 0.9971841$) but were significantly smaller compared to infested females from Mississippi (13.97 – 27.95 mm; mean = $19.03 \text{ mm} \pm 0.31$; $p = 0.0016$). The difference between infested females collected in the Ouachita NF and those caught in Mississippi was also significant ($p = 0.0025$). However, egg load of infested females was not significantly different among locations ($F = 0.112$, d.f. = 2, $p = 0.895$) (OZ: 100 ± 8.02 , OU: 102 ± 9.95 , MS: 93 ± 6.38) (Fig. 2b).

Percent sterilization was significantly different among locations ($\chi^2 = 16.966$, d.f. = 2, $p = 0.0002$) with the Ozark NF (85%) and Ouachita NF (90%) having similar rates of sterilization

and Mississippi (78%) having significantly lower percent sterilization. Percent sterilization negatively affected body length ($t = -6.71$, $p < 0.0001$) but explained little variation ($R^2 = 0.16$). Percent sterilization also negatively affected egg load ($t = -5.312$, $p < 0.0001$) but the predictive relationship was weak ($R^2 = 0.11$) (Fig. 3).

Discussion

Bergmann's rule states that, within a broadly distributed species, animal body sizes are positively correlated with latitude (Bergmann, 1848). Female *S. nigricornis* body size increased as latitude decreased, suggesting a converse Bergmann's rule which is regularly found in poikilothermic species (e.g. Blanckenhorn and Demont, 2004; Mousseau, 1997). When uninfested females are removed from the dataset this relationship is weaker, but still present.

Egg load, which should be related to body size, differs significantly among locations when uninfested and infested females are combined, but when considering only infested females egg load does not differ among locations. Percent sterilization also had a significantly negative effect on egg load. These results suggest that nematode infection introduces additional variability that is not seen when evaluating only differences related to location. The authors recognize that females were collected in baited traps and not reared from host material. Therefore, females may have oviposited prior to being collected and egg load was not necessarily representative of true potential fecundity. This aspect of *S. nigricornis* ecology should be explored further with laboratory-reared females to examine this trend in the absence of other environmental factors.

Nematode-infested females were both smaller and carried fewer eggs, indicating that virulence has negative impacts on female woodwasps in addition to the direct effect of nematode infection. Percent sterilization also differed significantly among regions, possibly suggesting

differences in nematode strains in these locations. Both the Ozark NF and Ouachita NF had more sterilized females as compared to Mississippi, but all regions had an average percentage sterilization of 75% or higher. This is different than previous reports of complete sterilization in Illinois, Louisiana, and South Carolina (Zieman et al., 2015). However, sequence analysis of the nematode COI gene indicates that all native *Deladenus* nematodes isolated from *S. nigricornis* are a single species and, potentially, a single breeding population (unpublished data, J.A. Hartshorn). Variation in virulence observed could be related to variation in another gene or to other factors not explored in this study (e.g. host species, timing of oviposition, woodwasp genetic diversity) and these factors need to be examined.

We propose that the native nematode, *D. proximus*, be considered as a biological control agent to target *S. noctilio* in North America. This new association biological control (Hokkanen and Pimentel, 1989, 1984) avoids previously described non-target effects associated with the introduction of a non-native, highly virulent strain of *D. siricidicola*. Both *D. proximus* and *D. siricidicola* are present in *S. noctilio* and *S. nigricornis* in New York and virulence of *D. siricidicola* appears to be low, as it has not been found sterilizing female *Sirex* in North America (Kroll et al., 2013). Conversely, *D. proximus* appears to be highly virulent in native *S. nigricornis* populations (Zieman et al., 2015) and may provide control of *S. noctilio*. It is necessary to inoculate trees in the northeastern United States with southern populations of *D. proximus* to determine if this virulence transfers to infection of *S. noctilio*. If this is the case, potential negative impacts associated with classical biological control can be circumvented with the use of a native nematode in biological control of *S. noctilio*.

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Figures and Tables

Figure 1. Map showing locations of all Arkansas and Mississippi sites used in current study, designated by management type.

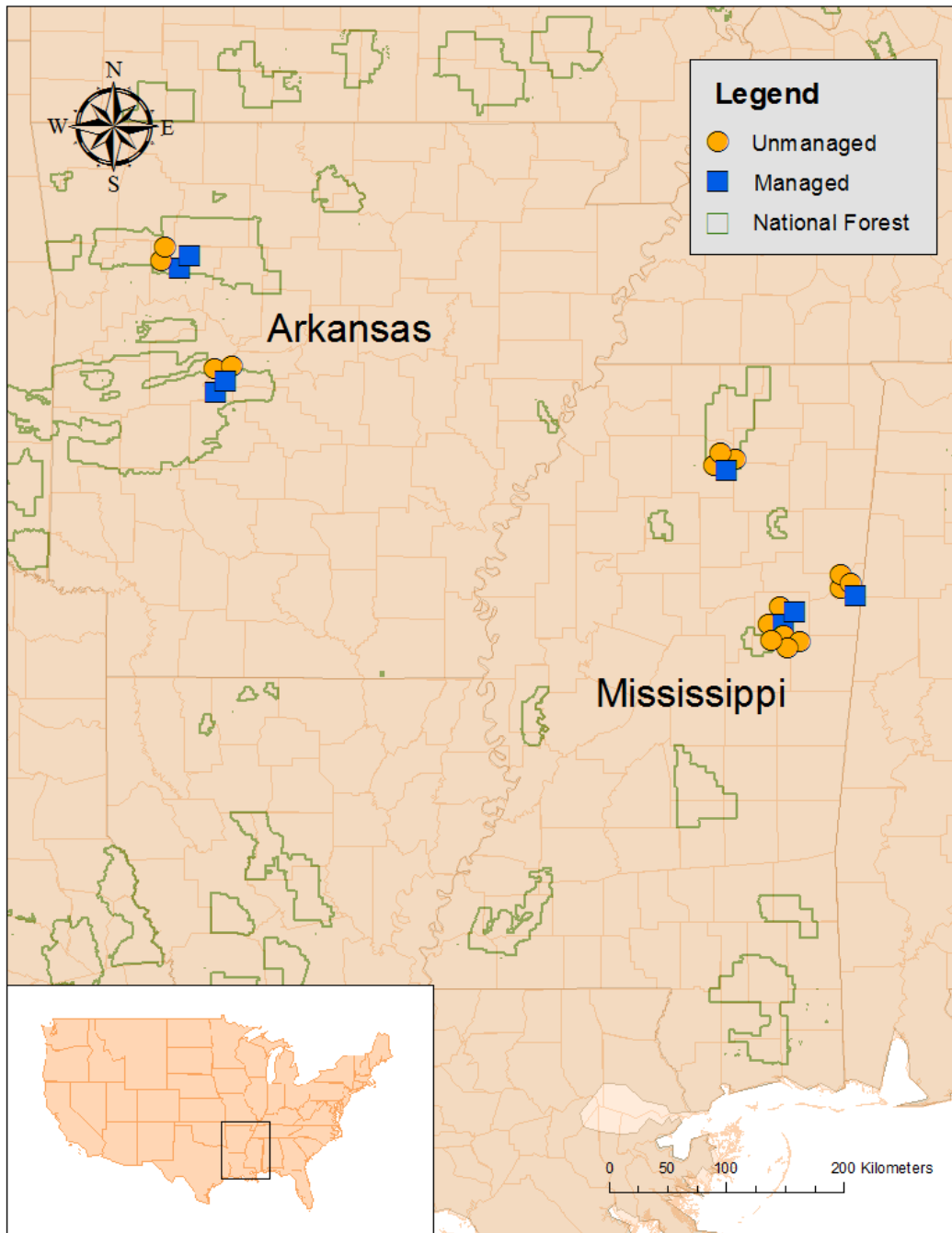


Figure 2. Non-transformed means of body length (mm) (a) and egg load (b) of total, infested, and uninfested females *S. nigricornis* among collection locations.

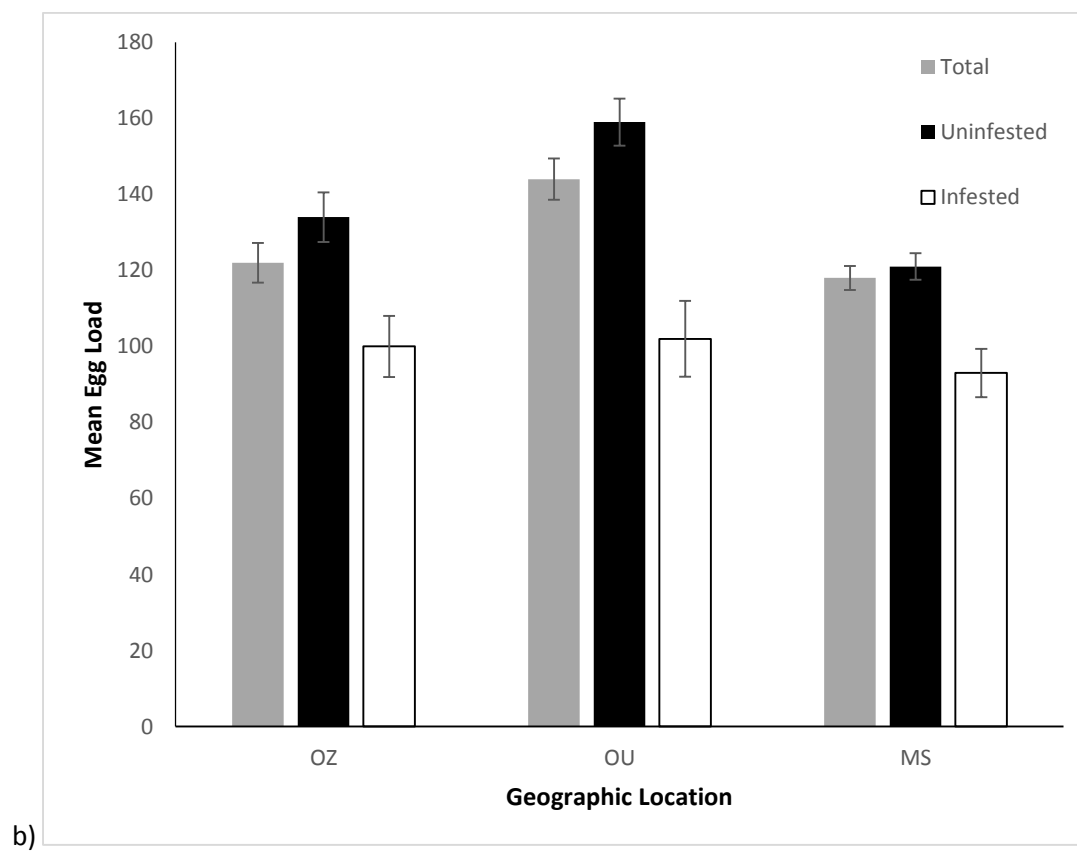
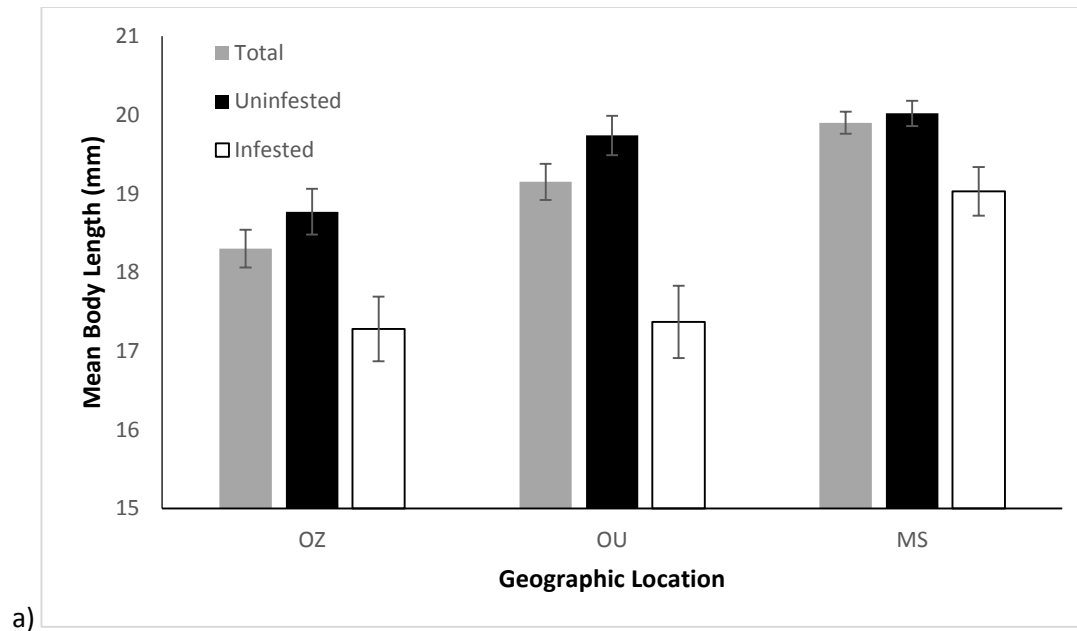
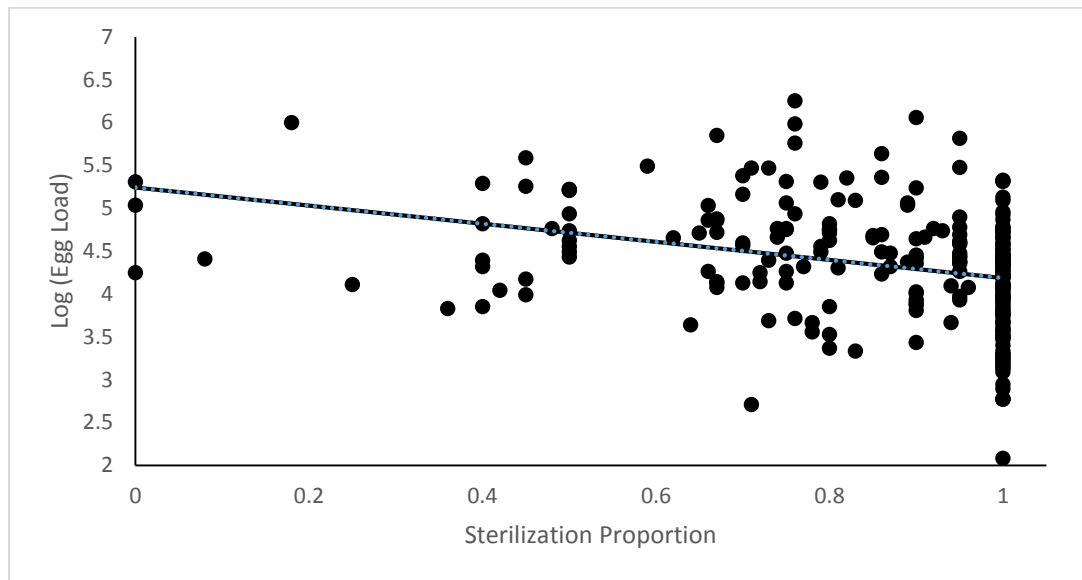


Figure 3. Scatterplot with linear model overlaid ($\text{Log}(\text{Egg Load}) = 128.272 - 10.012 \times \text{sterilization proportion}$) showing relationship between sterilization proportion and log-transformed egg load of infested female *S. nigricornis*.



Chapter 5 – Conclusions

Based on previous introductions into commercial pine plantations along with the knowledge that *S. noctilio* can oviposit and develop in North American pines, *S. noctilio* undoubtedly has the ability to spread into the southeastern United States and cause considerable injury to economically and ecologically important pine stands. Effective monitoring to track the spread and establishment of this woodwasp is essential. Additionally, the use of native parasitic nematodes may be beneficial to slowing the spread of *S. noctilio* or preventing it from becoming a significant pest. With this dissertation I propose 1) the use of local temperatures to determine the most effective monitoring time and 2) the use of native nematodes to control *S. noctilio*. Monitoring traps should be deployed when maximum and minimum temperatures, and the difference between these temperatures, begin to decrease. Emergence coinciding with drops in temperature was consistent for both *S. nigricornis* in the Southeast and Northeast and *S. noctilio* in Ontario. *Deladenus proximus* are able to significantly reduce the size and fecundity of *S. nigricornis* in Arkansas and Mississippi. They are also able to use *S. noctilio* as a novel host in the northeastern United States. In the southeastern United States we find a much higher infestation rates with higher sterilization proportions, potentially indicative of a virulent strain of nematode. This virulence may cause significant reductions to *S. noctilio* female fitness while not causing additional stress on native woodwasp females. Research to test their viability in field trials is essential to ensure the success of this new association biological control program.