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Effects of Associated Subcortical Beetles on Oviposition Behavior and Early-stage Survival of Sirex Nigricornis F. (Hymenoptera: Siricidae)

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EFFECTS OF ASSOCIATED SUBCORTICAL BEETLES ON OVIPOSITION BEHAVIOR AND EARLY-STAGE SURVIVAL OF *SIREX NIGRICORNIS* F. (HYMENOPTERA: SIRICIDAE)

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ABSTRACT

Sirex (Hymenoptera: Siricidae) woodwasps develop within xylem of host conifers. *Sirex* females drill through the bark, phloem and into xylem tissues where they deposit eggs along with a symbiotic *Amylostereum* fungus. The presence of *Amylostereum* is necessary for successful development of *Sirex* immatures as the larvae are unable to derive adequate nutrition from xylem in the absence of the fungus. The Eurasian woodwasp, *Sirex noctilio* F., was discovered in northeastern North America in 2004. *Sirex noctilio* has caused significant economic damage in *Pinus radiata* D. Don plantations of the southern hemisphere, but is of little economic significance in its native range. It is unknown how *S. noctilio* will impact pine forests in the southeastern United States. The presence of associated subcortical insects in *S. noctilio's* native range and their absence in the southern hemisphere has led researchers to speculate that these associate species may inhibit *S. noctilio* population expansion. I investigated interactions between a native woodwasp, *S. nigricornis* F., and its associated subcortical insect-complex with the goal of understanding how woodwasp populations are impacted by these interactions. The objectives of my research were to: 1) identify the relative timing of host colonization between *S. noctilio* and its associated species; 2) investigate how tornado disturbance affects the relative abundance of *S. nigricornis*; and 3) determine if associated insects affect oviposition behavior and survival of *Sirex*. The most common insect inhabitants of downed pine trees are subcortical beetles (e.g. Cerambycidae and Scolytidae) which normally colonized host substrate before *S. nigricornis*. *Sirex nigricornis* abundance was similar at high and low levels of host material while more beetles were trapped in areas with more host material. *Sirex nigricornis* females drilled into host material with similar frequency regardless of the presence of associated beetles. There was evidence of less *S. nigricornis* oviposition on beetle-colonized as opposed to non-colonized bolts. *Sirex nigricornis* mortality estimates were higher on beetle-colonized than non-

colonized bolts. These results suggest that *Sirex* are negatively affected by the presence of associated insects.

This thesis is approved for recommendation
to the Graduate Council.

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CHAPTER 1 – INTRODUCTION

OVERVIEW

Sirex nigricornis Fabricius (= *S. edwardsii*) (Hymenoptera: Symphyta: Siricidae: Siricinae) is a pine colonizing woodwasp native to eastern North America (Goulet 2012). Likely due to its economic insignificance, *S. nigricornis* has not been well studied. A majority of siricid research attention has been paid to *S. noctilio* due to its invasiveness, economic importance in the southern hemisphere and because it is the only siricid known that has the capability to kill pine trees (Ciesla 2003, Slippers et al. 2012a).

Discovery of established *Sirex noctilio* populations in Ontario, Canada and New York in 2004 (Hoebeke et al. 2005, de Groot et al. 2006) has spurred research on *Sirex* in North America. Much of this research is being undertaken in an effort to understand the potential economic and environmental impacts of *S. noctilio* and how to best manage this species in North America (Borchert 2007, Dodds et al. 2007, Yemshanov et al. 2009b, Dodds et al. 2010, Zylstra et al. 2010, Dodds and de Groot 2012). Of particular interest is how *S. noctilio* will impact the southeastern United States (Borchert 2007). The southeast is the world's largest producer of softwood timber products, accounting for more than $8 billion annually and this output is projected to increase (Borchert 2007). Given its ability to fly long distances [e.g. up to 49 km in a day; (Bruzzone et al. 2009)], its potential to spread naturally 30 – 40 km per year (Carnegie et al. 2006) and its aptitude to be transported anthropogenically, there is a high potential for *S. noctilio* to establish in southeastern pine forests (Carnegie et al. 2006, Yemshanov et al. 2009a). Furthermore, because of frequent tropical storms and hurricanes there is often a buildup of stressed host material (Borchert 2007). Improper stand management can also lead to a wealth of

suitably stressed trees. An abundance of suitable host material increases the likelihood an invading *S. noctilio* population will successfully establish and that an established population will reach outbreak levels in which relatively healthy trees are attacked (Liebhold et al. 1995, Farji-Brener and Corley 1998, Borchert 2007, Corley and Villacide 2012).

Central to addressing its impact, is the need to understand how *S. noctilio* will assimilate into our native pine forests and interact with the existing rich community of pine colonizing organisms (Ryan et al. 2011a, Dodds and de Groot 2012). One goal of this thesis is to strengthen our understanding of how native subcortical pine colonizing insects and their associated fungi affect the behavior and survival of *Sirex* woodwasps. To accomplish this, interactions that subcortical pine colonizing organisms have with *S. nigricornis* in Arkansas are investigated.

This thesis also aims to address the paucity of information on *S. nigricornis* by comparing and contrasting elements of its behavior, biology and life history to the well documented *S. noctilio* with which it is assumed to be similar. Anecdotal evidence suggests the two species are similar and this is supported by CO1 sequence homology (Wilson and Schiff 2010), overall morphology (Goulet 2012), host species attacked (Schiff et al. 2006), and an obligate association with a fungal symbiont in the genus *Amylostereum* Boidin (Basidiomycetes) (Martin 1992, Slippers et al. 2003). Yet their evolutionary histories are not exactly similar, likely giving rise to differences in their behavior, biology and life histories. For example, in ranges where the species co-occur, *S. noctilio* emerges earlier than *S. nigricornis* (Long et al. 2009, Zylstra et al. 2010); therefore adult activity of the two species may occur under different environmental and ecological conditions. Another possible difference between *S. noctilio* and *S. nigricornis* is the condition of host material colonized. In studies among European species, *S. noctilio* is thought to have an expanded niche compared to other pine colonizing siricids because

it is the only species that prefers and successfully colonizes relatively physiologically active pines (Spradbery 1973, Spradbery and Kirk 1978, 1981, Dodds et al. 2010). The ability of *S. noctilio* to overwhelm living host trees while other Siricinae cannot is thought to be due, in part, to its comparatively larger mucus glands and mucus storage reservoir which likely affords it the ability to deliver a comparatively higher dose of mucus at one time than other Siricinae (Spradbery 1973, 1977).

It is prudent to collect behavioral, biological and life history information on *S. nigricornis* in Arkansas before *S. noctilio* invades. Assuming *S. noctilio* invades Arkansas' pine forests (and likely all pine forests where *S. nigricornis* occurs) in the future, the opportunity will never arise again to study *S. nigricornis* in the absence of *S. noctilio*. By collecting behavioral, biological and life history information on *S. nigricornis* now, before *S. noctilio* invades, a unique opportunity will be afforded to look at whether the establishment of *S. noctilio* alters the behavior, biology or life history of *S. nigricornis*.

SIREX-AMYLOSTEREUM COMPLEX

Sirex nigricornis has an intimate mutualism with *Amylostereum* fungi to such an extent that the two have evolved a stable obligate symbiosis (Martin 1992, Wermelinger and Thomsen 2012). *Amylostereum* benefits from the association by gaining protected transportation to host substrate inside the wasp's mycangia and the fungus benefits *S. nigricornis* by providing accessible nutrition to the developing larvae (Martin 1992). *Amylostereum chailletii* (Persoon: Fries) Boidin is the most common fungal symbiont found inside the mycangia of *S. nigricornis* (Slippers et al. 2003). It was originally thought *A. chailletii* was the exclusive symbiont of *S.*

nigricornis, but there is growing evidence that *Amylostereum areolatum* (Fries) Boidin can also serve as a mutualist to *S. nigricornis* (Nielsen et al. 2009, van der Nest et al. 2012).

The mutualistic association with *Amylostereum* is not unique to *S. nigricornis*, the mycangial relationship being shared among all Siricinae with the exception of *Xeris spectrum* Linnaeus (Buchner 1965, Morgan 1968, Talbot 1977, Wermelinger and Thomsen 2012) and although *X. spectrum* does not harbor *Amylostereum* in mycangia, its development is contingent on *Amylostereum* being inoculated into trees by other Siricinae (Fukuda and Hijii 1997). Confirmation that *Amylostereum* relies on Siricinae for distribution is the fact that it rarely reproduces sexually (Gilbertson 1984) and spread is primarily vegetative with clonal lineages expanding over wide geographic areas (Vasiliauskas et al. 1998, Vasiliauskas and Stenlid 1999, Slippers et al. 2001, Wermelinger and Thomsen 2012).

The inability to derive sufficient nutrition for successful development from wood in the absence of *Amylostereum*, indicates that larval *Sirex* are wholly dependent on the presence of this obligate symbiont (Stillwell 1966, Martin 1992). *Sirex* have anatomical features and behavioral adaptations that ensure the continued relationship with *Amylostereum* (Talbot 1977). Female *Sirex* have invaginated intersegmental sacs, mycangia, that protrude into the body where *Amylostereum* is cultured and carried (Francke-Grosman 1939, Boros 1968, Talbot 1977). Each mycangium covers an organ termed the club organ (Boros 1968). Secretions released from unicellular glands inside the club organs empty via wide ducts into the mycangium and these secretions stimulate and enhance the growth of *Amylostereum* (Boros 1968, Titze and Turnbull 1970). Mycangia are connected by ducts to the anterior end of the ovipositor (Boros 1968, Talbot 1977). During host colonization, *Sirex* females deposit arthrospores of *Amylostereum* along with mucilaginous secretion into the wood (Morgan 1968, Coutts and Dolezal 1969,

Madden 1974, Talbot 1977). The mucus deposited concurrently with *Amylostereum* is produced in secretory glands and is stored in a reservoir where a duct connects it to the base of the wasp's oviduct (Boros 1968). The mucus has been shown to release the fungus from wax packets and to stimulate growth (Boros 1968, Morgan 1968), but it also appears to play a role in reducing the defenses of living host material (Coutts 1969, Talbot 1977).

Amylostereum deposited by *Sirex* females grows into the wood surrounding oviposition holes and larval tunnels (Cartwright 1938, Parkin 1942, King 1966). The presence of *Amylostereum* surrounding eggs stimulates their eclosion (Coutts 1965, Madden 1981) and female larvae re-acquire the fungus in specialized organs, likely from tunnel walls (Talbot 1977). These organs, termed hypopleural organs, are deep folds in the cuticle between the first and second abdominal segments specialized for carrying *Amylostereum* oidia in a waxy matrix (Parkin 1942, Gilmour 1965, Talbot 1977). It is not certain how female larvae acquire *Amylostereum* in their hypopleural organs [see (Talbot 1977) for possibilities], but the fungus is transferred from one instar to the next and the cuticle from the last larval molt is retained as a cap over the terminal abdominal segments of the pupa (Parkin 1941, Boros 1968, Morgan 1968, Talbot 1977). Before leaving her pupal chamber an adult female acquires *Amylostereum* by twisting her abdomen against the retained larval cuticle and the fungus is probably transferred to her mycangia via her ovipositor (Cartwright 1938, Francke-Grosman 1939, Morgan 1968, Talbot 1977). Growth of *Amylostereum* up the ovipositor and into the mycangia may be stimulated by secretions of the club and secretory glands (Talbot 1977). These behavioral, physiological and anatomical adaptations of *Sirex* ensure a continuous association of the wasps with *Amylostereum* (Talbot 1977, Martin 1992).

SIREX NUTRITION AND DEVELOPMENT

Sirex adults do not feed, relying on stored fat reserves acquired during the larval stage (Morgan 1968, Taylor 1981, Hoebeke et al. 2005, Ryan and Hurley 2012). However, exactly how *Sirex* larvae obtain nutrients inside host xylem is uncertain (Talbot 1977, Ryan and Hurley 2012).

There are conflicting reports in the literature as to whether *Sirex* can derive nutrients from the wood itself. Parkin (1942) and Boros (1968) both stated that the guts of larval Siricinae were simpler than those of larvae that usually digest wood. In a review of Siricidae, Morgan (1968) claimed wood fragments were passed along the outside of the body and that no wood is found in larval alimentary canals. However, staining the guts of actively feeding larvae with phloroglucinal showed that lignin was present in the midgut (Madden 1981) indicating wood fibers are at least ingested.

Also equivocal is whether the larvae feed on *Amylostereum* when inside the host. Cartwright (1929) observed that a newly hatched *S. cyaneus* Fabricius larva survived for three weeks on a pure culture of *A. chailletii* [misidentified as *Stereum sanguinolentum* (Albertini & von Schweiniz) Fries; see Stillwell (1966) and Slippers et al. (2003)] and that a more mature larva definitely fed on *A. chailletii* for three months. Further support that Siricinae larvae consume *Amylostereum* is that digestive fluids of *S. juvencus* Linnaeus and *Urocerus gigas* Linnaeus readily degrade fungal hyphae but not cellulose or wood fibers (Francke-Grosman 1939). Interestingly and despite an earlier contradicting report by Clark (1933), mycelium are thought to be absent from the gut of the larvae (Parkin 1942, Boros 1968, Talbot 1977).

It is possible that mycelium may not be ingested, but dissolved nutrients or enzymes from the fungi could be (Morgan 1968). *Sirex cyaneus* acquires cellulases and xylanases from *A.*

chailletii and these enzymes can break down plant polysaccharides affording larvae the ability to more efficiently extract nutrients from woody xylem by increasing their ability to digest cellulose and hemi-cellulose (Kukor and Martin 1983, Martin 1992). Some researchers who have done extensive work on the life history of *S. noctilio* report that second instar larvae feed exclusively on *Amylostereum* and that later instars feed on fungal-penetrated wood (Madden and Coutts 1979).

Clearly there is not a consensus on how *Sirex* larvae derive nutrition inside their host. Regardless of the exact role *Amylostereum* plays in providing larval nutrition, the presence of the fungi is necessary for successful larval development (Ryan and Hurley 2012, Wermelinger and Thomsen 2012). Evidence that *Sirex* require their fungal symbiont for successful development comes from research on *S. cyaneus* [misidentified as *S. juvencus*; see Talbot (1977) and Goulet (2012)] by Stillwell (1966). Stillwell created aposymbiotic females by removing females from their pupal chambers before *A. chailletii* was inoculated into their mycangia. He then allowed aposymbiotic females and normal females containing *A. chailletii* to oviposit into logs. Eclosion and development of offspring from females containing *A. chailletii* was successful while none of the offspring from aposymbiotic females developed successfully, although many larvae emerged from eggs.

EFFECT OF *AMYLOSTEREUM* AND ADULT SIZE ON OFFSPRING PERFORMANCE

The successful establishment of *Amylostereum* on host material is a requisite for the successful development of *Sirex* offspring, but growth of the fungus also impacts offspring size. It appears that where conditions are optimal for the growth of *Amylostereum*, larger adult *Sirex* emerge (Madden and Coutts 1979, Madden 1981, Ryan and Hurley 2012).

Larger females have comparatively higher potential fecundity (Madden 1974, Neumann and Minko 1981, Fukuda et al. 1993, Fukuda and Hijii 1997) and realized fecundity (Madden 1974, Fukuda et al. 1993, Fukuda and Hijii 1996, 1998) than smaller females. In addition, larger *Sirex* have longer life spans (Madden 1974) and they have the ability to disperse further (Bruzzone et al. 2009, Corley and Villacide 2012).

LIFE HISTORY OF *SIREX NIGRICORNIS* IN ARKANSAS

As mentioned in the introduction, little work has been done directly on *S. nigricornis*, most of its life history being extrapolated from the life history of *S. noctilio* and a few other siricids (Morgan 1968). A portion of the research work done for this thesis is to determine if *S. nigricornis* behaves similarly to *S. noctilio* and to note where some behavioral and biological differences occur. Since there is almost no published information on *S. nigricornis*, portions of this section will be written about *Sirex* and Siricinae in general.

In Arkansas, *S. nigricornis* adult emergence occurs from October to December with activity peaking around the beginning of November (Keeler 2012). Emergence of *Sirex* appears to be related to climactic conditions (Madden 1974, Neumann et al. 1987). Falling barometric pressure and above average temperatures led to maximal emergence of *S. noctilio* adults in Tasmania (Taylor 1981). In addition, when conditions were adverse some adults remained in the wood for several days even though they had already chewed exit holes (Taylor 1981).

Male *Sirex* emerge a few days to weeks before females based on observations of *S. juvencus* (Stillwell 1966) and *S. noctilio* (Rawlings 1948, Morgan and Stewart 1966b, Taylor 1981). Males aggregate in the upper branches of trees (Morgan and Stewart 1966b, Madden 1988) and are joined by newly emerged photopositive virgin females (Taylor 1981, Madden

1988). Pheromones released by males are attractive to other males and virgin females enhancing aggregation (Cooperband et al. 2012). *Sirex* adults are sexually mature at emergence and the females are parthenogenetic (Morgan 1968, Ryan and Hurley 2012). Unfertilized eggs develop into males and fertilized eggs develop into females (Peacock and Gresson 1931, Rawlings 1953). Mated females can oviposit both fertilized and unfertilized eggs, while unmated females oviposit only unfertilized eggs (Rawlings 1953, Morgan 1968, Ryan and Hurley 2012).

After mating and/or initial flight, female *S. noctilio* locate potential pine hosts for oviposition (Madden 1988, Ryan and Hurley 2012). Physiologically stressed trees releasing monoterpenes and oxygenated compounds are the most attractive to *S. noctilio* (Simpson and McQuilkin 1976, Madden 1988). When a female *S. noctilio* lands on a potential host, she locates a place to probe by dragging her abdomen over the bark and palpating with her antennae (Francke-Grosman 1939, Ryan et al. 2011c), after which she probes through the bark and into the xylem to assess host suitability for oviposition (Madden 1988). Host suitability can be determined after one or two probes (Madden 1988).

When a suitable host is found, a female will start to oviposit. Eggs of most siricid species are oviposited two to fifteen mm into the xylem although at least one species, *S. areolatus*, has been reported ovipositing into both wood and bark (Morgan 1968). The depth of oviposition depends on the wasp's ovipositor length and has been recorded reaching a depth of 19mm into the xylem (Morgan 1968, Ryan and Hurley 2012).

From the bark surface, oviposition sites containing eggs and probing sites without eggs look similar; both appearing as a single circular hole roughly 0.5 mm in diameter, although size of the hole will vary depending on ovipositor girth (Coutts and Dolezal 1969, Spradbery 1977).

Since on the bark surface it is impossible to distinguish a probed site from an oviposition site, they will both be referred to as drill sites. Drill sites of *S. noctilio* and *S. juvencus* where oviposition has occurred usually have multiple tunnels diverging in the xylem from a common entrance hole in the bark (Coutts and Dolezal 1969, Spradbery 1977). The maximum number of tunnels per drill site recorded for *S. noctilio* is six, although this occurs with a very low frequency (Madden 1988). Single tunnel drill sites are usually probe sites that do not receive eggs, but do get *Amylostereum* (Coutts and Dolezal 1969, Spradbery 1977, Neumann and Minko 1981, Ryan and Hurley 2012). In multiple tunnel drill sites, eggs are oviposited into most tunnels except the last tunnel which receives *Amylostereum* (Coutts and Dolezal 1969, Madden 1974, Spradbery 1977, Baxter et al. 1995). It follows logically and is shown empirically that as the number of tunnels increases, so too does the number of eggs laid (Coutts 1965, Madden 1974, Spradbery 1977, Madden and Coutts 1979). Interestingly, the oviposition behavior of the nearctic *S. cyaneus,* which is established in Europe, appears different to the two European *Sirex, S. juvencus* and *S. noctilio* (Morgan 1968, Spradbery 1977). *Sirex cyaneus* is more likely to limit the number of tunnels per drill site it creates to two, however, it is more likely to oviposit into single tunnels and it will often deposit multiple eggs per tunnel (Stillwell 1966, Morgan 1968, Spradbery 1977). Information on drill site architecture (i.e. number of tunnels per drill) and average number of eggs oviposited at drill sites with different architecture are lacking for *S. nigricornis*.

The condition of host material affects drill site architecture and number of eggs laid for most siricids (Morgan 1968, Spradbery and Kirk 1981) For *S. noctilio* the number of tunnels per drill site, and therefore eggs per drill site, is related to drill site conditions (Morgan 1968, Ryan and Hurley 2012). If osmotic pressure exceeds 18×10^5 Pascals, only single tunnels without

eggs are created (Madden and Coutts 1979) and as osmotic pressure in the phloem sap decreases the number of tunnels per drill site increases (Madden 1974).

Ryan et al. (2011c) provide some evidence that *S. noctilio* detect and may avoid trees colonized by some species of ophiostomatoid fungi (discussed in greater detail in later sections). This ability to discern drill site condition is likely shared among all *Sirex*, as their ovipositors contain sensillae and are similar in structure (Vilhelmsen 2000, Goulet 2012). That said, exactly what they discern may be different due to their evolutionary histories, preferred hosts and preferred host conditions.

Eclosion of *S. noctilio* larvae occurs after about two to four weeks (Morgan and Stewart 1966b, Neumann and Minko 1981) and is preceded by growth of *Amylostereum* (Madden 1981). If conditions impede fungal growth, then eclosion may be delayed (Madden 1981). The range of preferred moisture content for oviposition (40 to 75 %), also favors the growth of *Amylostereum* (Coutts 1965, Coutts and Dolezal 1965) and this moisture content likely favors eclosion and early larval development, although there are no published studies that detail this (Morgan and Stewart 1966b, Talbot 1977, Neumann and Minko 1981, Ryan and Hurley 2012).
Sirex noctilio has between six to twelve instars (Madden 1981). Early instar *S. noctilio* larvae feed on either fungus or wood (see preceding section on *Sirex-Amylostereum* complex for discussion of this apparent feeding discrepancy) and larvae mine parallel to the wood grain (Morgan 1968, Madden 1988). After reaching the third or fourth instar larvae penetrate deeper into the sapwood (Taylor 1981). Pupation occurs about 5 cm from the bark's surface and, including the prepupal stage, lasts about 8 weeks (Morgan and Stewart 1966b, Taylor 1981).

NATURAL ENEMIES OF *SIREX*

HYMENOPTEROUS PARASITOIDS

In North America there are several parasitoids, primarily Hymenoptera in the families Ibaliidae and Ichneumonidae (*Rhyssa* species), that attack *Sirex* (Borchet et al. 2007, Long et al. 2009, Eager et al. 2011, Cameron 2012, Ryan et al. 2012b). The hypothetical combined parasitism rate of *Sirex* by species of *Rhyssa* Gravenhorst, *Megarhyssa nortoni* Cresson and *Ibalia leucospoides* Hochenwarth in eastern North America and Ontario is estimated to be between 15 to 28 % with a majority of the parasitism being attributed to *I. leucospoides* (Long et al. 2009, Ryan et al. 2012b). The parasitism rate is deemed hypothetical because these parasitoids were not reared directly from *Sirex* specimens and parasitoids that emerged from trees could have parasitized the eggs and larvae of subcortical organisms other than *Sirex* (Ryan et al. 2012b). Based on rearing parasitoids from *Sirex* larvae, Eager et al. (2011) calculated a parasitism rate of *Sirex* in New York to be approximately 16% , with *I. leucospoides* accounting for a majority of the parasitism. Many of these neartic parasitoids and other related paleartic parasitoids have been released for control of *S. noctilio* in the southern hemisphere. In some cases rates of parasitism by these hymenopteran parasitoids have reached high levels of greater than 70% in the southern hemisphere, but in general they are considered insufficient for adequately controlling outbreaks of *S. noctilio* populations on their own with the average combined parasitism rate by all parasitoids being around 30% (Hurley et al. 2007, Cameron 2012). It is unknown what the rate of parasitism of *S. nigricornis* is in the southeastern United States or how these parasitoids affect their population dynamics.

NEMATODES

Deladenus (=*Beddingia*) Thorne (Tylenchida: Neotylenchidae) nematodes infest the ovaries of siricids and can limit the population growth of *Sirex* (Bedding and Iede 2005, Hurley et al. 2007, Bedding 2009). *Deladenus siricidicola* Bedding originally collected from Sopron, Hungary has been used with varying degrees of success as a biological control agent of *S. noctilio* in the southern hemisphere (Hurley et al. 2007, Slippers et al. 2012b). Many species and strains of *Deladenus* existed for use, but the Sopron strain of *D. siricidicola* and subsequent re-isolations from Kamona forest in Tasmania (Kamona strain), were selected for use as a biological control agents of *S. noctilio* because they usually caused complete sterilization of infected *S. noctilio* and had a strong potential to be dispersed by infected wasps (Bedding 1972, Bedding and Akhurst 1978, Hurley et al. 2007, Collett and Elms 2009) However, the ecological ramifications of releasing *D. siricidicola* as biological control agent for *S. noctilio* in North America are still being investigated (Williams et al. 2012). Interestingly, *D. siricidicola* is established in North American populations of *S. noctilio*; it likely was transported here within the invading *S. noctilio* population (Yu et al. 2009, Williams et al. 2012). However, the strain of *D. siricidicola* in North America (neither Sopron nor Kamona) does not cause sterility of *S. noctilio* and whether it will have an impact on *S. nigricornis* populations is unknown (Ryan et al. 2012b, Williams et al. 2012).

There is a native nematode, *Deladenus proximus* Bedding, that parasitizes *S. nigricornis* (Bedding 1974, Bedding and Akhurst 1978). Parasitism by *D. proximus* reportedly results in complete sterilization of infected *S. nigricornis* females (Bedding 1974). This assertion is based on the assumption that all eggs of infected *S. nigricornis* females are penetrated by *D. proximus*

juveniles (Bedding 1974). Contrarily, nematodes in the ovaries of infested Arkansas *S. nigricornis* rarely penetrate all of the eggs (Keeler 2012).

BIRDS

Birds have also been shown to prey on siricids. Woodpeckers prey on larvae (Marshall 1967, Spradbery 1990) and aerially hunting birds consume adults (Madden 1982). Birds undoubtedly contribute to woodwasp mortality, but their overall impact on a large scale, although hard to quantify, is likely minimal (Marshall 1967, Madden 1982, Spradbery 1990).

INTERACTIONS WITH SUBCORTICAL ASSOCIATES

There are many insects other than woodwasp that colonize xylem and phloem of damaged pine trees (USDA-FS 1985, Hanula 1993). Both direct and indirect interactions may occur between these subcortical insects and *Sirex* (Ryan et al. 2011a). Examples of direct interactions are competition and predation. Examples of indirect interactions include tree partitioning and augmentation of host characteristics.

There is likely little direct competition for space between *Sirex* and other insects. *Sirex* develop within the xylem, a relatively spacious part of the host that is not colonized by many other insects (Hanula 1993, Ryan et al. 2011a). Interestingly, few studies have looked at competition among insects for tree xylem, but many authors ascribe the colonization of wood xylem by insects as an escape from the intense competition that occurs in phloem [e.g. (Farrell et al. 2001, Harrington 2005)].

Facultative predation of *Sirex* larvae by other subcortical larvae may occur. Cerambycid larvae have been shown to prey on larvae of other subcortical insects (Victorsson and Wikars 1996, Dodds et al. 2001, Ware and Stephen 2006, Schoeller 2011). This predation is generally

regarded as facultative, in that the cerambycid larvae normally are not actively seeking out their prey (Dodds et al. 2001). The larvae of other subcortical insects including buprestids and tenebrionids have also been considered to be facultative predators (Goyer and Smith 1981, Gindin et al. 2009, Richardson et al. 2010). The rate of facultative predation of one larval insect on another relates to many factors, but density within host substrate and the relative size of the larvae involved appear to play a strong role (Richardson et al. 2010). The extent to which facultative predation affects *S. nigricornis* is unknown.

An indirect interaction will occur if an insect changes its oviposition and colonization behavior because host material has been previously colonized. The change in these behaviors can either be increased or inhibited by the presence of other insects. Increases in oviposition or colonization will occur if host substrate is deemed more suitable by a secondary colonizer than it would be in the absence of previous colonizers (Kaplan and Denno 2007). Indeed, many subcortical insects start to colonize a host after it has been compromised by feeding or attacks from earlier colonizers (Stephen and Dahlsten 1976, Spradbery and Kirk 1978, Klepzig et al. 1991, Schroeder 1997, Aukema et al. 2004, Kaplan and Denno 2007, Smith et al. 2011, Stephen 2011). Spradbery and Kirk (1978) detail several examples of defoliators (e.g. *Choristoneura murinana* (Hübner)) or beetles (e.g. *Ips*, *Hylobius* and *Pissodes*) stressing trees to the point that they became particularly susceptible to attack by *S. juvencus* and *S. noctilio*. Mass outbreaks of two European *Sirex* species were associated with previous attacks on host trees by *C. murinana* (Spradbery and Kirk 1978).

Besides compromising a host that makes it more susceptible to attacks, other subcortical insects may change the attractiveness of host material. Almost all subcortical insects show a preference for wood of a certain moisture content or stage of decay and these changes in host

composition are often mediated by earlier colonizers (Graham 1925, Speight 1989, Stevens 1997, Grove 2002, Jacobs et al. 2007). As mentioned in preceding sections, *S. noctilio* shows a preference for oviposition depending on moisture content and these changes in moisture can be brought on by other colonizers (Coutts 1965, Morgan and Stewart 1966b, Speight 1989). Like their vectors, the presence of fungi inoculated by subcortical insects may facilitate and increase oviposition and colonization by secondary colonizers either through weakening host defenses (Spradbery and Kirk 1978, Paine et al. 1997) or through increasing attractiveness of the host (Belmain et al. 2002, Johansson et al. 2006). The woodwasp *X. spectrum*, which has no fungal symbiont of its own, oviposits more on logs previously colonized by other siricids and therefore containing *Amylostereum* than on trees without the fungus (Fukuda and Hijii 1997).

Inhibition of oviposition and colonization also may exist among *Sirex* and other subcortical insects. For instance, vertical partitioning of host resources in the presence of competitors is well documented for the bark beetle guild (Paine et al. 1981, Rankin and Borden 1991, Schlyter and Anderbrant 1993, Ayres et al. 2001, Stephen 2011). Such partitioning of a tree is often mediated by chemical feedback mechanisms, acoustic communication, visual inspection and niche preference all of which should function to reduce interspecific competition (Birch et al. 1980, Flamm et al. 1987, Byers 1989b, a, Schlyter and Anderbrant 1993). However, it does not appear that *Sirex* avoid any sections of the tree bole as *S. noctilio* adults emerge from all sections of the tree bole regardless of the presence of potential competitors (Wermelinger et al. 2008, Ryan et al. 2011a). This suggests that *Sirex* do not exhibit vertical resource partitioning. This is likely due to the fact that *Sirex* inhabit the relatively spacious, competition free xylem while the majority of other subcortical insects predominately spend their time feeding in phloem (Farrell et al. 2001, Harrington 2005, Ryan et al. 2011a). *Sirex* might not be inhibited

by the presence of other insects, but suboptimal moisture content or stage of decay unsuitable for oviposition and colonization of host material can be facilitated by insects and their associated fungi (Graham 1925, Speight 1989, Grove 2002).

Although *Sirex* may not be inhibited by the presence of other insects, the woodwasps may avoid ovipositing on hosts that have been inoculated by fungi associated with these insects (Ryan et al. 2012a). During colonization subcortical beetles and their phoretic mites inoculate Ophiostomatoid fungi into host material (Moser 1985, Wingfield 1987, Paine et al. 1997, Jankowiak and Rossa 2007, Hofstetter 2011) and wind-born fungal spores colonize larval galleries (Kukor and Martin 1986, Speight 1989, Martin 1992, Siitonen 2001). These fungi when present in host material may inhibit oviposition of *Sirex.* There are anecdotal accounts of *Sirex* avoiding or reducing oviposition activity on areas of hosts that contain fungi other than *Amylostereum* (herein termed non-*Amylosterum* fungi unless a more descriptive term for the fungus involved is used) (Hanson 1939, Spradbery and Kirk 1978, Fukuda and Hijii 1996), but only one study provides evidence that *Sirex* change their behavior in the presence of potentially antagonistic fungi, although it is still unknown if the non-*Amylostereum* fungi caused a reduction in oviposition (Ryan et al. 2012a). Avoiding hosts with potentially antagonistic fungi would be beneficial to the offspring of *Sirex* if these fungi cause a decrease in development or survivorship. No studies have looked directly at the effect non-*Amylostereum* fungi have on *Sirex* survival, but some of these fungi reportedly cause offspring mortality (Hanson 1939, Rawlings 1953, Coutts 1965, Morgan and Stewart 1966a, Neumann and Minko 1981).

IMPACTS OF NON-*AMYLOSTEREUM* FUNGI ON DEVELOPING *SIREX*

Subcortical insects interact with fungi in a variety of ways and the ultimate impact of this interaction depends on the organisms involved (Klepzig et al. 2001, Six and Wingfield 2011). Already discussed in the preceding section is how fungi can condition host material to make it more or less suitable to colonizing insects (Graham 1925, Speight 1989).

Fungi will have a more direct impact on these insects if the two co-occur within host material. Many ophiostomatoid fungi are known to redistribute nitrogen, vitamins and other nutrients, concentrating them in their mycelia and this provides increased nutrient consumption to the beetle larvae that feed on fungi (Ayres et al. 2000, Harrington 2005, Bleiker and Six 2007). Developmental benefits derived from consuming these fungi include reduced developmental times, larger body size, higher fecundity and a higher likelihood of successful development (Bleiker and Six 2007, Six and Wingfield 2011). Incidental consumption of non-mycangial fungi by cerambycid beetles (e.g. *Monochamus marmorator* Kirby feeding on *Trichoderma harzianum* Rifai) has been shown to increase the efficiency of food utilization, primarily the digestion of cellulose, and this is linked to increased rates of growth and development of their larvae (Kukor and Martin 1986, Kukor et al. 1988, Martin 1992).

In contrast to the benefits some non-mycangial fungi confer to developing subcortical larvae, some species of fungi are antagonistic to insect offspring that develop in their presence. The presence of *Ophiostoma minus* (Hedgcock) H. & P. Sydow is correlated with slowing the growth and development of southern pine beetle, *Dendroctonus frontalis* Zimmerman, larvae and in some cases can even cause larval mortality (Barras 1970, Goldhammer et al. 1990, Hofstetter et al. 2006, Klepzig and Hofstetter 2011). The negative impact that *O. minus* has on developing southern pine beetles is attributed to the phoretic *O. minus* outcompeting the beetles' mycangial

fungi, *Ceratocystiopsis ranaculosa* Perry & Bridges and *Entomocorticium* sp. A (Klepzig and Wilkens 1997, Hofstetter et al. 2006). Ophiostomatoid fungi may impact *Sirex* by competing with their mycangial fungi. Ophiostomatoid fungi are usually inoculated into the phloem of host material by phoretic mites associated with colonizing bark beetles (Bridges and Moser 1983, Moser and Bridges 1986), but the fungi readily grow into the rays and tracheids of sapwood (Ballard et al. 1984, Six and Wingfield 2011). It is in the xylem sapwood where ophiostomatoid fungi would interact with *Sirex* and their fungal symbiont, *Amylostereum*. Ryan et al. (2011b) investigated the interaction between *A. areolatum* and two subcortical beetle vectored ophiostomatoid fungi, *O. minus* and *Leptographium wingfieldii* Morelet, on PDA and *Pinus sylvestris* Linnaeus woodchips. They demonstrated that both beetle vectored fungi were usually able to capture more uncolonized resources than *A. areolatum* regardless of temperature or substrate (Ryan et al. 2011b). They also found that some strains of the ophiostomatoid fungi could capture *P. sylvestris* substrate from live *A. areolatum* cultures, but that *A. areolatum* never colonized space occupied by living cultures of the ophiostomatoid fungi (Ryan et al. 2011b).

The growth of *Amylostereum* is reportedly hindered by fungi other than ophiostomatoid fungi, although the reports are only anecdotal. *Trichoderma* instead of *Amylostereum* have been recovered in areas where care was taken to inoculate only *Amylostereum* into host trees (Tabata and Abe 1999) and *Trichoderma* as well as *Sphaeropsis sapinea* (Fries) Dyko & B. Sutton supposedly cause death of *Amylostereum* hyphae on agar cultures (King 1966). These results suggest that at least some fungi vectored or aided in establishment by subcortical beetles can impact the growth of *Amylostereum*. This in turn could have an impact on the successful development and survival of *Sirex* offspring because they are, at least during the early larval stages, dependent on *Amylostereum* for nutrition and the larvae may starve if growth of

Amylostereum is inhibited (Stillwell 1966, Coutts and Dolezal 1969, Madden and Coutts 1979, Martin 1992, Ryan et al. 2011b). In addition, where conditions for the growth of *Amylostereum* are optimal, larger more fecund siricid adults emerge (Madden 1974). If competition from non-*Amylostereum* fungi limit the growth of *Amylostereum*, then this may affect the size and fecundity of emerging *Sirex* adults (Madden 1974, 1981, Neumann and Minko 1981, Ryan et al. 2011b). No studies have directly examined the effect non-*Amylostereum* fungi have on *Sirex* offspring, but some of these fungi reportedly cause offspring mortality (Hanson 1939, Rawlings 1953, Coutts 1965, Morgan and Stewart 1966a, Neumann and Minko 1981).

SUMMARY

The recent discovery in New York and Ontario of a Eurasian woodwasp, *Sirex noctilio*, has initiated research on *Sirex* species in North America. *Sirex noctilio* has caused considerable damage to some pine plantations in the southern hemisphere, but it is not considered a serious pest in its native range. Pine is introduced into the southern hemisphere and as a consequence there are few subcortical insects or other endemic organisms that inhabit those plantations. In contrast, there is a wealth of insects and other organisms that inhabit pine stands and plantations in the northern hemisphere where pine is native. *Sirex* species undoubtedly interact with other subcortical insects, but there is a lack of knowledge on these interactions or how these species impact *Sirex*. *Sirex* may alter their oviposition behavior in the presence of these co-habitants. The presence of subcortical insects and their associated fungi could also affect *Sirex* development and survival.

Sirex have an obligate relationship with *Amylostereum* fungi, its presence being required for successful development of their offspring. Not only is *Amylostereum* required for successful

offspring development, but it can also affect adult body size. Larger wasps emerge in conditions where growth of *Amylostereum* is favored and they have comparatively greater potential and realized fecundities, dispersal ability and longevity.

In general, all *Sirex* have similar biology and behavior, yet there are differences among species. Much more is known about *S. noctilio* and other Eurasian species than North American *Sirex* species. There is a particular lack of published information on the biology and behavior of the *Sirex* species that occurs in the southern United States, *S. nigricornis*.

In order to better understand and predict the impact that *S. noctilio* will have in southern pine forests, more research needs to be conducted on how *Sirex* species interact with subcortical insects that inhabit this forest. Acquiring information on the biology and behavior of *S. nigricornis* will allow for better comparisons and contrasts to be made between it and *S. noctilio* and the factors that impact these species.

RESEARCH OBJECTIVES

The objectives of this research were to investigate how subcortical insects, primarily beetles, interact with and influence oviposition behavior and early stage survival of *S. nigricornis* offspring. Active trapping was used to determine the relative phenology and potential interactions of *S. nigricornis* with subcortical beetles (Chapter one). Choice tests between beetle-colonized bolts (=logs) and non-colonized bolts were used to determine if *S. nigricornis* changes its oviposition behavior in the presence of these beetles (Chapter two). To determine the impact that subcortical beetles have on early stage survival of *S. nigricornis*, beetle-colonized bolts and non-colonized bolts that *S. nigricornis* oviposited into were dissected and compared (Chapter three). Also addressed throughout this research are gaps in knowledge about the

specific biology and behavior of *S. nigricornis*. Information gained from addressing these objectives increases our understanding of community interactions as well as factors that affect *Sirex.*

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CHAPTER 2 – INVESTIGATION INTO INTERACTIONS BETWEEN *SIREX NIGRICORNIS* AND ASSOCIATED SUBCORTICAL BEETLES IN TORNADO-DISTURBED PINE STANDS OF ARKANSAS' OZARK NATIONAL FOREST

ABSTRACT

The woodwasp *Sirex nigricornis* Fabricius colonizes the bole of stressed and damaged pine trees and these trees commonly are also colonized by subcortical beetles. These subcortical beetles will interact with *S. nigricornis* if they both attempt to colonize the same host substrate. Exactly how these insects interact with *S. nigricornis* will depend, in part, on the timing of host colonization by the species in question. Identification of potential *S. nigricornis* associates and their relative flight activity was investigated by setting up traps baited with pine volatiles and *Ips* pheromones at ten different plots in Arkansas' Ozark National Forest. Five plots had been recently disturbed by tornadoes. Results from this trapping study indicate that subcortical inhabiting beetles in the families Cerambycidae, Buprestidae and Scolytidae are likely to interact with *S. nigricornis*. Buprestids and cerambycids were trapped with greater frequency at sites that had been disturbed by tornadoes 4-6 months prior to trapping than at non-disturbed sites. *S. nigricornis* was trapped with similar frequency at disturbed and non-disturbed sites. In general, peak flight of subcortical beetles ended before *S. nigricornis* flight reached its apex, affording these beetles and their associated fungi the opportunity to colonize host material in advance of *S. nigricornis* and its fungal symbiont. The presence of these beetles and their associated fungi can affect oviposition behavior of *S. nigricornis* adults. In addition, survival of *S. nigricornis* offspring may be altered by the presence of these beetles via direct or indirect interactions.

INTRODUCTION

Sirex Linnaeus (Hymenoptera: Siricidae) woodwasps colonize xylem in the bole of declining and damaged conifer trees. In general, these woodwasps colonize only physiologically stressed trees that are in a weakened condition (Spradbery and Kirk 1978, Dodds et al. 2010). Members of the genus *Sirex* in their home ranges are considered secondary pests because they are rarely implicated in causing large-scale tree mortality (Spradbery and Kirk 1978, Wermelinger and Thomsen 2012). Interestingly, a Eurasian species, *S. noctilio* Fabricius, sometimes causes extensive tree mortality where it has invaded plantations of *Pinus radiata* D. Don planted in the southern hemisphere [(Neumann and Minko 1981, Madden 1988, Hurley et al. 2007); but see chapters 13-18 in (Slippers et al. 2012)]. Pine trees are not native to the southern hemisphere and thus relatively few pine-inhabiting insects are found in these plantations (Wingfield et al. 2008). In contrast, there are numerous insects that colonize declining and recently damaged pine in the northern hemisphere and many of these insects, a majority of which are beetles, co-occur on the bole of the tree with *Sirex* (USDA-FS 1985, Hanula 1993, Gandhi et al. 2007, Wermelinger et al. 2008, Ryan et al. 2011a). This has led researchers to speculate that the unprecedented damage caused by *S. noctilio* in the southern hemisphere is due, in part, to a lack of insect associates exerting negative pressure on burgeoning populations; affording them the ability to reach levels high enough to cause considerable tree mortality (Dodds et al. 2010, Ryan et al. 2011a). However, more research needs to be conducted on *Sirex* populations in endemic habitats to understand how community associates impact the wasp (Dodds et al. 2010).

For many forest insects that periodically cause wide-scale damage, reaching epidemic outbreak levels often involves breeding on compromised or damaged host material stressed by

poor stand conditions or environmental disturbances (Bouget and Duelli 2004, Wermelinger 2004, Dodds et al. 2007, Gandhi et al. 2007, Schowalter 2012). Such disturbances and poor stand management have been linked to outbreaks of *S. noctilio* (Morgan 1968, Neumann and Minko 1981, Madden 1988, Hurley et al. 2007). Of concern in the southeastern United States is that frequent disturbances, such as tornadoes, hurricanes, ice storms, and lightning that create an increase in host material by damaging and uprooting trees will increase the likelihood that *S. noctilio* will successfully establish and become economically damaging (Borchert 2007, Dodds and de Groot 2012).

These debilitated host trees go through three phases of decomposition: the colonization phase, then the decomposition phase and finally the humification phase (Speight 1989). These phases of decomposition are initiated by insects, their phoretic mites and associated fungi which invade intact wood during the colonization phase (Speight 1989). *Sirex* belong to this first phase of colonizers (primary saproxylics) and it is other primary saproxylics that are the most likely to interact with *Sirex*. Primary saproxylic insects are predominately beetles (Speight 1989, Siitonen 2001, Grove 2002, Jacobs et al. 2007). Frequent and abundant groups of primary saproxylic beetles include bark beetles (Curculionidae: Scolytinae), long-horned beetles (Cerambycidae) and flat-head borers (Buprestidae) (Speight 1989, Siitonen 2001, Wermelinger et al. 2002).

Sirex could be impacted by the presence of co-occurring subcortical beetles in numerous ways. Subcortical larvae sometimes feed facultatively on one another (Goyer and Smith 1981, Dodds et al. 2001, Richardson et al. 2010) and they may compete for host resources (Coulson et al. 1976, Paine et al. 1981). However, colonization of xylem is thought to be an escape from the intense competition that occurs in phloem (Farrell et al. 2001, Harrington 2005). Therefore, *Sirex* may not be greatly impacted by direct competition from these beetles; most of which spend

a predominant amount of time in the phloem (Hanula 1993, Siitonen 2001). Impacts of these beetles on *Sirex* are more likely to occur via indirect interactions of associated fungi (Ryan et al. 2011a).

All *Sirex* have an obligate association with decay fungi in the genus *Amylostereum* (Morgan 1968, Martin 1992, Wermelinger and Thomsen 2012). Eggs will hatch when *Amylostereum* is excluded from host material, but no larvae will develop into pupae (Stillwell 1966); strongly suggesting that *Sirex* development is contingent on the presence of their fungal symbiont. In addition, when conditions are optimal for the growth of *Amylostereum*, larger more fecund *Sirex* emerge (Madden 1974, Madden and Coutts 1979, Madden 1981, Neumann and Minko 1981, Fukuda et al. 1993). Therefore, factors affecting the establishment and subsequent growth of *Amylostereum* on host material could affect population dynamics of *Sirex*.

During colonization of host material, primary saproxylic beetles and their phoretic mites inoculate fungi, most notably ophiostomatoid fungi, into the wood (Bridges and Moser 1983, Wingfield 1987, Speight 1989, Paine et al. 1997, Jankowiak and Rossa 2007). Holes and galleries created in the wood by these beetles are also frequently colonized by airborne fungi and other opportunistic microbes (Kukor and Martin 1986, Martin 1992, Siitonen 2001). The impact that these non-*Amylostereum* fungi have on *Sirex* larvae could be positive or negative. Some ophiostomatoid fungi concentrate nitrogen and other nutrients in their mycelia and larvae of subcortical insects that opportunistically feed on these fungi derive developmental benefits such as shorter developmental time, larger body size, higher fecundity and higher likelihood of successful development (Ayres et al. 2000, Harrington 2005, Bleiker and Six 2007, Six and Wingfield 2011). Facultative ingestion of fungi can benefit developing larvae in other ways too. Larvae of subcortical insects that facultatively feed on decay fungi may acquire digestive

enzymes that increase their ability to digest constituents of wood (e.g. cellulose and hemi-cellulose) and this too has been linked to developmental benefits (Kukor and Martin 1986, Kukor et al. 1988). *Amylostereum* already confers many of these benefits to *Sirex*; such as the acquisition of enzymes necessary for digesting wood (Kukor and Martin 1983) and there is some evidence that it affords *Sirex* access to more nitrogen (Gu 2010), although this has not been tested directly. Therefore accidental ingestion of non-*Amylostereum* fungi may not be of a great benefit to *Sirex* since *Amylostereum* already fills nutritional roles.

It is more likely that non-*Amylostereum* fungi are antagonistic to *Sirex.* The presence of *Ophiostoma minus* (Hedgcock) H. & P. Sydow is correlated with slowing the growth and development of southern pine beetle, *Dendroctonus frontalis* Zimmerman, larvae and in some cases can even cause larval mortality (Barras 1970, Goldhammer et al. 1990, Hofstetter et al. 2006, Klepzig and Hofstetter 2011). The negative impact that *O. minus* has on developing southern pine beetles is attributed to the phoretic *O. minus* outcompeting the beetles' mycangial fungi, *Ceratocystiopsis ranaculosa* Perry & Bridges and *Entomocorticium* sp. A (Klepzig and Wilkens 1997, Hofstetter et al. 2006). Similarly, ophiostomatoid fungi may impact *Sirex* by competing with their mycangial fungi. Ophiostomatoid fungi are usually inoculated into the phloem of host material, but the fungi readily grow into the rays and tracheids of sapwood (Ballard et al. 1984, Six and Wingfield 2011). It is in the xylem sapwood where ophiostomatoid fungi would interact with *Sirex* and their fungal symbiont, *Amylostereum.* Ryan et al. (2011b) investigated the interaction between *A. areolatum* and two subcortical beetle vectored ophiostomatoid fungi, *O. minus* and *Leptographium wingfieldii* Morelet, on PDA and *Pinus sylvestris* Linnaeus woodchips. They demonstrated that both beetle vectored fungi were usually able to capture more uncolonized resources than *A. areolatum* regardless of temperature or

substrate (Ryan et al. 2011b). They also found that some strains of the ophiostomatoid fungi could capture *P. sylvestris* substrate from live *A. areolatum* cultures, but that *A. areolatum* never colonized space occupied by living cultures of the ophiostomatoid fungi (Ryan et al. 2011b). The growth of *Amylostereum* is reportedly hindered by fungi other than ophiostomatoid fungi, although the reports are only anecdotal. *Trichoderma* instead of *Amylostereum* have been recovered in areas where care was taken to inoculate only *Amylostereum* into host trees (Tabata and Abe 1999) and *Trichoderma* as well as *Sphaeropsis sapinea* (Fries) Dyko & B. Sutton supposedly cause death of *Amylostereum* hyphae on agar cultures (King 1966). These results suggest that at least some fungi vectored or aided in establishment by subcortical beetles can impact the growth of *Amylostereum*.

Understanding the phenology of these subcortical beetles in relation to the phenology of *Sirex* is important because the interactions that occur and the subsequent impacts on *Sirex* relate to the timing these species colonize host material (Ryan et al. 2011a). Insects that colonize host material first change the composition of the host substrate for subsequent colonizers (Speight 1989, Smith et al. 2011). A benefit conferred to insects with relatively later adult activity is the ability to avert interspecific competition by avoiding already colonized host material [e.g. (Paine et al. 1981, Flamm et al. 1987, Byers 1989, Rankin and Borden 1991, Schlyter and Anderbrant 1993, Ayres et al. 2001)]. In contrast, the presence of previous colonizers may facilitate host colonization for later arriving insects; sometimes with the potential negative effects of interspecific competition being balanced-out by positive effects the presence of the first colonizer confers on the second (Spradbery and Kirk 1978, Schroeder 1997, Smith et al. 2011). In addition, the outcome of interactions between *Amylostereum* and beetle vectored fungi depends, in part, on the timings of inoculation (Klepzig et al. 2009, Ryan et al. 2011b).

The objectives of the research conducted in this chapter were to: 1) identify the large subcortical insects that are most likely to interact with a native woodwasp *Sirex nigricornis*; 2) determine the relative phenology of these insects to *S. nigricornis*; 3) determine how these insects and *S. nigricornis* respond to varying abundances of host material. Information gained about the interactions between *S. nigriconis* and other subcortical insects will strengthen our knowledge about how woodwasp populations interact with their community associates and this can aid in predicting the extent and severity of *S. noctilio* establishing in the southeastern United States. To investigate these objectives baited panel traps were deployed in the fall of 2011 across a wide range of recently damaged host material throughout Arkansas' Ozark National Forest to trap for *S. nigricornis* and associated subcortical insects.

MATERIALS AND METHODS

TRAPS

Traps were created by directly joining commercially available flight intercept panel traps (APTIV Intercept™) to the snap-on lid of 33 gallon Rubbermaid® trashcans (dimensions: 66 x 56 x 72 cm) which served as receptacles for live insects. A square hole (~ 15 x 15 cm) slightly larger than the bottom funnel portion of the panel trap was removed from the center of a trashcan lid. The portion of the panel trap which normally supports a collection container was set inside the square hole on the outside top of the trashcan lid and affixed to the lid by riveting 6.25 cm semi-circles of plastic, cut out of the trashcan, to each side of the funnel and then to the top of the lid. Duct tape sealed the space surrounding the point where the panel trap entered the trashcan lid from the underside, while silicone caulk was used to seal any gaps around the point of entry on top of the trash can lid. The bottom funnel portion of the panel trap and the lid of the trashcan

were then one apparatus (Figure 1). This allowed the lid apparatus to be snapped onto the receptacle of the trashcan and the panel-trap to be set up on top, effectively creating one trapping unit (Figure 2) with insects flying into the panel being funneled into the trashcan.

The receptacle of the trashcan was modified in the following ways. Four 8 mm holes were drilled into the base of the trashcan with a drill bit to allow water to drain. Fourteen holes were cut from the side of the receptacle with a 6.7 cm hole-saw to increase airflow. The holes were cut in three rows: six holes ~ 6 cm from the top of the trashcan, 4 holes ~ 30 cm from the top and 4 holes ~ 56 cm from the top. Lumite® mesh was placed over all of the holes and hot-glued in place to keep trapped insects from escaping. Weather stripping was adhered to the top of the rim of the receptacle to create a tighter seal between the receptacle and the lid. Withford Dispersion #1 (Withford Corp., Frazer, PA), a polytetrafluoroethylene, was applied with a paint brush to the inside of the funnel. Additional Withford Dispersion #1 was applied to the inside of the lid and around the rim of the trashcan to deny purchase to escaping insects. The trashcan and lid, but no parts of the flight intercept trap were spray painted white to reduce the amount of heat absorbed by the trashcan. This had the added benefit of allowing notes (e.g. collection dates, lure change dates, trap ID, etc.) to be written on the trashcan.

In the field, the trashcan receptacle was placed on the ground, the lid apparatus snapped in place and then the remaining part of the panel trap joined to the funnel. Sticks collected on the ground were placed in the holes for connecting the funneling base of the trap to the panel in lieu of plastic clips normally used, which joined the vane to the entire trap apparatus. The panel trap was supported from the top as it would normally be from a bent pole of 1.9 cm electrical metal tubing (EMT) with a hole in the end. The EMT pole was attached with baling wire to a steel T-post driven ~ 25 cm into the ground. Supporting the panel trap in this manner allows one to

service the trap receptacle and remove insects, replace bolts, etc. while keeping the lid and vanes suspended above the ground at the appropriate height (Figure 3). To keep the trap from blowing over in the wind, a handle of the trashcan was tied to the T-post. Pine bolts or boughs were placed inside the container to provide refuge to trapped insects.

A collection funnel was created out of a trashcan lid and a plastic polyethylene sheet (~ 70 X 40 cm, and 2 mm thick; Figure 4) to collect insects from the receptacle. The polyethylene sheet was made into a funnel by wrapping one edge over the other and then riveting it in place. The top of the funnel had a hole approximately 8 cm in diameter and the base of the funnel had a hole approximately 38 cm in diameter. 2 cm deep cuts approximately 10-15 cm wide were cut out of the base of the funnel leaving eight flanges (~ 2 cm deep by 8 cm wide). These flanges were then bent and riveted to the outside top of the trashcan lid. Silicone caulk was used to seal the base of the funnel to the top of the lid. After removing pine bolts or bows inside the trashcan receptacle and brushing insects clinging to substrate back into the receptacle, the funnel could be snapped onto the lid of the trashcan (Figure 4). The entire unit could then be inverted to funnel insects into a dry collection bag.

TRAPPING LOCATIONS

A total of ten trapping sites were selected in stands containing *Pinus echinata* Miller in two regions (OZ and WED) of Arkansas' Ozark National Forest in the fall of 2011 (Figure 5). The OZ region and WED region were considered to be spatially separated from each other with little forest habitat between the two regions. It is therefore assumed that populations of insects in one region do not easily move to the other region. Of the ten sites, five 'disturbed' sites were purposefully selected for their abundance of windthrown pine. Five control sites were also selected for having similar topographical attributes (aspect, altitude, slope face, etc.) and forest

composition to a disturbed site, but without an abundance of windthrown material (Table 1 and Table 2). One other clear difference between disturbed and undisturbed sites was canopy cover. Many of the dominant trees in disturbed sites that were contributing to the canopy were no longer contributed after being blown down. The loss of these trees from the canopy is reflected in the lower, on average, basal area in disturbed sites compared to undisturbed sites (Table 2).

Eight sites in the Ozark region, four control (OZ1con, OZ2con, OZ3con, OZ4con) and four disturbed (OZ1tor, OZ2tor, OZ3tor, OZ4tor), were selected for trapping near the towns of Ozark and Ozone in the Pleasant Hill Ranger District (Figure 5, Table 1). The general region is mountainous with a large continuous forest (> 500,000 ha) containing small bits of farmland interspersed throughout. All sites were located approximately 3 to 12 km inside the southern edge of the larger forested area. Forest habitat was pervasive and relatively contiguous between these sites, although the hardwood/softwood composition varied. Given the continuity of forest between OZ sites, it was assumed that target insect populations could travel freely throughout the region. Every site was oriented on the south side of a mountain. Sites were at least 1 km away from the next nearest site except for OZ3tor and OZ4tor which were ~ 160 m apart, and OZ3con and OZ4con which were ~ 300 m apart. The windthrown pine in disturbed sites of the OZ region were presumed to be the result of EF3/EF4 tornadoes occurring on May 24th and 25th, 2011 (NOAA-NWS 2011, Taylor 2011).

One disturbed site (WEDtor) and one control site (WEDcon) in the WED region were selected for trapping near Lake Wedington in the Boston Mountain Ranger District (Figure 5c; table 1). The region is relatively flat compared to the OZ sites. The forested area containing these sites is roughly 55,000 ha and is surrounded by farmland. Both sites are situated along the southwestern side of the forested area. Each site is inside forested area, but is located less than

100 m from the forest edge. The two sites are separated by at least 4 km. Continuous forest habitat connecting the two sites led to the assumption that target insect populations could travel freely between the two sites. The windthrown pine at WEDtor is presumed to be the result of an EF3 tornado passing through the area on December 31st, 2010 (NOAA-NWS 2010, Sullivan 2011, Vreeland 2011).

CHARACTERIZATION AND DESCRIPTION OF TRAPPING SITES

Plots for estimation of total basal area, pine basal area and downed coarse woody pine (CWP) volume were adapted from FIA protocols for estimating coarse (=downed) woody debris (CWD or DWD) using fixed radius plots (USDA-FS 2011; Figure 6). Plots used for these estimations consisted of four subplots. Subplot 1 was placed in the direct center of a site. The center of the remaining three subplots were placed 37 m from the center of subplot 1. The azimuth for subplot 2 was 0˚, subplot 3 was 120˚ and subplot 4 was 240˚.

Diameter at breast height (dbh) of all trees within a 7.3 m radius of subplot centers was measured with tree calipers. Only trees with a dbh greater than 10 cm were recorded and used in calculations. Total basal area and pine basal area for a site (Table 2) was calculated by averaging the estimated values from the four subplots.

For estimating CWP volume, two 18 m long transects originating from the center of every subplot were established; totaling eight transects per site. Transect orientation differed depending on subplot number (Figure 6). The azimuths of transects for subplots 1 and 4 were 150˚ and 270˚. The azimuths of transects for subplots 2 and 3 were 30˚ and 150˚. Pine trees used in estimating CWP volume needed to be downed (leaning more than 45 degrees from vertical) and needed to be greater than 10 cm at the point of intersection with a transect. The diameter of the largest end and smallest end of downed pine intersecting a transect was measured

with tree calipers. If the smallest end was less than 10 cm in diameter, then the diameter was marked down as 10 cm. The length of bole between the two diameter measurements was measured with measuring tape. Coarse woody pine volume contributed by a given downed pine tree was calculated as if it were the frustum of a cone. Estimated CWP volume (Table 2) for a site was calculated as the sum of CWP volume contributed by each downed pine tree.

TRAPPING METHODOLOGY

At each site, three traps were placed 15 m apart at azimuths 120˚ from each other. Each trap was 8.66 m from the center of subplot 1. Traps were baited with pine volatiles (EtOH and α-pinene) and *Ips* pheromones (ipsenol, ipsdienol, lanierone) lures (Synergy Semiochemical, Burnaby, BC, Canada). Lures were changed once around October 18th. Traps were erected the second week in September and they were collected on a weekly basis starting the third week in September through the third week of November. Final collection of traps occurred on November 30th at the Wed-sites and December 8th at the Oz-sites. Trap samples were initially placed in -4˚C to kill living insects after which they were stored in 4˚C until processing.

INSECT IDENTIFICATION, COUNTING AND SEXING

Only species in target families were counted. Target families included Siricidae, Buprestidae and Cerambycidae. These families were selected based on their known association with pine trees and the relative size of the specimens which allowed for easy and accurate counting. Bark beetles (Scolytidae) are known to be common associates of physiologically stressed pine trees, but they were not counted owing to their small size and the amount of time required to accurately count and separate species. All non-target subcortical pine colonizing insects discovered when processing trap catches were recorded, but not counted. It is possible that small, rare species were missed and thus excluded from the record; however, if a species is

rare then the interactions it will have with *Sirex* is limited and hence of little concern regarding this study.

Siricidae were identified to species using Schiff et al. (2006) with appropriate taxonomic revisions according to Goulet (2012) being accounted for. Cerambycidae were identified to species using Yanega (1996) and Arnett et al. (2002). The taxonomic characters used to separate *Monochamus carolinensis* Olivier from *Monochamus titillator* Fabricius are unreliable and the two species are broadly sympatric in pine stands (Linsley and Chemsak 1984, Yanega 1996, Miller et al. 2011). Due to the uncertainty of distinguishing between these two species, the designation by Miller et al. (2011) of placing *M. titillator, M. carolinensis* and any possible hybrids into a *Monochamus* spp. was adopted. The gender of *M. titillator* specimens was determined by the presence (females) or absence (males) of anal pubescence (Linsley and Chemsak 1984). The gender of two other cerambycids, *Acanthocinus obsoletus* Olivier and *A. nodosus* Fabricius, was determined by the conspicuously extruded ovipositor on females (Linsley and Chemsak 1995). Buprestidae were identified using Hansen (2010). All other specimens were identified by referencing with previous lab collections and by using Arnett et al. (2002) and Arnett and Thomas (2001). Whenever possible, taxonomic names and authorities were checked using ITIS (2012).

Trap catch numbers were collected on all target families (Siricidae, Cerambycidae and Buprestidae). Presence of Curculionidae and other subcortical pine colonizing insects was noted, but abundance was not recorded. Phenology curves for total cerambycid, buprestid and *Sirex* trapped where summed across all traps and sites.

ANALYSIS

All statistical analysis was performed using JMP Pro 9 (SAS Institute Inc., Carey, NC). Trap catch data were summed over all dates for individual traps. Comparisons were made with sites being the sample unit and each trap at a site being a measurement. Analyses of trap catches were performed only on data from the OZ region. Data from the WED region and OZ region were not combined for analysis because of differences in the dates of tornado disturbances and because there were clear differences in the mean number (*t*-test: $t_{28} = 2.43$, $P = 0.0215$) and mean ranks (Kruskal-Wallis test: $\chi^2 = 9.2$, df = 1, $P = 0.0024$) of total target specimens trapped between the two regions. Analysis was not performed on data within the WED region due to lack of replication. Trap catch data on number of *Sirex*, number of cerambycids and on number of individual cerambycid species with sufficient numbers ($N \geq 50$; (Miller et al. 2011) were compared between disturbed and undisturbed sites within the OZ region using a *t*-test which assumes unequal variances. A *t*-test has the added benefit of not being particularly sensitive to deviations from normality, especially when testing for differences in means of equal numbers of observations (Bartlett 1935) as is the case in this study. Deviations from normality are hard to gauge in the trap catch data due to small sample sizes; however, the number of observations is equal between disturbed and undisturbed sites within the OZ region. Buprestidae were not subjected to statistical analysis due to the low numbers (zero in many cases) trapped in undisturbed sites. Logistic regression was performed on total number of *Monochamus* spp. trapped over the entire trapping period plotted against CWP with each site being a sample unit.

Basal area of all trees and pine basal area between disturbed and undisturbed sites in the OZ region were compared using a *t*-test with each sites estimate of basal areas being the sample unit. Average dbh of all trees and average dbh of only pine trees between disturbed and

undisturbed sites in the OZ region were compared using a *t*-test in which each sites average dbh is the sample unit. Estimated CWP was compared between disturbed and undisturbed sites in the OZ region using a *t*-test in which each sites estimate of CWP was the sample unit.

RESULTS

Stand characterizations: Within the OZ region, undisturbed sites had a higher mean basal area than disturbed sites ($t_{5.3} = 2.95$, $P = 0.030$), but there was no significant difference in mean pine basal area between disturbed and undisturbed sites ($t_{4.1} = 2.65$, $P = 0.056$). The mean average dbh of all trees within the OZ region was higher at undisturbed than disturbed sites ($t_{5.0} = 3.3$, $P = 0.022$), but there was no significant difference in the mean average dbh of pine trees between disturbed and undisturbed sites ($t_{5.0} = 0.11$, $P = 0.92$). Mean estimated CWP was higher at disturbed sites than undisturbed sites within the OZ region ($t_{3.3} = 4.50$, $P = 0.016$).

Subcortical insect composition: A variety of subcortical pine colonizing insects were trapped (Table 3). A total of 7735 specimens from target families were trapped (Table 4). Cerambycidae was the most abundant target family with 7481 individuals represented by *Monochamus* spp. and 7 other species. Abundant ($n \geq 50$) Cerambycidae were *Monochamus* spp., *A. obsoletus, A. nodosus,* and *Xylotrechus sagittatus* Germar. *Monochamus* spp. was the most abundant longhorn beetle accounting for 81% of total Cerambycidae and 78% of total target specimens trapped. Eighty-eight Buprestidae specimens were caught represented by three species. *Buprestis lineata* Fabricius was the only buprestid caught in abundant numbers. One hundred sixty-six female Siricidae were caught represented by 3 species. No male specimens were trapped. *Sirex nigricornis* was the only siricid caught in abundant numbers accounting for 94% of all Siricidae trapped, but only 2% of total target specimens trapped.

Flight activity: *Sirex nigricornis* activity lasted from mid-October till early-December (Figure 7). *Sirex nigricornis* activity reached its apex in late-October and substantial activity remained throughout November. Overall cerambycid activity was already high in late-September when trapping commenced and remained so throughout the trapping period. Peak cerambycid activity occurred in early to mid-October. *Monochamus* spp., *A. nodosus*, and *A. obsoletus* were active at the beginning of the trapping period and activity for all three cerambycids peaked in early-October (Figure 7). *Monochamus* spp. activity remained substantial throughout the trapping period. *Xylotrechus sagittatus* activity was relatively constant throughout the trapping period although activity did peak in late-October (Figure 7). Overall buprestid activity occurred throughout the trapping period, but was highest when trapping commenced and started to decline there after (Figure 7).

Abundance of Target Species Trapped in Disturbed and Undisturbed Sites:

Traps at the disturbed sites in the OZ region caught more target specimens than traps at undisturbed sites ($t_{15.1} = 3.45$, $P = 0.0036$). *Sirex nigricornis* trapped did not differ significantly between disturbed and undisturbed sites within the OZ region ($t_{21.8} = 0.30$, $P = 0.76$). More cerambycids were trapped at disturbed sites than undisturbed sites within the OZ region ($t_{15.1} = 3.37$, $P = 0.0041$). *Monochamus* spp., the most abundant target insects, were trapped more at disturbed sites than undisturbed sites within the OZ region ($t_{16.4} = 3.53$, $P = 0.0027$). The number of *Monochamus* spp. trapped had a significant relationship with the estimated amount of CWP contained in a site ($F_{1,6} = 20.1$, $P = 0.004$, $r^2 = 0.77$; Figure 8). Like *Monochamus* spp. trapped, *A. nodosus* and *A. obsoletus* were trapped more at disturbed sites than undisturbed sites within the OZ region ($t_{11.1} = 2.85$, $P = 0.016$ and $t_{13.2} = 3.07$, $P = 0.0088$ for each species respectively). *Xylotrechus sagittatus* showed a different response than the other cerambycids

with more *X. sagittatus* being trapped at the undisturbed sites than disturbed sites within the OZ region ($t_{19.4} = 5.56$, $P < 0.0001$).

Sex Ratio of Selected Cerambycid Species: In total, 52% of the *Monochamus* spp. trapped were female (Table 5). The percent of *Monochamus* spp. that were female at individual sites ranged from 48% to 58% (Table 5). The phenology curve of *Monochamus* spp. males and females are similar (Figure 9a), indicating the two sexes may emerge congruently.

In total, 51% of *A. nodosus* trapped were female (Table 5). At individual sites, the percent of *A. nodosus* trapped that were female ranged from 33% to 83% (Table 5). *Acanthocinus nodosus* abundance was relatively low at some sites and at these sites the percentage *A. nodosus* trapped that were female deviated the most from the overall total of 51%. Sites with a relatively greater abundance of *A. nodosus* had percentages of female *A. nodosus* trapped that were closer to 51%. More male *A. nodosus* were trapped at the beginning of the trapping period than female *A. nodosus* (Figure 9b). Three weeks later female *A. nodosus* were more abundant than males and this trend continued throughout the remainder of the trapping period.

In total, 42% of *A. obsoletus* trapped were female (Table 5). The percent of *A. obsoletus* trapped that were female ranged from 0% to 67% at individual sites (Table 5). Only 5 *A. obsoletus* were trapped at WEDcon which is the site where 0% of *A. obsoletus* trapped were female. If the WEDcon site is excluded, the percent of *A. obsoletus* trapped that were female ranged from 31% to 67% at individual sites. More male *A. obsoletus* were trapped at the beginning of the trapping period than female *A. obsoletus* (Figure 9c). Both sexes were trapped at relatively the same abundance for the remainder of the trapping period.

DISCUSSION

Traps baited with pine volatiles and *Ips* pheromones caught specimens in all three target families. The fact that *S. nigricornis* is caught in traps baited with *Ips* pheromones provides evidence that this wasp will at least approach host material containing *Ips* spp. (Curculionidae: Scolytinae; alternatively Scolytidae). *Sirex nigricornis* was the only Siricidae collected in abundant numbers (n = 156) and at every locale. Two other Siricidae, *Tremex columba* and *Urocerus cressoni* were collected in relatively low numbers (n = 5 in both cases). These species are known to be collected in analogous traps baited with similar lures (de Groot et al. 2006, Coyle et al. 2012, Keeler 2012), so these results are not surprising. However, it is interesting to note that the described range of *U. cressoni* includes Arkansas (Schiff et al. 2006), but that no specimens were previously trapped despite trapping for Siricidae in previous years (Keeler 2012). No *U. cressoni* specimens from Arkansas were present in the University of Arkansas' arthropod collection either. Also worth noting is that *T. columba*, which is predominately considered a hardwood species, was found drilling into a pine bolt placed inside one of the traps. *Tremex columba* females have previously been observed ovipositing into pine (Schiff et al. 2006).

Of the target beetle families trapped, Cerambycidae was much more abundant than Buprestidae. *Monochamus* spp. comprised the majority, 78%, of target insects trapped. *Acanthocinus nodosus, A. obsoletus,* and *X. sagitattus,* all cerambycids, were the only other beetles that comprised more than 1% of target insects trapped, with proportions equaling 3%, 6% and 8% respectively. The species and relative proportions of these cerambycids are similar to other studies conducted in the southeastern United States (Dodds and Stephen 2002, Miller et al. 2011).

Sirex nigricornis and subcortical beetles being trapped in the same traps suggests that these organisms are attracted to, or at least not repelled by, similar volatile mixtures. The mixture of pine volatiles and *Ips* pheromones used in this experiment are commonly released from physiologically stressed and damaged pine trees after they have been colonized by *Ips* (Hughes 1974, Simpson and McQuilkin 1976, Kimmerer and Kozlowski 1982, Teale et al. 1991, Smith 2000, Ayres et al. 2001, Byers and Birgersson 2012). Since *S. nigricornis* and these subcortical beetles are both attracted to similar volatiles commonly released from host material, (Simpson 1976, Simpson and McQuilkin 1976, Miller and Asaro 2005, Miller 2006, Miller et al. 2011, Coyle et al. 2012), it is likely that *S. nigricornis* will come in contact with the same substrate as these subcortical beetles. Indeed, many subcortical beetles and *Sirex* spp. emerge from the same host material indicating these species are attracted to the same host material and hence they likely interact with one another (Wermelinger et al. 2008, Ryan et al. 2011a).

Peak trap catch numbers of *Monochamus* spp., *A. nodosus*, and *A. obsoletus* occurred between September 26th and October 10th, although *Monochamus* spp. trapped remained relatively high throughout the study. *Sirex nigricornis* numbers peaked around October 31st, approximately 4 weeks after total cerambycid numbers peaked. *Sirex nigricornis* trap catch numbers peaking in late October in the Ozarks is in agreement with trapping conducted during the prior two years (Keeler 2012). Trap catch numbers of one cerambycid, *X. sagitattus*, peaked at the same time as *S. nigricornis*. The highest number of buprestids trapped occurred on September 26th, the first day traps were collected and numbers declined thereafter.

Because most of the cerambycid and buprestid activity peaked relatively earlier than *S. nigricornis* activity, *S. nigricornis* likely arrives to hosts that have already been colonized by these beetles. It is also known that many bark beetles, *Ips* spp. and *Dendroctonus* spp., have

multiple generations a year and are abundant throughout the season before *S. nigricornis* emerges (Connor and Wilkinson 1983, Hain et al. 2011), so they too have the potential to be in host substrate before *S. nigricornis* arrives. Arriving second affords *S. nigricornis* the ability to accept or reject host material already colonized by these beetles. *Sirex nigricornis* might avoid host material containing these beetles and their associated fungi in an effort to mitigate interspecific competition. There is some evidence that a related *Sirex* species, *S. noctilio,* augments its oviposition behavior in the presence of beetle vectored ophiostomatoid fungi although it is not known if oviposition was augmented (Ryan et al. 2011c). It is currently unknown how the presence of these beetles and their fungi affect the oviposition behavior of *S. nigricornis*.

Another implication of these beetles colonizing host material before *S. nigricornis* is it affords beetle vectored fungi (e.g. ophiostomatoid fungi) an opportunity to colonize host material before *S. nigricornis'* symbiont, *Amylostereum*, is inoculated into the host. This could have a profound effect on the development and survival of *S. nigricornis* offspring because developing *Sirex* larvae require consumption of *Amylostereum* in order to successfully develop (Stillwell 1966 , Martin 1992, Wermelinger and Thomsen 2012). Ophiostomatoid fungi not only get inoculated into host substrate first, but ophiostomatoid fungi typically colonize more substrate than *Amylostereum* when both fungi are inoculated concurrently (Ryan et al. 2011b). In addition, *Amylostereum* doesn't establish on substrate occupied by ophiostomatoid fungi, but some ophiostomatoid strains can establish on substrate colonized by *Amylostereum* (Ryan et al. 2011b). Clearly, *Amylostereum* is a poor competitor with at least the ophiostomatoid species *Leptographium wingfieldii* and *Ophiostoma minus* (Ryan et al. 2011b) and in general, most

ophiostomatoid fungi seem well suited to outcompete decay fungi like *Amylostereum* (Brown and Webber 2009).

The sex ratio of females:males trapped was in the range of 1:1 for *Monochamus* spp., *A. nodosus,* and *A. obsoletus*. The sex ratio of 1:0.94 for *Monochamus* spp. females:males trapped is almost exactly the same as the emergence sex ratio of 1:0.95 for *M. carolinensis* (Linit 1985). Knowing female abundance of these cerambycids is important because females likely contribute more to altering host material, due to their role in oviposition, than males. Females of these cerambycids chew egg niches into the bark of host material where they oviposit single to multiple eggs per niche (Webb 1909, Walsh and Linit 1985, Dodds and Stephen 2000, Dodds et al. 2002). These niches, plus galleries created by cerambycid offspring create access points for opportunistic airborne fungi to establish and grow (Kukor and Martin 1986, Martin 1992, Siitonen 2001). Furthermore, beetle larvae feeding in the phloem change the composition of host material and hasten degradation which may affect oviposition behavior or offspring survival of *S. nigricornis* (Graham 1925, Speight 1989).

Within the OZ region, significantly more target insects were trapped in areas disturbed 4-6 months prior that contained an abundance of windthrown pine compared to undisturbed areas. Not surprisingly since it comprises 97% of the target insects trapped, this statistical difference was also seen when comparing Cerambycidae trapped between disturbed and undisturbed sites within the OZ region. This difference was reflected below the family level for *Monochamus* spp., *A. nodosus* and *A. obsoletus*. Interestingly, *X. sagittatus* was trapped significantly more in undisturbed sites than disturbed sites within the OZ region. Although not looked at statistically, buprestids were trapped more at disturbed than undisturbed sites within the OZ region. An

increase in cerambycid and buprestid numbers on windthrown material is well documented in other studies too(Wermelinger et al. 2002).

Unlike these subcortical beetles, *S. nigricornis* did not show a preference for either disturbed or undisturbed sites within the OZ region. This suggests that *S. nigricornis* and presumably other pine colonizing *Sirex* may not be strongly attracted to an influx of host material like many associated subcortical beetles are. This is possibly caused by their life history. Female *Sirex* are initially photopositive, flying to the tops of trees close to where they emerged to mate with males who have already aggregated there (Morgan and Stewart 1966, Taylor 1981, Madden 1988). Besides being photopositive, virgin females may be further attracted to the tops of trees by male produced pheromones (Cooperband et al. 2012). After mating, the photopositive response of females is replaced by a host-location response (Madden 1988). Thus newly emerged female *Sirex* are initially concentrated near the place they emerged in areas primed for mating, not for ovipositing. How far mated females commonly disperse is unknown, but the majority of the population likely doesn't venture far from the site of emergence if attractive volatiles are being released in the area (Madden 1988, Corley and Villacide 2012). Indeed, at least as it pertains to a multi-year study on an expanding population of *S. noctilio* in Argentina, half of newly attacked trees are found within a 45 m radius and 90% within a 130 m radius of trees attacked the previous season (Corley and Villacide 2012). This indicates *Sirex* populations may not be influenced by a disturbance event unless it occurs near the source population because mating and host location both appear to occur within close proximity to the place of emergence.

What if a disturbance event does occur near a source population of *Sirex*? *Sirex* densities have been shown to increase locally in windthrown areas with an abundance of host material

(Wickman 1965). Such disturbances have been linked to outbreaks of other forest pests which build up population densities on windthrown host material to levels high enough to overwhelm defenses of adjacent, physiologically active trees (Wermelinger et al. 2002, Wermelinger 2004, Gandhi et al. 2007). Yet *S. nigricornis* and other *Sirex* in endemic habitats have rarely, if ever, been reported causing tree mortality after a windthrow disturbance event.

Concern has been raised about how pine stands in North America, particularly the southeastern United States, will be impacted by the recently introduced *S. noctilio* (Borchert 2007, Yemshanov et al. 2009b, Dodds et al. 2010, Dodds and de Groot 2012). There is a high potential for *S. noctilio* to establish in pine forests of the southeastern United States (Carnegie et al. 2006, Yemshanov et al. 2009a) and frequent windthrow disturbances in the region, if left unmanaged, could result in an abundance of host material for *S. noctilio* (Borchert 2007). However, unlike in the southern hemisphere where *S. noctilio* outbreaks are notorious, accumulation of compromised host material in the United States is accompanied by an increase in competitors, particularly subcortical beetles and their associated fungi.

CONCLUSION

Abundance of *Sirex nigricornis* trapped was similar between tornado disturbed and undisturbed sites. Disturbed sites had an abundance of downed pine trees, a comparatively defenseless resource compared to trees that are still physiological active, and this may favor an increase in *Sirex* offspring survival and development; hence local population growth. However, competition from subcortical beetles and their associated fungi may have reproductive consequences on developing *Sirex* offspring and these beetles were more abundant in tornado disturbed sites, exhibiting a positive response to accumulations of host material. Generally

speaking, subcortical cerambycids and buprestids are active a few weeks prior to *S. nigricornis*, affording them and associated fungi the ability to colonize suitably stressed host material first. Impact of these beetles on *Sirex* could be important in regulating population expansion of the woodwasp, especially in areas with an abundance of favorable host resources. Further studies need to be conducted to investigate how subcortical beetles affect the behavior and survival of *Sirex*.

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FIGURES

Figure 1. Trap lid made by affixing panel trap funnel to trashcan lid

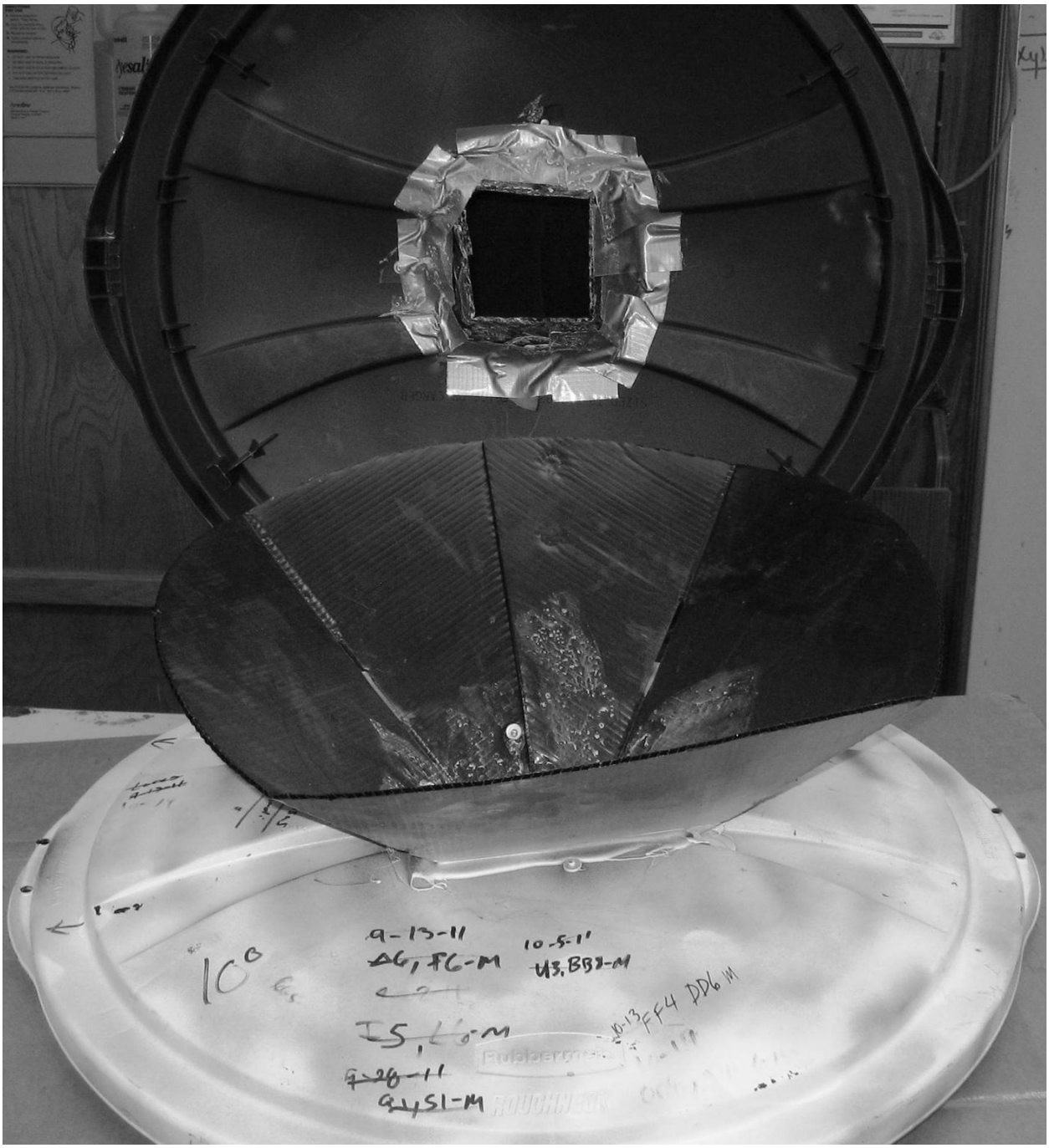


Figure 2. Entire trapping unit

Figure 3. Trap set up in field

Figure 4. Funnel for removing insects from trap

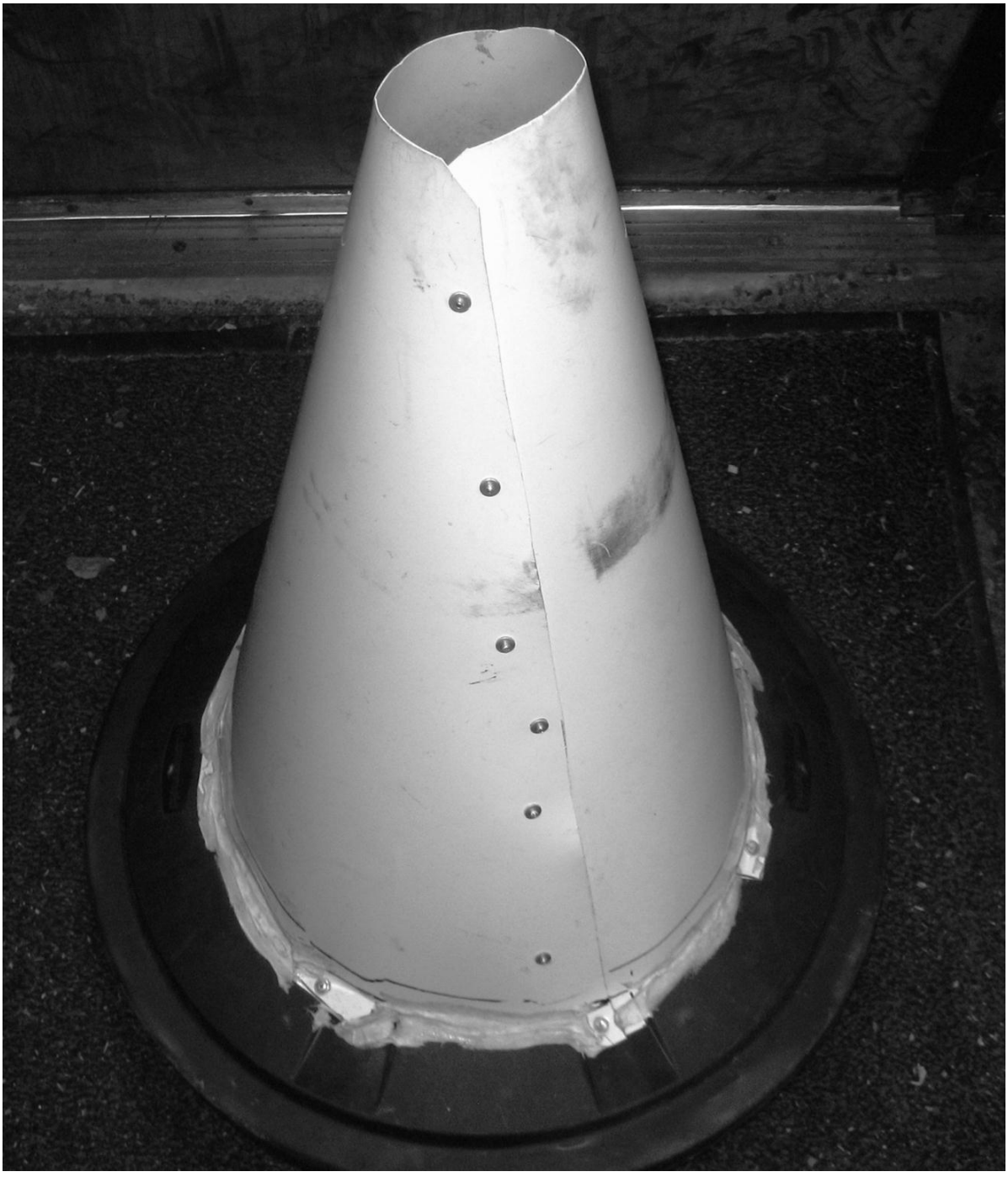


Figure 5. Locations of trapping regions and sites in Arkansas' Ozark National Forest

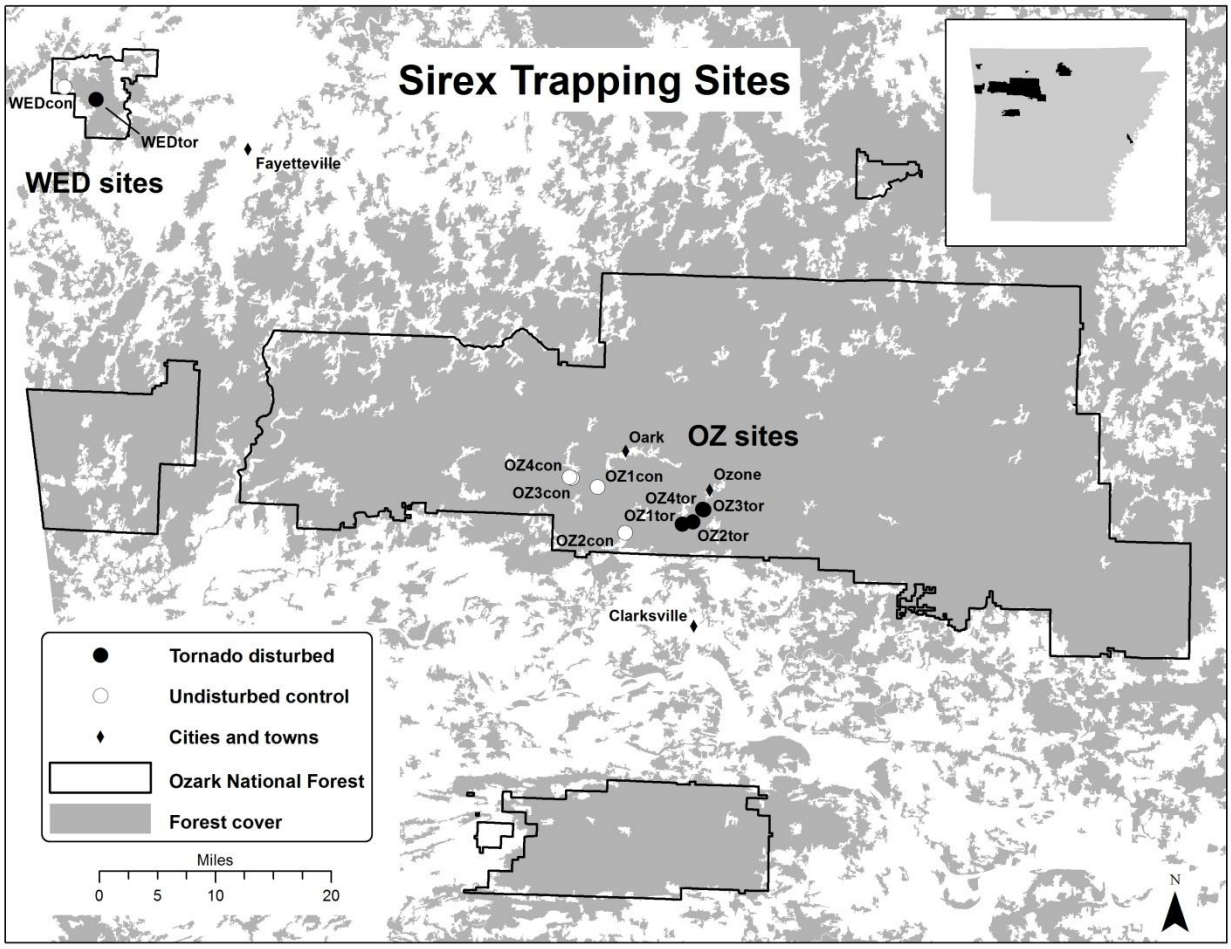


Figure 6. Layout of FIA plot for measuring coarse woody pine and basal area

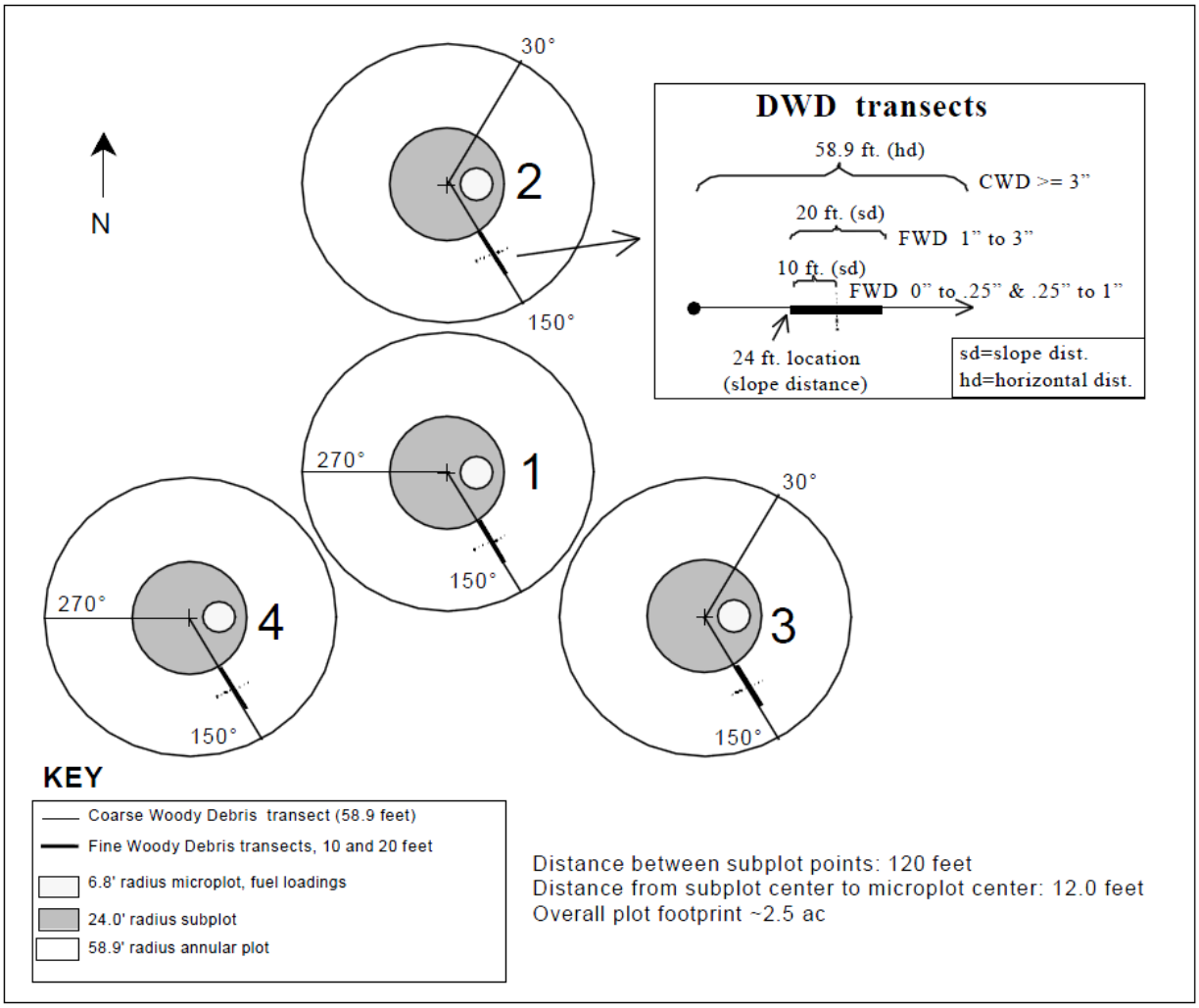
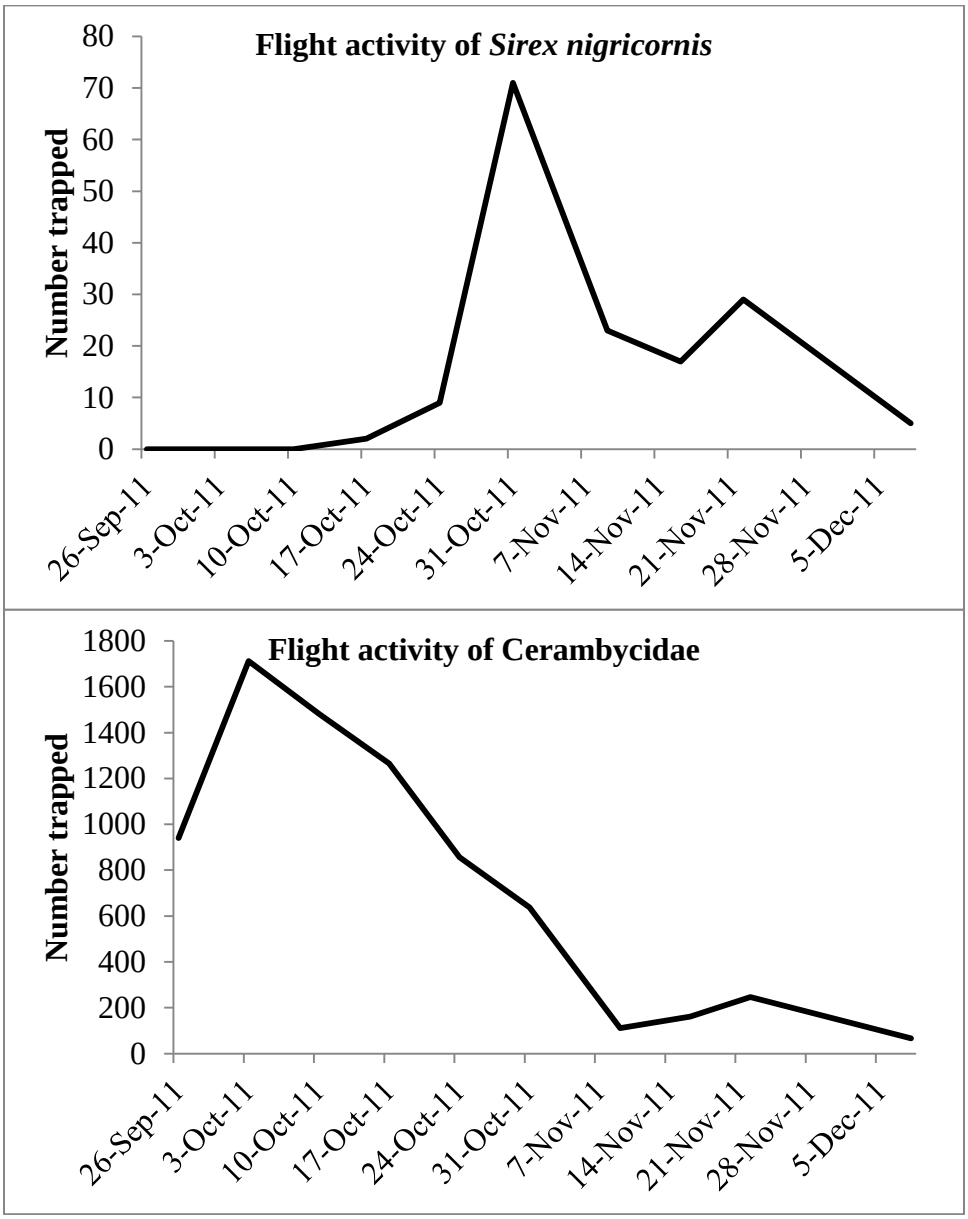
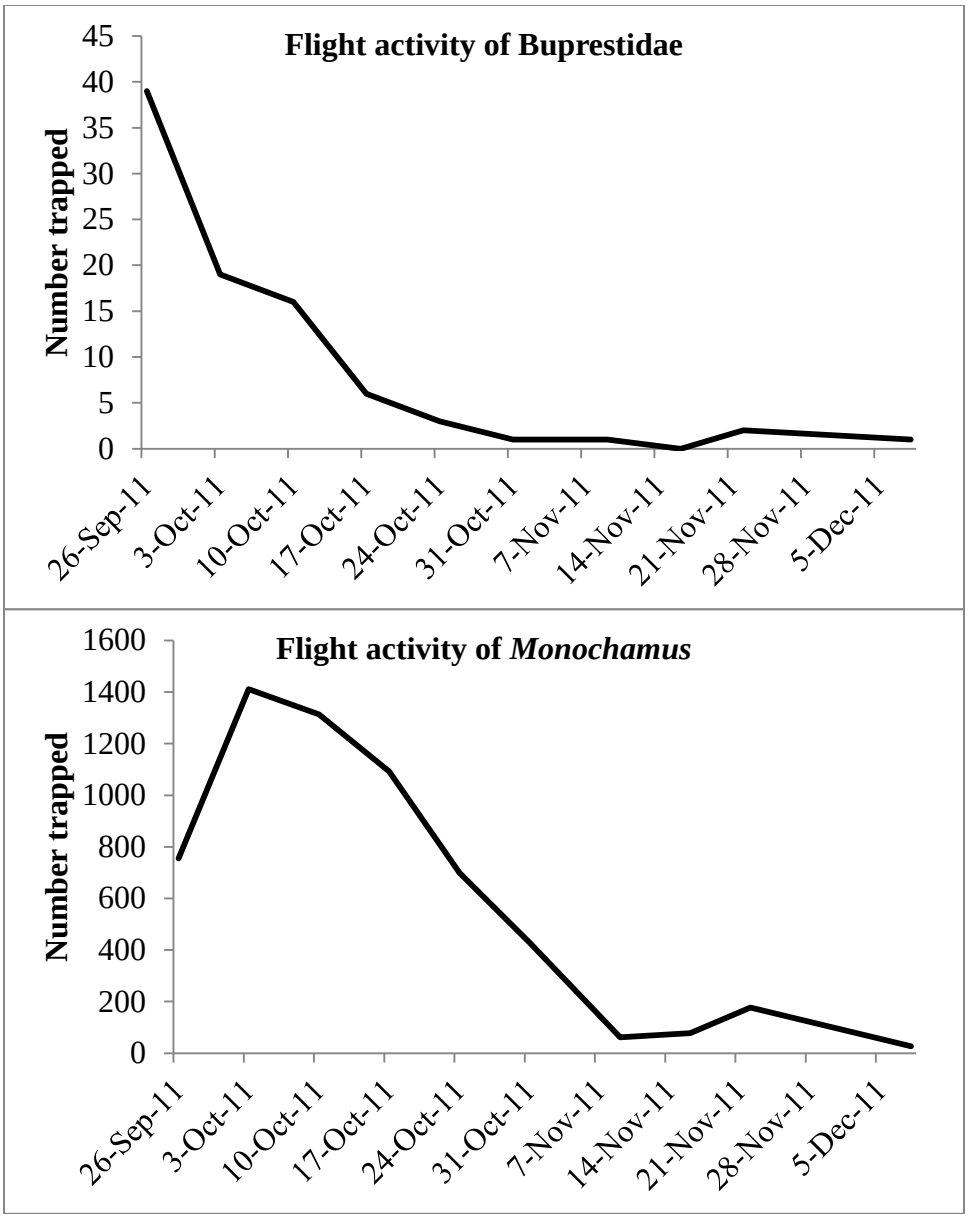


Figure was taken from USDA-FS (2011)

Figure 7. Activity of *Sirex*, Buprestidae, Cerambycidae, *Monochamus* spp., *Acanothcinus nodosus*, *Acanthocinus obsoletus* and *Xylotrechus sagittatus*




Flight activity of Buprestidae
Number trapped
45
40
35
30
25
20
15
10
5
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11
Flight activity of *Monochamus*
Number trapped
1600
1400
1200
1000
800
600
400
200
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11

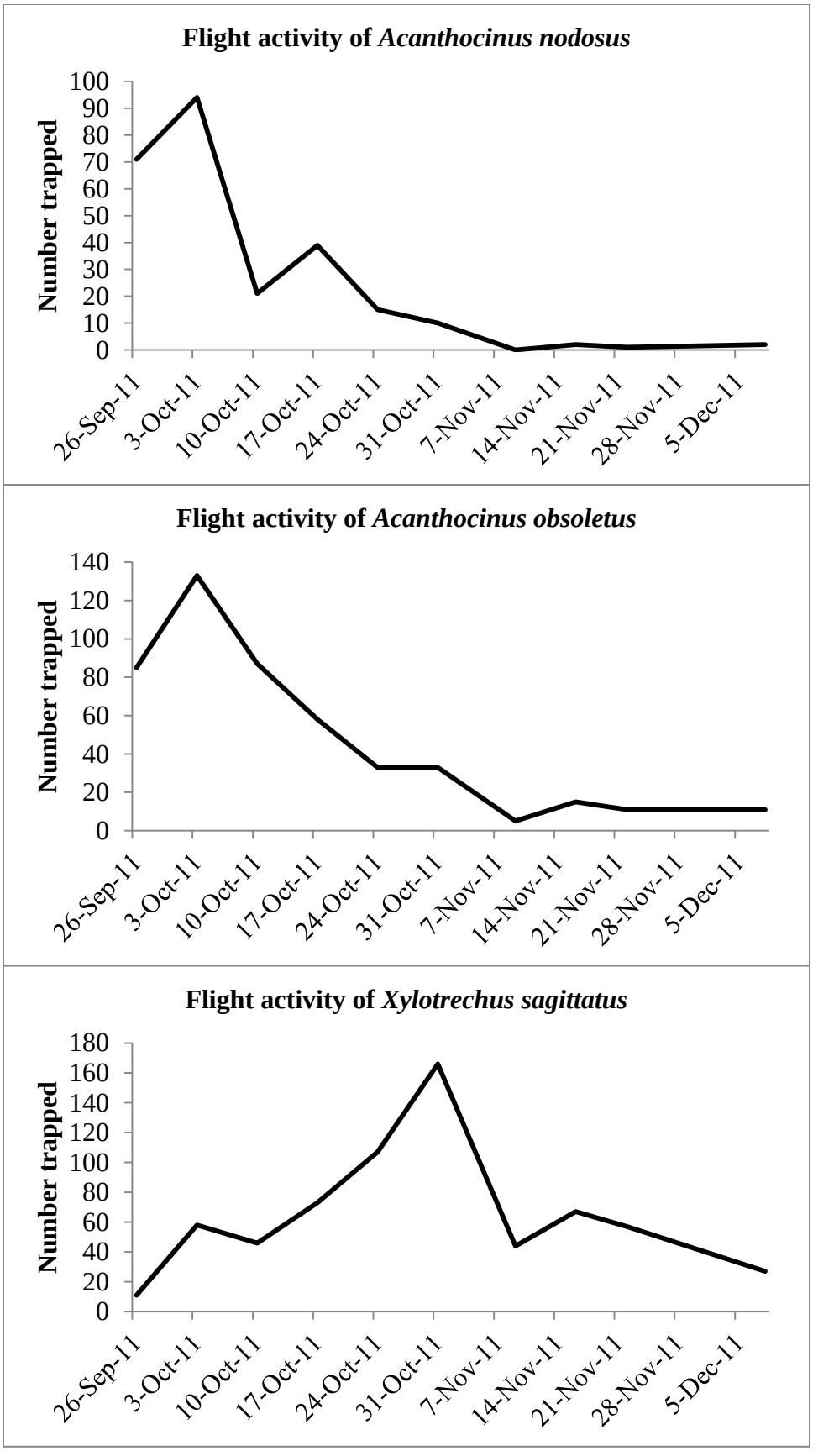

Flight activity of *Acanthocinus nodosus*
Number trapped
100
90
80
70
60
50
40
30
20
10
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11
Flight activity of *Acanthocinus obsoletus*
Number trapped
140
120
100
80
60
40
20
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11
Flight activity of *Xylotrechus sagittatus*
Number trapped
180
160
140
120
100
80
60
40
20
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11

Figure 8.

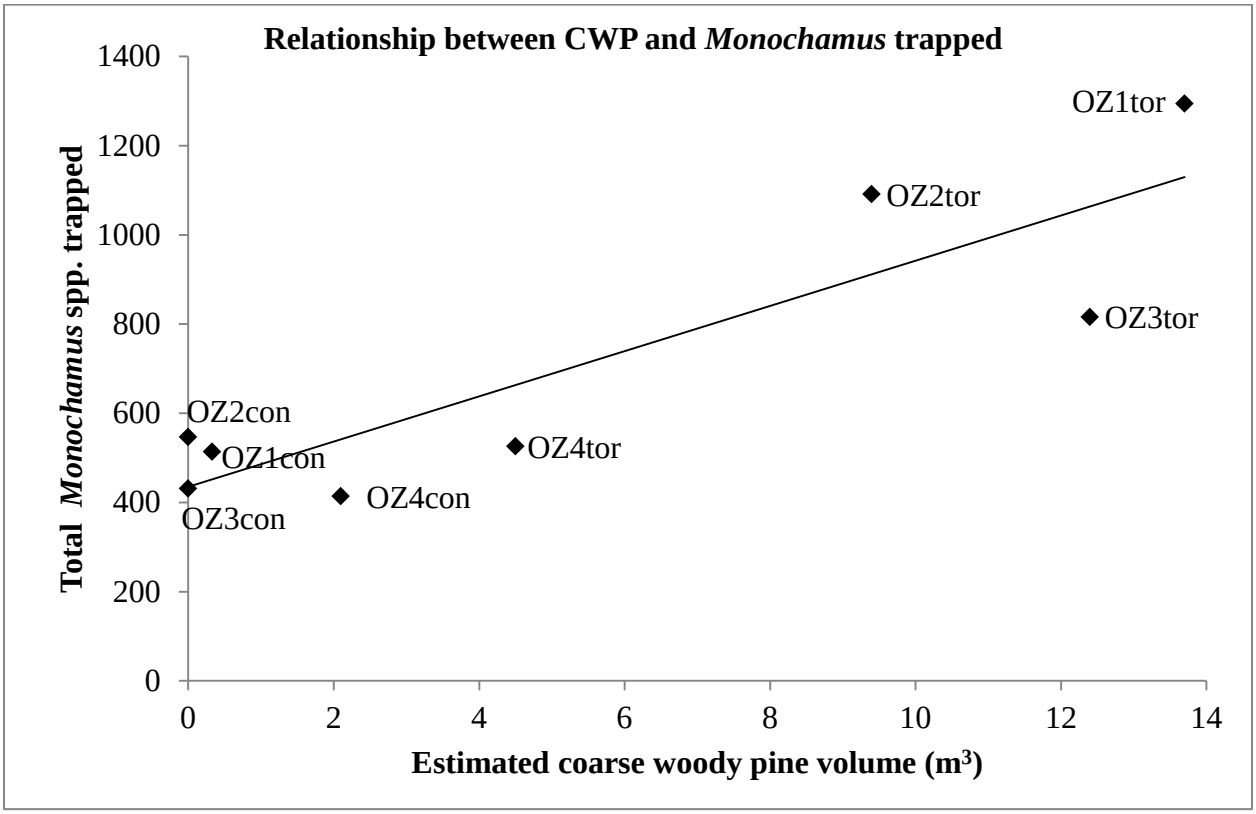
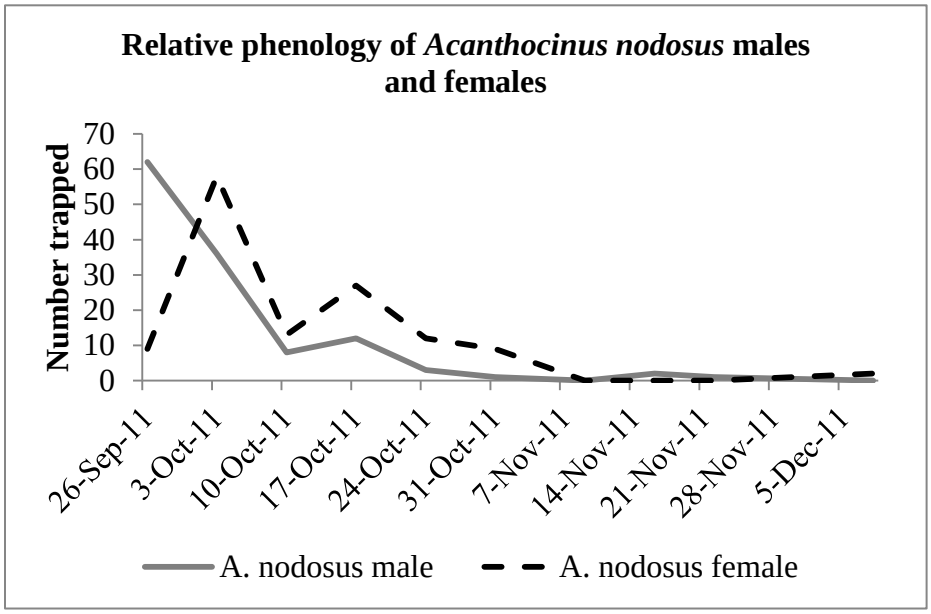
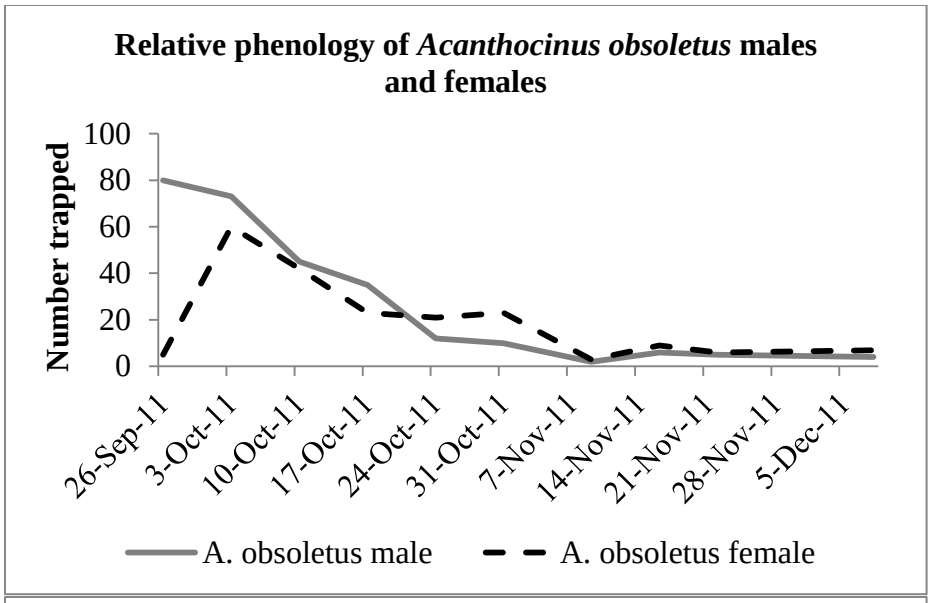
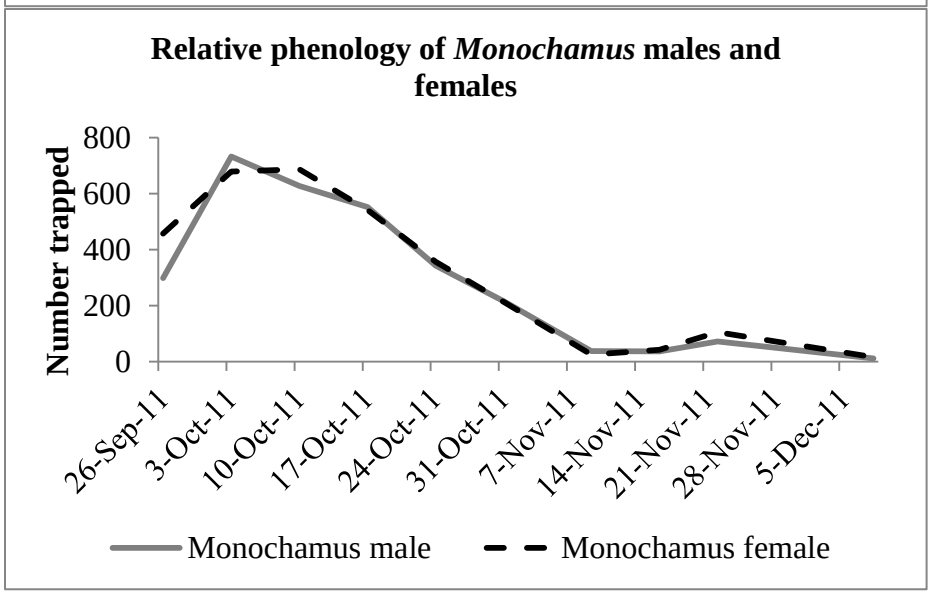

Relationship between CWP and *Monochamus* trapped
Total *Monochamus* spp. trapped
Estimated coarse woody pine volume (m³)
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
OZ1tor
OZ2tor
OZ3tor
OZ2con
OZ1con
OZ4tor
OZ4con
OZ3con

Figure 9. Relative phenologies of select cerambycid males and females




Relative phenology of *Acanthocinus obsoletus* males and females
Number trapped
100
80
60
40
20
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11
A. obsoletus male
A. obsoletus female


Relative phenology of *Monochamus* males and females
Number trapped
800
600
400
200
0
26-Sep-11
3-Oct-11
10-Oct-11
17-Oct-11
24-Oct-11
31-Oct-11
7-Nov-11
14-Nov-11
21-Nov-11
28-Nov-11
5-Dec-11
Monochamus male
Monochamus female

TABLES

Table 1. Site location, site description, tree species recorded more than twice in basal area (BA) measurement, proportion BA that is made up of pine

Ranger District	Site ID	UTM 15N	Disturbance Date	Aspect	Altitude (m)	% Pine BA	Tree species	Description of area
Boston Mountain	WEDtor	0374640 3998395	12/31/2010	Flat	389	42%	*Pinus echinata,Quercus alba, Carya* spp.	Field south. Predominately hardwood in other directions.
Boston Mountain	WEDcon	0370200 4000118	None	Flat	348	18%	*Quercus alba*, Q. *stellat, Q. rubra, Pinus echinata, Carya crodiformis*	Field south. Hardwood to east and west before becoming fields. Suppressed pine stand to north.
Pleasant Hill	OZ1tor	0456024 3939533	5/24/2011	Ridge	498	39%	*Quercus alba, Pinus echinata, Liquidambar styraciflua, Q. rubra, U. alata*	Steep slope to the south. Mix of hardwood and pine in surrounding area.
Pleasant Hill	OZ2tor	0457463 3939872	5/24/2011	Valley	287	43%	*Quercus alba, Pinus echinata*	Mix of mature pine and hardwood. Salvage

								operations occurred in surrounding area.
Pleasant Hill	OZ3tor	0459018 3941546	5/24/2011	Bench	545	24%	*Quercus alba*	Steep slope south. Few standing trees. Surrounding area mixed pine and hardwood.
Pleasant Hill	OZ4tor	0458866 3941603	5/24/2011	Bench	552	29%	*Pinus echinata*, *Liquidambar styraciflua*, *Quercus rubra*, *Prunus serotina*, *Acer rubrum*	Steep slope south. Healthy looking pine stand to west. Mixed harwood and pine to east and north.
Pleasant Hill	OZ1con	0444251 3944661	None	Mountain top	549	21%	*Nyssa sylvatica*, *Carya* spp., *Pinus echinata*, *Acer rubrum*, *Quercus alba*	Mix of hardwood and pine in all directions. Relatively flat.
Pleasant Hill	OZ2con	0448075 3938327	None	Valley	330	79%	*Pinus echinata*, *Quercus alba*, *Q. rubra*	Mixed stand of hardwood and mature pine. Lots of undergrowth. Suppressed pine stand to southwest.

Pleasant Hill	OZ3con	0440670 3945954	None	Bench	437	65%	*Pinus echinata*, *Quercus alba*, *Acer rubrum*, *Carya* spp.	Predominately pine with a slight leveling off to south before sloping steeply.
Pleasant Hill	OZ4con	0440378 3946043	None	Bench	412	43%	*Pinus echinata*, *Quercus alba*, *Liquidambar styraciflua*	Slopes steeply to south. Mixed hardwood and pine in surrounding area.

Table 2. Average diameter at breast height (dbh) for all trees, average dbh of pine, basal area (BA) of all trees species in plot, BA of all pine trees in plot and estimated coarse woody pine (CWP) for all sites

Region	Site ID*	Avg dbh (cm)	BA (m^2/ha)	Avg pine dbh (cm)	Pine BA (m^2/ha)	Estimated CWP (m^3)
WED	WEDtor	26.0 ± 2.2 (27)	25.3	35.0 ± 3.3 (7)	10.6	12 (15)
WED	WEDcon	23.5 ± 1.8 (29)	22	31.0 ± 8.7 (3)	3.9	0
OZ	OZ1tor	20.2 ± 1.9 (29)	17.5	30.5 ± 7.9 (5)	6.9	13.7 (22)
OZ	OZ2tor	21.4 ± 2.6 (12)	12.2	35.8 ± 9.5 (3)	5.2	9.4 (7)
OZ	OZ3tor	21.9 ± 4.4 (5)	3.3	26 (1)	0.8	12.4 (15)
OZ	OZ4tor	19.5 ± 1.6 (41)	23.4	19.1 ± 1.3 (15)	6.8	4.5 (12)
OZ	OZ1con	24.3 ± 1.6 (44)	35.9	32.2 ± 3.4 (6)	7.7	0.3 (1)
OZ	OZ2con	26.9 ± 2.7 (28)	30.1	29.8 ± 3.7 (18)	23.7	0
OZ	OZ3con	22.6 ± 2.1 (29)	21.6	29.2 ± 3.5 (12)	14	0
OZ	OZ4con	22.2 ± 1.4 (44)	30.1	22.0 ± 2.7 (18)	12.9	2.1 (1)

Numbers in parentheses are the total number of trees that went into the calculation. Basal area measurements used same trees as average dbh measurements for calculation. *Sites with tor were disturbed and sites with con were undisturbed.

Table 3. List of pine colonizing species recorded in traps

Buprestidae	*Buprestis lineata* Fabricius
	Chalcophora sp.; likely *Chalcophora virginiensis* Drury
	Dicerca sp.
Cerambycidae	*Acanthocinus nodosus* Fabricius
	A. obsoletus Olivier
	Arhopalus rusticus Linnaeus
	Astylopsis sexgutatta Say
	Neoclytus mucronatus Fabricius
	Rhagium inquisitor Linnaeus
	Monochamus spp.
	Xylotrechus sagittatus Germar
Cleridae	*Thanasimus dubius* Fabricius
Colydiidae	*Colydium* sp.
	Lasconotus sp.
Curculionidae	*Dendroctonus terebrans* Olivier
	Hylobius pales Herbst
	Ips avulsus Eichhoff
	I. grandicollis Eichhoff
	Myoplatypus flavicornis Fabricius
	Pachylobius picivorus Germar
	Pissodes nemorensis Germar
	Xyleborus spp.
Cucujidae	*Cucujus clavipes* Fabricius
Elateridae	Unidentified spp.
Histeridae	*Platysoma* spp.
Ichneumonidae	*Ibalia leucospoides* Hochenwarth
Siricidae	*Sirex nigricornis* Fabricius
	Tremex columba Linnaeus
	Urocerus cressoni Norton
Staphylinidae	Unidentified spp.
Tenebrionidae	*Corticeus* sp.
Trogossitidae	*Temnochila virescens* Fabricius
	Tenebroides spp.

Table 4. Total target species trapped in disturbed and undisturbed sites of Arkansas' Ozark National Forest

	WED region		OZ region										
	Dist.	Undis.	Disturbed				Undisturbed					% of Family	% of Target
	WED	WED	OZ1	OZ2	OZ3	OZ4	OZ1	OZ2	OZ3	OZ4*	Total	Total	Total
Siricidae	11	10	20	13	28	8	13	7	33	23	166	100%	2%
Sirex nigricornis	8	7	20	13	27	7	12	7	32	23	156	94%	2%
Urocerus cressoni	2	1	0	0	0	1	1	0	0	0	5	3%	0%
Tremex columba	1	2	0	0	1	0	0	0	1	0	5	3%	0%
Cerambycidae	301	466	1562	1273	963	598	628	639	526	525	7481	100%	97%
Acanthocinus nodusus	12	0	88	81	36	18	8	6	0	6	255	3%	3%
A. obsoletus	26	5	166	92	61	40	26	37	9	9	471	6%	6%
Arhopalus rusticus	15	6	2	0	5	4	4	3	2	4	45	1%	1%
Monochamus spp.	208	205	1294	1091	816	526	514	547	431	414	6046	81%	78%
Xylotrechus sagittatus	40	250	10	8	45	10	74	46	83	90	656	9%	8%
Rhagium inquisitor	0	0	0	0	0	0	1	0	0	0	1	0%	0%
Neoclytus mucronatus	0	0	2	0	0	0	0	0	1	1	4	0%	0%
Astylopsis sexgutatta	0	0	0	1	0	0	1	0	0	1	3	0%	0%
Buprestidae	15	1	28	8	7	25	3	1	0	0	88	100%	1%
Buprestis lineata	12	1	24	6	5	23	1	1	0	0	73	83%	1%
Chalcophora sp.	0	0	2	1	1	0	0	0	0	0	4	5%	0%
Dicerca sp.	3	0	2	1	1	2	2	0	0	0	11	13%	0%
Total target specimens	327	477	1610	1294	998	631	644	647	559	548	7735	na	100%

% Total target specimens	4%	6%	21%	17%	13%	8%	8%	8%	7%	7%	100%	na	na

* On one collection date a trap was knocked over by a bear, but data was still recorded from the trap because the whole unit was still intact and the number of insects inside the trap appeared to be at normal levels when compared to the other two traps and to other dates.

Table 5. Percentage of select Cerambycidae trapped that are female

Monochamus spp.			
Site	Total trapped	Number female	% female
OZ1con	514	298	58%
OZ1tor	1294	637	49%
OZ2con	547	269	49%
OZ2tor	1091	560	51%
OZ3con	431	236	55%
OZ3tor	816	410	50%
OZ4con	414	239	58%
OZ4tor	526	268	51%
WEDcon	205	104	51%
WEDtor	208	100	48%
All sites	**6046**	**3121**	**52%**
Acanthocinus nodosus			
Site	Total trapped	Number female	% female
OZ1con	8	5	63%
OZ1tor	88	41	47%
OZ2con	6	2	33%
OZ2tor	81	45	56%
OZ3con	0	0	na
OZ3tor	36	16	44%
OZ4con	6	5	83%
OZ4tor	18	9	50%
WEDcon	0	0	na
WEDtor	12	7	58%
All sites	**255**	**130**	**51%**
Acanthocinus obsoletus			
Site	Total trapped	Number female	% female
OZ1con	26	14	54%
OZ1tor	166	71	43%
OZ2con	37	18	49%
OZ2tor	92	33	36%
OZ3con	9	5	56%
OZ3tor	61	24	39%
OZ4con	9	6	67%
OZ4tor	40	20	50%
WEDcon	5	0	0%
WEDtor	26	8	31%
All sites	**471**	**199**	**42%**

CHAPTER 3 – THE EFFECTS OF SUBCORTICAL BEETLES ON THE OVIPOSITION BEHAVIOR AND EARLY-STAGE OFFSPRING MORTALITY OF *SIREX NIGRICORNIS* FABRICIUS

ABSTRACT

Little is known about how community associates affect the oviposition preference and offspring performance of woodwasps, particularly woodwasps in North America. This chapter seeks to address this lack of knowledge.

Sirex nigricornis did not avoid drilling into host material that contained other subcortical insects, but females did appear to limit their oviposition activity on bolts colonized by subcortical insects. They created fewer tunnels per drill site on bolts colonized by subcortical insects compared to bolts that had not been colonized and more tunnels per drill site relates to more eggs being oviposited. In addition, *S. nigricornis* offspring mortality was highest on bolts colonized by other insects compared to bolts that had not been colonized by these insects. Lastly, subcortical insects hastened the rate at which sapwood desiccated and this could have affected the development and survival of *S. nigricornis* offspring.

INTRODUCTION

For a majority of insects, where a female oviposits has a profound effect on her offspring's growth, survival and reproduction (Thompson 1988). Her choice in oviposition sites and how she allocates her time, energy and eggs, can also greatly influence her own total fitness (Doak et al. 2006). The relationship between where a female oviposits to her total fitness and offspring performance is, generally speaking, the most pronounced in non-grazing

holometabolous insects because they have relatively immobile juveniles that complete development in close proximity to where they were oviposited (Thompson 1988, Doak et al. 2006). For insects like woodwasps (Siricidae) that colonize the phloem and/or xylem of trees (subcortical insects), this is especially true.

Offspring of a female who oviposits into a poor host or site on a host will, on average, have reduced performance compared to offspring of a female who oviposits at a more optimal site. It follows via natural selection that females will evolve mechanisms that increase the likelihood they oviposit into material conducive to their offspring's development. This not only entails locating a suitable host and often a particular area on the host that affords their offspring access to necessary nutrients, but it also entails locating a place that will buffer their offspring from inter and intraspecific competition as well as other biotic and abiotic mortality factors. Ultimately, the life history strategy of the species or population in question will influence which factors are most influential on the oviposition preference of a female.

A majority of subcortical insects are monophagous or oligophagous, colonizing only specific tree species or genera (USDA-FS 1985). This is true for most subcortical insects that colonize the bole of pine trees (USDA-FS 1985). Many species of bark beetles, long-horn beetles, flat-head borers and woodwasps utilize pine almost exclusively (USDA-FS 1985, Zhang and Schlyter 2004); although some on rare occasions may colonize related trees, but often with a marked reduction in offspring performance (Spradbery and Kirk 1981, Zhou and Togashi 2006). In North America, with the exception of eruptive populations of bark beetles, these subcortical insects avoid high mortality associated with attacking vigorous physiologically active pine trees by colonizing stressed, moribund and damaged pines whose constitutive and induced defenses have been compromised by external factors (drought, fire, flooding, mechanical damage by ice,

wind, machines, lightning, poor stand management, or other insects) (Gandhi et al. 2007, Schowalter 2012). Debilitated pine trees like these, with compromised defenses, are an ephemeral and relatively rare resource. Therefore, subcortical pine colonizing insects must attempt to colonize the same host substrate as their associates, which may lead to high levels of interspecific competition. Mechanisms have evolved in many of these insects to mitigate the negative force imposed on them by interspecific competition. Many subcortical insects will colonize separate parts of a debilitated tree, thus avoiding interspecific competition. For instance, vertical partitioning of host resources in the presence of competitors is well documented for the bark beetle guild (Paine et al. 1981, Rankin and Borden 1991, Schlyter and Anderbrant 1993, Ayres et al. 2001, Stephen 2011). Such partitioning of a tree is often mediated by chemical feedback mechanisms, acoustic communication, visual inspection and niche preference, all of which should function to reduce interspecific competition (Birch et al. 1980, Flamm et al. 1987, Byers 1989b, a, Schlyter and Anderbrant 1993). *Sirex* woodwasps do not appear to show this vertical resource partitioning. *Sirex* adults will emerge from every section of the tree bole regardless of the presence of competitors (Wermelinger et al. 2008, Ryan et al. 2011a). The utilization of most parts of the tree bole by *Sirex* may be due to the fact that they inhabit the relatively spacious, competition free xylem while the majority of other subcortical insects predominately spend their time feeding in phloem (Hanula 1993, Farrell et al. 2001, Harrington 2005, Ryan et al. 2011a) and therefore selective pressures constraining *Sirex* to colonize a particular vertical section of host material just because it has other insects may be minimal. However, *Sirex* still may be impacted by, and ovipositing females may detect, differences in localized areas (microsites).

In general, the insect arriving second will have the opportunity to accept or reject a host in the presence of competitors. In Arkansas' pine forests, *Sirex nigricornis* Fabricius flight activity occurs late in the year, after the activity of many of its pine associates (chapter 2). This affords *Sirex nigricornis* the ability to accept or reject host material colonized by other insects. *Sirex nigricornis* may inhabit every section of the tree bole, but colonization of a particular host by other subcortical insects still may inhibit oviposition by the woodwasp. Both *S. noctilio* and *S. nitobei* Matsamura have been shown to augment their oviposition behavior based on host tree condition and host qualities such as moisture content (Morgan and Stewart 1966b, Madden 1974, Madden and Coutts 1979, Fukuda and Hijii 1996). Suboptimal moisture content or stage of decay unsuitable for oviposition and colonization of host material can be facilitated by insects and their associated fungi (Graham 1925, Speight 1989, Grove 2002). There is also some evidence that ophiostomatoid fungi associated with subcortical beetles affects the oviposition behavior of at least one woodwasp, *S. noctilio* Fabricius (Ryan et al. 2011c).

The avoidance by *Sirex* of hosts, or parts of hosts, that have suboptimal moisture content or the presence of potentially antagonistic fungi may relate to how these factors affect development and survival of their offspring (i.e. offspring performance). All *Sirex* have an intimate mutualism with *Amylostereum* fungi to such an extent that the two have evolved a stable obligate symbiosis (Martin 1992, Slippers et al. 2003, Wermelinger and Thomsen 2012). Female *Sirex,* carrying the fungus in their mycangia, deposit arthrospores of the fungus into host substrate during oviposition (Morgan 1968, Coutts and Dolezal 1969, Madden 1974, Talbot 1977). Exactly how *Amylostereum* provides *Sirex* access to nutrition is equivocal, but it is certain that the fungus is necessary for larval development (Morgan 1968, Wermelinger and Thomsen 2012). Although larvae develop, none become adults when *Amylostereum* is not

present (Stillwell 1966). In addition, where conditions for the growth of *Amylostereum* are optimal, larger *Sirex* emerge (Madden and Coutts 1979, Madden 1981, Ryan and Hurley 2012). Larger females have comparatively higher potential fecundity (Madden 1974, Neumann and Minko 1981, Fukuda et al. 1993, Fukuda and Hijii 1997) and realized fecundity (Madden 1974, Fukuda et al. 1993, Fukuda and Hijii 1996, 1998) than smaller females. Plus, larger *Sirex* have longer life spans (Madden 1974) and they have the ability to disperse further (Bruzzone et al. 2009, Corley and Villacide 2012). Thus, the performance of *Sirex* offspring appears to be directly related to the presence and quality in growth of *Amylostereum*.

Amylostereum grows better in wood with a moisture content around or below 70% saturation than at higher moisture contents (Coutts and Dolezal 1965). This moisture content, it turns out, correlates well with the preferred oviposition moisture content of 40-75% for *S. noctilio* (Morgan and Stewart 1966b, Morgan 1968). Thus, ovipositing into preferred moisture content likely increases the performance of *Sirex* offspring.

Two ophiostomatoid fungi, *Ophiostoma minus* (Hedgcock) H. & P. Sydow and *Leptographium wingfieldii* Morelet, have been shown to be better at capturing uncolonized resources than *Amylostereum* and some strains of these fungi have been shown to capture host substrate from *Amylostereum* while the converse is not true (Ryan et al. 2011b). Further, it appears that most ophiostomatoid fungi are well suited to outcompete decay fungi like *Amylostereum* (Brown and Webber 2009). Although only anecdotal, the growth of *Amylostereum* is reportedly also hindered by other fungi commonly isolated from trees. *Trichoderma* instead of *Amylostereum* has been recovered in areas where care was taken to inoculate only *Amylostereum* into host trees (Tabata and Abe 1999) and *Trichoderma* as well as *Sphaeropsis sapinea* (Fries) Dyko & B. Sutton supposedly cause death of *Amylostereum* hyphae

on agar plates (King 1966). Host substrate containing these fungi may influence the performance of a female's offspring and could influence her oviposition behavior.

There are, however, other benefits that discriminating insect females may garner to their own total fitness and to the performance of their offspring that does not relate to a hosts ability to provide optimal nutrition (Thompson 1988, Doak et al. 2006). Site discrimination can lead to reduced negative effects of predators, parasitoids and competitors on developing offspring (Thompson 1988). As mentioned earlier, the direct impact of competition from insects on *Sirex* is likely low due to *Sirex* colonizing the relatively spacious, competitor free xylem (Farrell et al. 2001, Harrington 2005). This does not, however, mean that *Sirex* will not avoid the presence of these insects. It just means that these insects are not, directly, competing with *Sirex*. Predators of *Sirex* larvae, which are mainly woodpeckers, likely have minimal impact on *Sirex* too (Marshall 1967, Spradbery 1990). Therefore, selective pressure from competitors and larval predators on the oviposition preference of *Sirex* females should be minimal. Unlike predators and competitors, hymenopterous parasitoids and parasitic nematodes of *Sirex,* which are routinely isolated from specimens and trees containing *Sirex,* have been shown to have some impact on these wasps (Bedding and Akhurst 1978, Hurley et al. 2007, Bedding 2009, Long et al. 2009, Eager et al. 2011, Cameron 2012, Ryan et al. 2012). This is more likely to place a selective force on the preference of ovipositing *Sirex*. Indeed, there is evidence that *Sirex* females will allocate a greater percentage of female offspring to the bottom portion of a tree bole and that this correlates with reduced parasitism by *Ibalia leucospoides ensiger* Norton (Ibaliidae) (Long et al. 2009). It has been hypothesized, but remains untested, that oviposition by *I. l. ensiger* could be limited by some feature of larger diameter wood (e.g. bark thickness) or by height in the tree (Long et al. 2009).

Benefits may be conferred to *Sirex* offspring by their mother discriminating among oviposition sites, but this can come at a cost to her total fitness. Discriminating among hosts, or sites on a host, decreases the rate of oviposition and increases search time. This, in turn, increases the probability a female dies with a lower realized fecundity due to her extended search time. Aerially hunting birds are known to prey on *Sirex* (Madden 1982) and longer search time, plus the act of exposing herself while flying to a new host, increase a females chance of being eaten before she oviposits all of her eggs.

General life history of *Sirex* with emphasis on oviposition biology and terminology

Sirex Linnaeus (Siricidae: Siricincae) are semelparous, proovigenic and parthenogenetic with unfertilized eggs developing into males and fertilized eggs developing into females (Peacock and Gresson 1931, Morgan 1968). All *Sirex* colonize and develop in conifer trees, although the preferred host species and the success of offspring developing in that host depends on the *Sirex* species in question (Morgan 1968, Spradbery and Kirk 1981, Schiff et al. 2006). *Sirex* are generally univoltine although pupation may be delayed one to two years under certain circumstances (Morgan 1968, Taylor 1981, Fukuda et al. 2007). *Sirex* emergence is protandrous and appears to be related to climactic variables such as falling barometric pressure and above average temperatures (Madden 1974, Taylor 1981, Neumann et al. 1987). Male *Sirex* aggregate near the crowns of trees they emerged from (Morgan and Stewart 1966b, Madden 1988) and mating takes place a few days later when the males are joined by females who are attracted to light and a pheromone released by the males (Taylor 1981, Madden 1988, Cooperband et al. 2012). After mating and/or initial flight, female *Sirex* locate potential pine hosts for oviposition (Madden 1988, Ryan and Hurley 2012). Physiologically stressed trees releasing monoterpenes

and oxygenated compounds are attractive to *Sirex* (Simpson and McQuilkin 1976, Madden 1988, Coyle et al. 2012).

When a *Sirex* female lands on a potential host, she locates a place to probe by dragging her abdomen over the bark and palpating with her antennae (Francke-Grosman 1939, Ryan et al. 2011c), after which she probes through the bark and into the xylem to assess host suitability for oviposition (Morgan 1968, Madden 1974, 1988). If the host material is suitable, a female will start to oviposit. Eggs of most siricid species are oviposited two to fifteen mm into the xylem although at least one species, *S. areolatus* Cresson, has been reported ovipositing into both wood and bark (Morgan 1968). The depth of oviposition depends on the wasp's ovipositor length and has been recorded reaching a depth of 19 mm into the xylem (Morgan 1968, Ryan and Hurley 2012).

From the bark surface, oviposition sites (contains eggs) and probing sites (no eggs deposited) look similar (Coutts and Dolezal 1969, Spradbery 1977); both appearing as a single circular hole roughly 0.5 mm in diameter, although the size of the hole will vary depending on ovipositor girth. Since on the bark surface it is impossible to distinguish a probed site from an oviposition site, they will both be referred to as drill sites. *Sirex* drill sites have between 1 to 5 and on rare occasions 6 tunnels diverging in the xylem from a common entrance hole in the bark [(Morgan 1968, Coutts and Dolezal 1969, Spradbery 1977, Madden 1988), Figure 1] Single tunnel drill sites are usually probe sites that do not receive eggs, but do get *Amylostereum* (Coutts and Dolezal 1969, Spradbery 1977, Neumann and Minko 1981). In multiple tunnel drill sites, eggs are oviposited into most tunnels except the last tunnel which receives only *Amylostereum* (Coutts and Dolezal 1969, Madden 1974, Spradbery 1977, Baxter et al. 1995). It follows logically and is shown empirically that as the number of tunnels increases, so too does

the number of eggs laid, at least for palearctic *Sirex* (Coutts 1965, Madden 1974, Spradbery 1977, Madden and Coutts 1979). Interestingly, the oviposition behavior of a nearctic species, *S. cyaneus*, which is established in Europe, appears different to the two European *Sirex* species studied (Morgan 1968, Spradbery 1977). *Sirex cyaneus* is more likely to limit the number of tunnels per drill site it creates to two. *Sirex cyaneus* also more likely to oviposit into single tunnels and it will often deposit multiple eggs per tunnel (Stillwell 1966, Morgan 1968, Spradbery 1977). Information on the architecture of *S. nigricornis* oviposition sites is currently lacking.

Research objectives

The main goal of this chapter is to enhance the understanding of *Sirex* oviposition behavior and its relation to offspring performance. The primary objective is to investigating the relatively understudied *Sirex nigricornis*' oviposition behavior and offspring mortality in the presence of subcortical insects. A secondary objective of this chapter is to address the gap in knowledge about the specific oviposition biology of *S. nigricornis* and to compare *S. nigricornis'* biology to information already obtained about *Sirex* species

MATERIALS AND METHODS

Morphometrics and fecundity

Pinus echinata Miller trees approximately 12.7 cm in diameter at breast height (dbh) were felled in the fall of 2009 and 2010, prior to emergence of *Sirex nigricornis*. These trees were left in the field during flight and oviposition of *S. nigricornis* so they could be colonized by the woodwasps. The following spring, these trees were cut into ~ 0.75 m bolts that were brought back to the University of Arkansas farm. The ends of these bolts were covered with

paraffin wax to reduce desiccation and then the bolts were placed in rearing containers made out of plastic trashcans. Rearing containers were checked on a nearly daily basis during the emergence period of *Sirex nigricornis* (Keeler 2012) to collect newly emerged females. Female *S. nigricornis* were assumed to have not oviposited any eggs before being collected; their only places to oviposit being the plastic trashcan or bolt from which they emerged. Females collected in the fall of 2010 were stored in a walk-in cooler with the temperature set around 4˚C. Females remained in the walk-in cooler until they were measured and dissected. Females collected in the fall of 2011 were killed and stored in 95% EtOH until they were measured and dissected.

Body length was estimated to the nearest 0.5 mm by placing a female dorsal side up on top of a ruler placed under a microscope. The body length of all specimens was measured from the vertex of their head just in front of the eyes to the tip of their cornus (referred to as body length to cornus tip). A second body length measurement, from the vertex of the head to the tip of the ovipositor (referred to as body length to ovipositor tip), was taken on 2011 specimens because that is how Keeler (2012) measured *S. nigricornis* body length. Ovipositor sheath length was measured from the first valvula to the tip of the sheath with digital calipers. Pronotal width was measured with digital calipers.

After body measurements were taken, females were dissected under a stereomicroscope. Ovaries were removed and placed on a 7.6 X 7.6 cm Office Depot® self-stick note that was placed inside a Petri® dish. Eggs were spread out on the self-stick note using forceps and probes to a thickness of approximately one (egg) layer thick. The body cavity of the female was then checked for any remaining eggs and the number remaining in the body cavity was tallied and written on the top of the self-stick note. Lines were drawn to separate the eggs on the self-stick note into columns. The number of eggs from each column was summed and the number of eggs

counted in the body cavity was added to this number to give an estimate of the total number of eggs a female contained. This process was repeated twice and the average of the two counts was recorded as a female's potential fecundity.

The body cavity and ovaries of females were also checked for nematodes. This was done because the presence of these nematodes in the ovaries of *Sirex* specimens may affect their average size and potential fecundity (Keeler 2012, Bedding and Iedes 2005).

Oviposition behavior and early stage offspring survival

Bolts were treated to examine the effect of host substrate on oviposition behavior and survival of *S. nigricornis*. Treatments involved bolts with shaved bark (bark study), bolts that were colonized by *Ips* (*Ips* study) and bolts colonized in the field (field study). The handling of bolts and specimens, measurements taken and general methods used in these three different studies are detailed below.

Bark study**:** Bolts were prepared from *P. echinata* trees approximately 12.7 cm in diameter at breast height that were harvested on October 22nd and November 30th, 2010. The trees were bucked into 1 m sections and stored in a walk-in cooler until being caged with *S. nigricornis* specimens. Before being caged the bolts were cut to a length of 0.5 m and 50% of their bark surface was shaved to a thickness of about 0.25 to 0.75 mm. Unshaved bark was approximately 1 to 3 mm thick. Bark was shaved in five patterns (Figure 2) to minimize the effect of females preferring to drill in one area of a standing bolt (e.g. top being preferred over bottom).

Female woodwasps used in this study were reared from trees colonized the previous fall. Trees were felled during the flight season of *S. nigricornis* (Keeler 2012). The range of time between when a *S. nigricornis* female emerged and when she was caged with bolts ranged from

zero to twenty-six days (Table 3). Females older than one day were refrigerated before being caged.

A total of 14 bolts were exposed to *S. nigricornis* females. The bolts were placed standing upright in wooden framed mesh cages. Bolts remained caged until all *S. nigricornis* in that cage died. Bolts were then removed and examined using a stereomicroscope to determine the number of drill sites in shaved and unshaved areas.

Ips study: Treated bolts (*Ips*-colonized bolts) used in these studies and tests were exposed to beetles from a colony of *Ips grandicollis*. Control bolts (non-colonized bolts) were treated in a similar fashion to *Ips*-colonized bolts except where noted.

In spring 2011, a laboratory colony of *Ips grandicollis* was established inside a Lumite® cage erected within the confines of an insulated shed. In October 2011, *P. echinata* bolts (66 cm and 200 cm in length) were cut from living pine trees approximately 12.7 cm in diameter at breast height. Bolts cut to the same length were grouped accordingly, for a total of two groupings. Six bolts 200 cm in length (Group A) were harvested on the 3rd of October, 2011. Group A bolts were taken from three trees approximately 12.7 cm in diameter at breast height. These bolts were stored outside of the University of Arkansas forest entomology building for three days before being paired. Bolts were paired visually. Emphasis was placed on the bolts having similar girth and bark thickness. One bolt from each of the three pairs was randomly selected to be the *Ips*-colonized bolt while its partner served as the control bolt. Treatment bolts were stood upright inside the Lumite cage containing the *Ips* colony. Control bolts were placed inside a mesh tent that was also located inside the insulated shed. The mesh tent served to keep insects from colonizing control bolts.

The 66 cm long bolts (Group B) were originally cut for the field-colonized studies. Group B bolts were harvested the first and second week in October. After being brought back to the lab, these bolts had nails sunk in their bottoms and then they were stood upright on the nails for two to three weeks in a room kept at ambient temperature. Group B bolts were paired based on being cut on the same date and having similar volumes (see section on handling field-colonized bolts for more details). Group B contained a total of three bolt pairs. One bolt from each of the pairs was randomly selected to be the *Ips*-colonized bolt while its partner served as the control bolt. On October 31st, nails were removed from the bolts and then the bolts were placed either in the Lumite® cage containing *Ips* or inside the mesh tent in a manner similar to the bolts in group A.

Treatment bolts were assumed to only be colonized by *Ips* and their phoretic mites, plus fungi and bacteria associated with them. Control bolts placed inside the mesh tent were assumed to not be colonized.

After 3 - 6 weeks, a batch of bolt pairs were removed from their confines, cut into 30 cm sections and then the ends were covered with Anchorseal® (U-C Coatings Corporation, Buffalo, NY) to reduce desiccation. Bolts were left out on a table overnight (> 12 hrs) to allow Anchorseal® to dry before the bolts were to be caged with *S. nigricornis*. There were a total of three bolt batches. Batch one (Batch 1) included a single pair of bolts from Group A. Batch two (Batch 2) also contained a single pair of bolts from Group A. The third batch (Batch 3) involved two of the three bolt pairings from Group B. One batch of bolts at a time was removed, sectioned and caged with *S. nigricornis* females. Only after all *S. nigricornis* females caged with a particular batch of bolt sections had died was a new batch of bolt sections prepared.

The 30 cm sections from *Ips*-colonized bolts were paired with analogous sections from their non-colonized partner. That is, the basal section from an *Ips*-colonized bolt was paired with the basal section from its non-colonized partner, the next contiguous sections from each bolt being paired and so forth. Cages were built out of 45 gallon Sterelite® storage tubs. A plastic divider was placed in the middle of the storage tub to create two cages. Bolt sections were fastened to cages by sinking two screws through the bottom of the storage tub into the bolt bottom. When the first batch of bolt sections was used the plastic dividers were not sealed around the edges with caulk. Due to specimens crossing from one cage to the other through cracks between the divider and the floor of the storage tub, caulk was used to create a tighter seal when the experiment was run for the remaining two bolt batches. As they became available, 1- 2 *S. nigricornis* females were added to a cage. *Sirex nigricornis* specimens were either collected from rearing containers stocked with trap trees colonized in the fall of 2010 or from live field-collected specimens. Field-collected specimens were trapped inside traps made for collecting live specimens (Chapter 2). Trapped specimens were included because of low numbers of adults emerging from rearing containers and because mated specimens are believed to drill with greater frequency (Madden 1974). Specimens collected from rearing were assumed unmated. Although the virgin status of field collected specimens is unknown, most were likely mated because mating tends to preclude host search behavior (Madden 1988). Once all females in a study cage had died, the paired sections of bolts were removed and the number of drill sites on each was counted under a stereomicroscope.

After the number of drill sites created by *S. nigricornis* females was counted and recorded, drill sites were dissected under a stereomicroscope. A drill site was dissected, starting from the bark, with handheld woodcarving tools. Attempts were made to dissect every hole on a

bolt. Sometimes a drill site close to one being dissected would get cut into and in that case it was not dissected. The initial tunnel leading away from a drill site was recorded as either being in the bark or in the xylem. Drill site architecture (i.e. number of tunnels at a particular drill site) for all drill sites uncovered was recorded as either being a single, double, treble, quadruple, or quintuple tunneled drill site depending on if there were one, two, three, four, or five tunnels respectively, located at that drill site. It was fairly easy to distinguish a drill site in the xylem as being distinctive from other drill sites because when multiple tunnels were created at a drill site, the tunnels were distributed perpendicular to the longitudinal axis of the bolt. Thus even in areas with high densities of drill sites, unless the tunnels of drill sites were created at exactly the same height, they could be separated from each other. Even if drill sites were located close to each other and their tunnels were on the same horizontal plane they could be separated as distinctive drill sites by looking at the angles of tunnels in relation to each other. The center most tunnel almost always penetrates directly into the xylem with the remaining tunnels being created at ever more oblique angles as they move outward from the center tunnel (Coutts 1965, Spradbery 1977).

Initially the number of eggs or larvae per drill site was also going to be recorded. However, this was the first time that eggs and larvae were searched for during dissections. The eggs and larvae, which are less than 2 mm in size, were still in their initial tunnels (no larval galleries were leading away). I believed that many of the larvae or eggs were accidently removed by the wood carving tools during dissections, plus I was still learning what to look for. Thus counting and recording the number of eggs or larvae per drill site was aborted because it was thought that the number of false negatives (i.e. there was an egg or larvae, but it was missed) was high. However, eggs and larvae were observed in many of the drill sites.

Field study: Treated bolts (field-colonized bolts) used in these studies and tests were exposed to insects trapped in baited panel traps modified for this study (Chapter 2). Traps were baited with pine volatiles (ethanol and α-pinene) and *Ips* pheromones (ipsenol, ipsdienol and lanierone) and they were modified by affixing trashcans to serve as collection receptacles at the bottom of the panels. Trashcans retained trapped insects inside of them (Chapter 2) and they had enough space for two 66 cm bolts to be stood upright inside of them. Treatment bolts and control bolts were handled similarly except that control bolts were wrapped in fine fiberglass mesh while inside traps.

On a 7 to 10 day cycle starting in the beginning of September 2011, five to eight non-merchantable *P. echinata* trees approximately 12.7 cm in diameter at breast height were felled. Trees were then given a unique identifying letter or combination of letters before being bucked into 66 cm bolts. Bolts were labeled on their bottom cross sectional areas with the unique identifying letter given to the tree they came from and then a number that identifies their location on the tree starting with the basal bolt and working progressively up the bole of the tree. Bolts were then loaded in the back of a covered vehicle which transported them to the University of Arkansas forest entomology lab. The time between felling a tree and placing bolts in the covered vehicle was kept minimal to avoid unwanted colonization by insects. Bolts were unloaded into a room kept at ambient temperature.

Two perpendicular diametric lines were drawn on the top and bottom cross sectional areas of the basal bolt from each tree harvested. The diameter of the top and bottom cross sectional areas were estimated as the average length of the two diametric lines. Diameter measurements included bark. Bark thickness on each end of basal bolts was estimated as the mean of four measurements taken with digital calipers where the diametric lines met the bark.

Diameter and bark thickness measurements were estimated in a similar manner for the top of all remaining bolts. Diameter and bark thickness estimates for the bottom of remaining bolts were assumed to be equivalent to the measurements for the top of the bolt removed from below it. Using Microsoft Excel® the volume of each bolt was calculated as the frustum of a cone by applying the following formula:

$$\text{Volume} = \frac{1}{3}\pi(66\text{ cm})\left[\left(\frac{D_{Top}}{2}\right)^2 + \left(\frac{D_{Top}}{2} * \frac{D_{Bottom}}{2}\right) + \left(\frac{D_{Bottom}}{2}\right)^2\right]$$

where 66 cm is the length of the bolt, and D_{Top} and D_{Bottom} are the estimated diameters of the top and bottom cross sectional areas of the bolt.

Each week, thirty bolt pairs were created from a stock of around 70 to 80 bolts. Bolts were paired based on their volume; bolts of closest volume being paired together. Larger volume bolts were selected first. Bolts with physical abnormalities (e.g. chainsaw gash, missing bark, lots of knots, branching) were not used. If bolts had similar volumes but their bark thicknesses were different by more than ~ 5 mm, they were not paired together. In these circumstances it was usually evident which bolt had an unusual bark thickness based on visually comparing its bark thickness to other bolts of similar volume.

All paired bolts had three to four nails set into their bottoms so that the nails were sticking out about 1 to 4 cm. Nails served to keep bolts off the ground while they dried in a successful effort to abate the growth of fungi which often colonize the bottom of fresh resinous bolts lying on the floor. Bolts were stood up on the nails inside a room kept at ambient temperature for approximately one week to allow resin pressure to subside. Bolts were then taken out in the field to be placed in traps. One bolt from each pair was randomly selected to be the control bolt (non-colonized) and the other the treatment bolt (field-colonized). The control bolt was placed in a fiberglass mesh bag and the ends of the bag were tied shut with string. The

mesh bag served to keep insects from colonizing the control bolt. Both bolts were placed standing upright inside the trap on the nails sunk into their bottom ends. Bolt pairs were replaced approximately every week when the insects caught in the traps were removed. Mesh was removed from the control bolt when it was taken out of the trashcan and then the control bolt and its partner were transported back to the lab. Nails were removed from the base of bolts upon returning to the lab.

Within a day or two after being returned to the lab, the moisture content of the ends of bolts was calculated as the average of four measurements taken with a Protimeter® Timbermaster moisture meter (GE Sensing EMEA, Clare, Ireland) set at the appropriate calibration setting 'B'. Two measurements were taken on each end of a bolt's cross sectional area. Measurements were taken approximately 1.5 cm from the edge of the bark. After moisture content measurements were taken, the ends of the bolts were covered in Anchorseal® (U-C Coatings Corporation, Buffalo, NY) to reduce desiccation. Bolts were stored on their sides raised off the ground on tables or boards inside our lab until they were needed.

A subset of bolt pairs returned from traps in the field was selected to be used for further study. Bolt pairs removed from trashcans that contained any woodwasp species were not considered for future use. Bolt pairs were selected with emphasis being placed on the field-colonized bolt having cerambycid oviposition pits while the non-colonized control bolt should have fewer, preferably no cerambycid oviposition pits. Although rare, it was obvious that cerambycids had sometimes chewed holes through the mesh and created oviposition pits on a few non-colonized bolts. Another characteristic looked for when selecting bolt pairs to cage with *S. nigricornis* specimens was the bark thickness of bolt pairs. Thicker barked bolt pairs were selected over thinner barked bolt pairs because we wanted to increase the likelihood that

subcortical beetles were successfully developing in field-colonized bolts. Many subcortical beetles that utilize phloem develop faster, create more galleries, have higher brood densities and experience less mortality in thicker as opposed to thinner bark and phloem (Haack et al. 1987, Zhang and Zhang 1993). However, thick bark can inhibit the ability of *Sirex* to reach the xylem with their ovipositor and it limits the realized fecundity of small woodwasps presumably because their ovipositors are too short to reach xylem (Coutts 1965, Morgan 1968, Fukuda and Hijii 1998). Thus, caution was taken to not place thick barked bolts in cages with small specimens. Do to the selection criteria of rejecting bolt pairs taken from traps that contained woodwasps and wanting to select field-colonized bolts with high levels of cerambycid oviposition pits, bolts selected to be caged with *Sirex* all came from bolts placed out in the field between September 21st and October 19th, 2011. These dates correspond with peak cerambycid activity and the onset of *Sirex* activity (Chapter 2). The average bark thickness and volume of bolts that ended up being caged with *S. nigricornis* females was 2.9 mm (Range: 0.7 to 13.1 mm) and 5424 cm^3 (Range: 2381 to 11219 cm^3). The average number of cerambycid pits on the field-colonized bolts used was 57 (Range: 30 to 117).

Whenever *S. nigricornis* females became available, they were placed in a 32 x 30 x 76 cm wooden framed mesh cage containing a pair of bolts. A total of 27 replications were conducted. All replications were started between the 3rd and 22nd of November, 2011. The range of time from when a bolt pair was returned from the field and when the bolt pair was caged with *S. nigricornis* was between two and six weeks. *Sirex nigricornis* specimens were either collected from rearing containers stocked with trap trees colonized in the fall of 2010 or from live field-collected specimens. Field-collected specimens were trapped inside the traps made for collecting live specimens (Chapter 2). Trapped specimens were included because of low numbers of adults

emerging from rearing containers. One to two females were placed in each cage depending on the number of *S. nigricornis* and cages available. Two females were used when possible. Once all females in a cage had died, the pair of bolts was removed and the number of drill sites on each bolt was counted under a stereomicroscope. Care was taken to scrutinize cracks and crevices in bark for drill sites. All drill sites found had a box drawn around them so they could easily be relocated in the future.

After counting the number of drill sites on both bolts in a pair, the bolts were set aside for a few months. Bolts from replications in which no drill sites were found on either bolt were removed from further study (Cages 22 to 27; Table 5). Three bolt pairs were left at ambient temperature and the other 18 bolt pairs sat out at room temperature for one to three weeks before being placed in a walk-in cooler kept at approximately 4.5˚ C. Bolts were rested on their sides on counter tops or shelves to keep them off the ground. Although bolts from different replications were stored differently, bolt pairs were always handled in the same manner and stored in the same conditions.

On the 14th and 15th of February, 2012, approximately three months after the bolts had been exposed to *S. nigricornis*, bolts were cut in half from 66 cm to 33 cm (hereto referred to as a bolt section) with a laser guided miter saw. The top and bottom of each bolt section was labeled with the bolts unique identifier and their respective position (i.e. top or bottom). The moisture content of each bolt section was measured four times with a Protimeter® Timbermaster moisture meter set at the appropriate calibration setting, 'B'. Two measurements were taken on the freshly cut end of the bolt section (middle) and two measurements were taken on the other end (end) of the bolt section. All measurements were taken approximately 1.5 cm from the edge of the bark. The time between being halved into sections and measuring moisture content was

kept minimal. The number of drill sites on the top and bottom section of each bolt was tallied so that comparisons could be made between the top and bottom of bolts. It should be noted that the sum of the number of drill sites on the top and bottom of each bolt should match the total number of drill sites initially counted on the bolt. However, holes near the direct center of a bolt may be lost due to the saw cut; hence the sum of drill sites created on the top and bottom of a bolt may be slightly less than the total number of drill sites initially counted on the bolt. In the case of one bolt pair (Cage 20; Table 5) in which the non-colonized bolt had one drill site and the field-colonized bolt had none, the drill site on the colonized bolt was lost. This effectively left zero drill sites created in the cage to be dissected and used in further analyses.

Bolt sections were then placed on the ground, standing upright on the old end, until they were dissected. Dissections lasted from the first week in March till the first week in May, 2012. Dissections were done under stereomicroscopes. Hand-held woodworking tools and scalpels were used in dissection of drill sites. The first five bolt pairs dissected (both top and bottom sections) were dissected slightly differently than the remaining bolt pairs. The architecture and content of drill sites on the first bolts dissected was investigated by dissecting though the bark and 1 to 2 mm into the xylem. On these five bolt pairs, all sites possible were dissected; however, it was often necessary to remove excess bark and xylem surrounding a drill site and this could obscure the architecture of drill sites located in close proximity. Therefore, not all drill sites on these bolts were dissected. Shaving down through the bark proved to be extremely tedious and excessively destructive as it was often necessary to excise larger areas than would be expected if the bark was gone. Excising exceedingly large areas of bark was necessary for achieving acceptable angles of approach to dig further into the wood with the woodcarving tools. It was decided to shave the bark of the remaining bolts down to the xylem with a drawshave.

The number of drill sites relocated after shaving the bark did not differ significantly from the number of drill sites counted in the bark (ANOVA: $F_{1, 52} = 1.7$, $P = 0.19$). In some cases more drill sites were located in the xylem than were counted in the bark, but a majority of the time fewer drill sites were located in the xylem then were originally counted in the bark. Drill site architecture for all drill sites uncovered was recorded as either being a single, double, treble, quadruple, or quintuple tunneled drill site depending on if there were one, two, three, four, or five tunnels respectively, located at that drill site. It was fairly easy to distinguish a drill site in the xylem as being distinctive from other drill sites because when multiple tunnels were created at a drill site, the tunnels were distributed perpendicular to the longitudinal axis of the bolt. Thus even in areas with high densities of drill sites, unless the tunnels of drill sites were created at the same height up the bolt, they could be separated from each other. Even if drill sites were located close to each other and their tunnels were on the same horizontal plane they could be separated as distinctive drill sites by looking at the angles of tunnels in relation to each other. The center most tunnel almost always penetrates directly into the xylem with the remaining tunnels being created at ever more oblique angles as they move outward from the center tunnel (Coutts 1965, Spradbery 1977).

After inventorying the drill site types on a 33 cm bolt section, a subset of the drill sites were selected for further dissection to investigate their contents. Bolt sections from replications in which only one total drill site was created on both bolts in the cage were not dissected. If a bolt section had less than 12 drill sites, all drill sites on that section were dissected. If a bolt section had more than 12 drill sites, the drill sites for dissection were selected in the following way to avoid sampling bias. Two lines, 180˚ from each other, were drawn parallel to the longitudinal axis from one end of the bolt section to the other. This effectively demarked the

bolt section into two halves. Two circumscribing lines, one a third of the way and another two-thirds of the way up, were drawn on the bolt section. This demarked the bolt section into three vertical regions of equal proportions and when combined with the lines drawn along the longitudinal axis, six quadrats were formed. If possible, two drill sites were dissected in each quadrat. One drill site selected for dissection was located closest to the bottom right corner of the quadrat and the other was closest to the top left corner. Therefore, if every quadrat contained at least two drill sites, 12 drill sites would be dissected. If a quadrat had less than two drill sites, but other quadrats had extra drill sites, the extra drill sites would be dissected from another quadrat to make up the missing number until 12 drill sites were dissected on that bolt. "Borrowed" drill sites were not dissected until all quadrats had their two drill sites dissected. If possible, borrowed drill sites were selected from the adjacent (same height up bolt) quadrat. The drill site that was located closest to the area missing a drill site was selected. For example, if one of the middle quadrats only had one drill site and the drill site was located closer to the top left corner of the quadrat, then it would be missing a drill site from the bottom right corner. The drill site closest to the bottom left corner of the middle quadrat on the other side of the bolt would be dissected to make up for the missing drill site. When there was no extra drill site on an adjacent quadrat, the first extra drill site located by the dissector in any of the remaining four quadrats was utilized. At most, 12 drill sites were dissected on each 33 cm section of a bolt. Data from each 33 cm section of bolt were combined for the whole 66 cm bolt. Each 66 cm bolt could have up to 24 drill sites dissected in this manner. However, most bolts contained fewer than 24 drill sites dissected.

Four bolts had more than 24 drill sites dissected in an effort to collect more sample points. All four of these bolts were ones that had not been debarked when assessing the

frequency of the different architecture types. It is worth noting that these four bolts were from two bolt pairs. Each bolt in a pair had a similar number of drill sites to its partner. All drill sites that had not been obscured while making other dissections were dissected. Sampling bias (e.g. deciding to select a drill site of a given architecture type or in a given location) was of little concern as all drill sites possible were dissected. The number dissected from each bolt was 34 for a non-colonized bolt and 42 for its field-colonized partner; and 37 for the other non-colonized bolt and 44 for its field-colonized partner. This inflated the amount of drill sites dissected on field-colonized bolts compared to non-colonized bolts; however, the total number of drill sites dissected on non-colonized bolts was still slightly higher (213 compared to 195).

Drill sites were dissected until the ends of all tunnels at that site were reached. Larval feeding galleries leading away from tunnels were traced until either a larvae was found or until the gallery ceased to continue, but no larvae was found. When larval galleries ceased to continue, there was usually a hole where the body of the larvae used to be. On three occasions the larval feeding gallery was lost without reaching the end of the gallery. In these instances where a larval feeding gallery was lost, the data from that drill site was thrown out. The architecture of each drill site, number of eggs still in an oviposition tunnel, number of live and dead larvae still in an oviposition tunnel, number of larval galleries leading away from tunnels and the number of live larvae recovered at the end of a larval gallery were recorded.

ANALYSES AND CALCULATIONS

All statistical analyses were performed using JMP Pro 9 (SAS Institute Inc., Carey, NC) unless otherwise noted. Potential outliers which could bias the outcome of analyses were determined by looking at their multivariate distributions and at their jackknife distances.

Observations were considered as potential outliers if they occurred outside a 95% density ellipse in multivariate scatterplots, and/or if they had a relatively large jackknife distance. Differences between treatments were considered significant if the corresponding test statistic yielded a *P*-value less than 0.05.

Standard error (SE) estimates reported with means may differ from the standard error estimate used in analysis. Standard error estimates reported with means were calculated from the variance within a particular group or treatment while standard error estimates used in statistical calculations often required pooling the variances from the different groups or treatments. Therefore something may be statistically significant, but when looking at the reported means and standard errors it may appear as though the mean estimates are not significantly different at the 0.05 alpha level.

Morphometrics and fecundity

The relationships among body measurements and potential fecundity were investigated with linear regression. Correlation coefficients were estimated using restricted maximum likelihood.

Drill site frequency

Paired *t*-tests were used to test the hypothesis that the mean difference in drill sites created by *S. nigricornis* between paired observations is zero. A total of four different components of host material were analyzed in this manner. Comparisons were made between shaved and unshaved bark (Bark study), *Ips*-colonized and non-colonized bolts (*Ips* study), field-colonized and non-colonized bolts (Field study), and the tops and bottoms of bolts (Height on bolt). Statistical analysis was performed both including and excluding potential outliers. Potential outliers were considered in relation to the number of drill sites created or the difference

between drill sites created among pairs. The distribution of the difference in number of drill sites created between bolts approximated normality enough to satisfy this assumption of the paired *t*-test.

Bark study: The difference in number of drill sites created on shaved and unshaved portions of bark was analyzed with bolts as the sample unit. This difference between shaved and unshaved portions of bark was also analyzed with cages as the sample unit. There were a total of 14 observations when bolt was considered the sample unit and seven observations when cage was considered the sample unit.

Ips study: The difference in number of drill sites created on *Ips*-colonized and non-colonized bolts was analyzed with cage being the sample unit. The experiment was conducted on three separate batches of bolt pairings. Batch 1 contained five bolt pairs, but when it was discovered that one of the specimens utilized was a woodwasp other than *S. nigricornis* (specimen was *Urocerus cressoni*) data from that observation was removed from analysis; therefore Batch 1 only contained four observations. Batch 2 contained five bolt pairs and Batch 3 contained four bolt pairs. In total, 13 observations were included in analysis.

Field study: The difference in number of drill sites created on field-colonized bolts and non-colonized bolts was analyzed with cage being the sample unit. Six of the 27 cages (replicates) received no drill sites on either bolt. In addition to the six cages with no drill holes, three cages had a lone drill site made on one of the two bolts. Looking at the difference in number of drill holes between treatments when no or one drill site in a cage was made may be misleading in the results. No or one drill site created in a cage could symbolize that *S. nigricornis* females in that cage chose not to drill on either bolt, however this hardly shows a preference. Therefore analyses (both the paired *t*-test and outlier analysis) were conducted with

all replicates included, again with the six cages that received no drill sites excluded and then once again with the nine cages that received one or less drill sites excluded.

Height on bolt: The difference between the number of drill sites created on the tops and bottoms of bolts was analyzed with bolt and cage being the sample unit. Data used for this analysis comes from the field-choice study. Only the 20 cages with bolt pairs that were cut in half and retained drill sites were used in this analysis.

Drill site architecture and frequency

The architecture of drill sites on bolts from the *Ips* and field studies were investigated. A Fisher's exact test and/or a G-test for goodness-of-fit were used to test the hypothesis that there is no difference in the proportion of architecture types between various components of host material.

A one-way analysis of variance (ANOVA) was used when used to test the hypothesis that the mean number of tunnels per drill site on treated bolts (*Ips*-colonized bolts or field-colonized bolts) is equal to the mean number of tunnels per drill site created on control bolts (non-colonized bolts from respective studies). Levene's test was used to check that treatment variances were homoscedastic. If treatment variances were unequal, then a Welch's test was used to test the hypothesis that the mean number of tunnels per drill site on treated bolts is equal to the mean number of tunnels per drill site created on non-colonized control bolts, although the variance in the number of tunnels per drill sites may differ between treatments. Information regarding the Levene's test is only included when the variances between treatments are unequal. If an ANOVA is used, the Levene's test showed no significant difference in the treatment variances and the statistics for it are omitted.

Assumptions being made with these means comparisons tests are that females probe at random as it pertains to the suitability of host material. Once the ovipositor is inside host material, sensory pits on it are stimulated by some condition of the area drilled into (microsite) and either a female will find the site suitable and she will create more tunnels at that dill site or she will find the site unsuitable and will reject it after making no or few additional tunnels (Madden 1974).

A means comparison was done once on all the data from a study. The means comparison test tested if *S. nigricornis* females drilled more into colonized or non-colonized bolts. Of concern is that some bolts received very few drill sites and the average number of tunnels per drill site is taken from these low counts. Thus, analysis was run again only retaining observations in which both bolts in a pair received a minimum of five drill sites uncovered.

Ips study: The percentage of drill sites dissected that ended in the bark or xylem was calculated using Microsoft® Excel®. All further analyses were only conducted on drill sites that penetrated into the xylem. Each drill site was considered the sample unit.

Field study: Analysis was performed only on drill sites that penetrated into the xylem. Each drill site was considered the sample unit.

Height on bolt: Also investigated using data from the field study was if there was a difference between the mean number of tunnels per drill site on the top and bottom halves of bolts. Data from both bolts in a cage were pooled together for this analysis. That is, the number of tunnels per drill site on the top half of both bolts in a cage were added together and the number of tunnels per drill site on the bottom half of both bolts were added together and then compared. Analysis was only performed on tunnels that penetrated into the xylem. Each drill site was considered the sample unit.

Early stage offspring mortality

An estimate for number of eggs oviposited (estimated eggs laid) was determined by multiplying Madden's (1974) 'drill type-egg number' conversion factors (mean number of eggs per architecture type) by the frequency of that architecture type. For example, Madden calculated the mean number of eggs oviposited in double tunnel drill sites to be 0.7; multiplying this by the 151, the number of double tunneled drill sites uncovered, yields an estimate of approximately 106 eggs oviposited in all double tunnel drill sites. These conversion factors were, however, derived from *Sirex noctilio* drill sites. It is possible that *S. nigricornis* allocates its eggs differently than *S. noctilio*, however, dissections on drill sites in our lab look strikingly similar to those of *S. noctilio* and like *S. noctilio*, as the number of tunnels per drill site increases, so too does evidence of oviposition (egg found, larvae found, or larval gallery leading away from drill site; Table 8). Ovipositing more eggs as the number of tunnels per drill site increases appears to be a trait shared by all *Sirex* (Spradbery 1977, Spradbery and Kirk 1981). However, even within a species, the mean number of eggs per drill site may vary considerably depending on, among other factors, the age of the wasp and condition of host material (Coutts and Dolezal 1969, Madden 1974, Spradbery 1977, Spradbery and Kirk 1981). I feel that the drill type-egg number conversion factors provided by Madden (1974) and used by others (Neumann and Minko 1981) can be crudely applied to estimating the number of eggs oviposited by *S. nigricornis*. The conversion factors used were 0.04 eggs per drill site on single drills, 0.7 eggs per drill site on double drills, 1.6 eggs per drill site on treble drills and 2.3 eggs per drill site on quadruple and quintuple drills.

Sign of oviposition when dissecting drill sites was calculated as the sum of eggs in oviposition tunnels, larvae in oviposition tunnels and larval feeding galleries leading away from

tunnels. This number is not the same as number of eggs oviposited. Signs of oviposition will equal the number of eggs oviposited only if all eggs laid, larvae in oviposition tunnels (be they alive or dead) and larval galleries can be accounted for. Unfortunately, it is unknown if eggs that fail to develop or larvae that eclosed but died without feeding left much evidence (possibly minute larval mandibles) of being there. Thus, unless drill sites are dissected immediately after a female oviposits, it cannot be said unequivocally if an egg was deposited or not.

Estimated first instar larvae was calculated as the number of larval feeding galleries plus the number of larvae still in oviposition tunnels. This number includes dead larvae found inside oviposition tunnels, but does not include larvae who died in their oviposition tunnel leaving behind little or no detectable remnants.

The number of first instar larvae who started to feed (live feeding larvae) was assessed by tallying the number of galleries leading away from oviposition tunnels. *Sirex* galleries are easily diagnosed by the tightly packed sawdust leading away from oviposition tunnels (Madden 1981, Neumann and Minko 1981).

Percent mortality estimates were calculated various, sometime multiple ways depending on the stage in question. Percent egg mortality was calculated by the following equation:

$$\text{Percent egg mortality} = 1 - \frac{\text{EFIL}}{\text{EEL} - \text{NEOT}} \text{ X } 100\%$$

where EFIL = estimated first instar larvae, EEL = estimated eggs laid and NEOT = number of eggs in oviposition tunnels. Percent mortality of eggs and first instar larvae before they reach the feeding stage was calculated as:

$$\text{Percent mortality before feeding} = 1 - \frac{\text{LFG}}{\text{EEL} - \text{LLOT} - \text{NEOT}} \text{ X } 100\%$$

where LLOT = number of live larvae in oviposition tunnels and LFG = larval feeding gallery. This number disregards live larvae in oviposition tunnels which have yet to create visible feeding

galleries, but are not dead. If live larvae in oviposition tunnels are to be counted and they are assumed to feed enough to create a visible gallery before dying, then the equation will change to:

$$\text{Percent mortality before feeding including LLOT} = 1 - \frac{\text{LFG} + \text{LLOT}}{\text{EEL} - \text{NEOT}} \text{ X } 100\%$$

The percent of larvae which started a feeding gallery and were still alive at the time of sampling was calculated by the following equation:

$$\text{Percent mortality to feeding larvae at time of sampling} = 1 - \frac{\text{LFL}}{\text{LFG}} \text{ X } 100\%$$

where LFL = number of live feeding larvae. If it is assumed that live and dead larvae in oviposition tunnels have created feeding galleries that just went unseen, then the equation would change to:

$$\text{Percent mortality to feeding larvae at time of sampling including LLOT and DLOT} = 1 - \frac{\text{LLOT} + \text{LFL}}{\text{LFG} + \text{LLOT} + \text{DLOT}} \text{ X } 100\%$$

where DLOT = dead larvae in oviposition tunnels. The percent total estimated mortality at the time of sampling was calculated by the equation:

$$\text{Percent estimated total mortality till time of sampling} = 1 - \frac{\text{LLOT} + \text{LFL}}{\text{EEL} - \text{NEOT}} \text{ X } 100\%$$

Change in moisture content of field bolts

Moisture content estimates for the ends of bolts were taken as the average of four measurements; two from the top end of the bolt and two from the bottom end. Moisture content estimates for the middle of bolts were taken as the average of four measurements; two from each freshly cut end (middle).

The change in moisture content for field-colonized bolts and their controls was determined by subtracting the estimated moisture content on the ends of bolts taken

approximately three months after being returned from the field, from the moisture content measured on the ends of these bolts right after they were returned from the field. This was done on 20 of the 21 bolt pairs that were halved for dissection. The one bolt pair not included (Cage 20), was excluded because the moisture content of one of the bolts was accidently not taken.

A paired *t*-test was used to test the hypothesis that the mean difference in moisture content between paired observations is zero. The mean difference in moisture content on the ends of bolts immediately after they were returned from the field, on the ends of bolts about 3 months after they were returned from the field and in the middle of bolts about 3 months after they were returned from the field were compared between field-colonized and non-colonized bolts with this paired *t*-test. Also compared was the change in moisture content between field-colonized and non-colonized bolts. These comparisons were done on the 20 bolt pairs who had moisture content measurements taken 3 months later. No pairs of bolts were considered as outliers.

RESULTS

Morphometrics and fecundity

There was considerable variation in the body size and potential fecundity of females measured (Table 1). The average predicted body length measured to the tip of the ovipositor, based on the regression equation yielded by plotting body length to the tip of the ovipositor against body length to the tip of the cornus, was slightly larger than the calculated body length to the tip of the ovipositor (Table 1). All body measurements were strongly correlated with each other and with a female's potential fecundity (Table 2). No nematodes were discovered in any specimens dissected.

An equation could be derived for predicting a female's potential fecundity using any of these body measurements, but pronotal width was selected because it had the best linear correlation with a female's potential fecundity and because it is a comparatively rigid body part. Number of eggs was log transformed before the equation line was fit due to a significant lack of fit on the untransformed data (Figure 3).

Drill site frequency

Bark study: Cage 6 which contained two bolts (M and N) had no drill sites while all other cages and bolts contained at least one drill site (Table 3). The number of drill sites created on unshaved and shaved portions of bolts did not differ significantly when either bolts (*t*-test: $t_{13} = 2.09$, $P = 0.057$) or cages (*t*-test: $t_6 = 2.55$, $P = 0.055$) were considered the sample unit and all observations were included.

Bolt H, which was the sole bolts in Cage 2, received considerably more drills than any other observation and it was flagged as a potential outlier. The difference in the number of drill sites between the shaved and unshaved portion of Bolt H, 114, is relatively high when compared to the overall mean difference of 17.1 (SE = 8.2). In respect to cages, the difference in the number of drill sites created between shaved and unshaved portions of the bolt in Cage 2 was still 114, but the mean difference was 34.1 (SE = 14.4). Even though Bolt H (or Cage 6) showed the same general trend of the shaved area containing more drill sites than the unshaved area of bolt(s) (Figure 4), its large variation from the overall mean likely had a strong affect on the standard error. Redoing the analyses with the observations of Bolt H or Cage 6 removed yielded significant differences between the number of drill sites created on shaved and unshaved bark when bolt was considered the sample unit (*t*-test: $t_{12} = 2.63$, $P = 0.022$) and when cage was considered the sample unit (*t*-test: $t_5 = 3.26$, $P = 0.023$). With the influential outlier removed, the

number of drill sites created in shaved bark ($Mean_{bolts}$ = 20.0, SE_{bolts} = 5.2; $Mean_{cages}$ = 43.3, SE_{cages} = 12.4) was significantly higher than on unshaved bark ($Mean_{bolts}$ = 10.4, SE_{bolts} = 2.9; $Mean_{cages}$ = 22.5, SE_{cages} = 6.4) as it pertains to both bolts and cages being the sample units.

Ips study: Female *S. nigricornis* created drill sites on at least one bolt in all 13 cages, although there was considerably variation in the total number of drill sites created between bolts and between cages (Table 4). Some bolts in a pair received zero drills even though its partner was drilled on. There was no difference detected in the number of drill sites created between *Ips*-colonized and non-colonized bolts (*t*-test: $t_{12} = 0.41$, $P = 0.69$; Figure 5). Two observations, corresponding to cages H and L, were flagged as potential outliers do to the relatively large total number of drill sites created on one of the bolts in a pair. Removing these outliers did not change the result (*t*-test: $t_{10} = 0.75$, $P = 0.47$). The mean number of drill sites created on the non-colonized bolts with the outliers included (Mean = 25.2, SE = 8.7) and excluded (Mean = 16.5, SE = 3.9) was higher than on the *Ips*-colonized bolts with the outliers included (Mean = 20.2, SE = 8.3) and excluded (Mean = 12.9, SE = 3.7), but not significantly so. The effect of batch and the interaction of treatment by batch were not significant. Neither was the effect of group or the interaction of treatment by group.

Field study: There was a lot of variation in the number of drill sites created as it pertains to both bolts and cages (Table 5). Observations corresponding to cages 4, 5, and 10 were flagged as potential outliers when all 27 replications were analyzed. Only observations 4 and 10 were considered potential outliers when replications in which zero drill sites were excluded was analyzed and only observation 10 was flagged as a potential outlier (and barely) when all replications in which one or less drill sites were excluded was analyzed. Excluding cages with zero or one drill site did not change the result's significance when including or excluding

potential outliers. Therefore, only information regarding the all inclusive test with 27 replications is reported. There was no difference found between the number of drill sites created on field-colonized bolts and non-colonized bolts (*t*-test: $t_{26} = 1.63$, $P = 0.12$; Figure 6). The average number of drill sites created on non-colonized bolts (Mean = 23.6, SE = 6.9) was higher than on field-colonized bolts (Mean = 15.2, SE = 4.8), but no significantly so.

Height on Bolt: In general, more drill sites were created on the top halves of bolts than the bottom halves (Figure 7). This was reflected in cages as well, with more drill sites being created on the top halves of bolt pairs than on the bottom halves (Table 5). Five observations (5, 5F, 9, 10 and 17; Figure 7) were flagged as potential outliers when bolts were considered the sample unit. Two observations were flagged as potential outliers (Cages 4, 5 and 10; Table 5) when cages were considered the sample unit. Removing outliers did not change the significance of results, so only statistics that involve all observations are reported. The mean difference in number of drill sites created on the top and bottom halves of bolts was significant when either bolts (*t*-test: $t_{39} = 3.61$, $P = 0.0009$) or cages (*t*-test: $t_{19} = 3.54$, $P = 0.0021$) were considered the sample unit. The average number drill sites created on the top half of bolts (Mean = 16.8, SE = 3.5) was higher than the average number created on the bottom half of bolts (Mean = 8.4, SE = 1.8). Likewise, the average number of drill sites created on the top halves of bolt pairs (Mean = 33.5, SE = 8.4) was higher than the average number of drill sites created on the bottom halves of bolt pairs (Mean = 16.7, SE = 4.7).

Drill site architecture and frequency

Ips study: In total, the architecture of 204 drill sites was determined. A majority, 87% (177), penetrated into the xylem, while the remaining 13% (27) ended in the bark. Only the 177 drill sites that penetrated into the xylem were included in analysis of drill sites. Drill site

architecture ranged from 1 tunnel per drill site to 5 tunnels per drill site. The relative frequency of single, double, treble, quadruple and quintuple tunneled drill sites created on all bolts of both treatment types is 15%, 32%, 46%, 7% and 1% respectively (Table 6). Regarding just *Ips*-colonized bolts (87 drill sites dissected) the relative frequencies for the different architecture types were 17%, 40%, 43%, 0% and 0%, while for non-colonized bolts (90 drill sites dissected) they were 12%, 23%, 50%, 13% and 1% for single, double, treble, quadruple and quintuple tunneled drill sites respectively (Table 6). The proportion of various architecture types differed significantly between *Ips*-colonized and non-colonized bolts (G-test: G = 22.9, d.f. = 4, P = 0.0001; Fisher's exact test: table probability = $2.6e^{-7}$, P = 0.0003; Figure 8).

There was a significant difference in the average number of tunnels per drill site between *Ips*-colonized and non-colonized bolts (ANOVA: $F_{1, 175} = 11.84$, P = 0.0007; Figure 9) when all 177 observations were analyzed. The mean number of tunnels per drill site on the non-colonized bolt (Mean = 2.68, SE = 0.087, n = 90) was higher than that on the *Ips*-colonized bolt (Mean = 2.25, SE = 0.088, n = 87). Four replications (Cages B, E, I, and M; Table 4) only received drilling on one of the two bolts in a pair. The difference in the average number of tunnels per drill site was still significant (ANOVA: $F_{1, 122} = 9.75$, P = 0.0022) when bolts from the aforementioned replications were removed. In this instance, the mean number of tunnels per drill site on non-colonized bolts (Mean = 2.68, SE = 0.11, n = 60) was still higher than the mean number of tunnels per drill site on the *Ips*-colonized bolts (Mean = 2.20, SE = 0.11, n = 64). One additional cage contained a bolt in which only four drill sites were dissected (Cage C; Table 4). When bolts from this replication were also excluded from analysis, the significance of the result did not change (ANOVA: $F_{1,108} = 3.96$, P = 0.049) In this case the mean number of tunnels per

drill site on non-colonized bolts (Mean = 2.52, SE = 0.12, n = 50) was barely significantly higher than on the *Ips*-colonized bolts (Mean = 2.20, SE = 0.11, n = 60).

Field study: In total, the architecture of 569 drill sites was determined. No drill sites were found in the xylem of either bolt in 6 bolt pairs (Cages 1, 11, 12, 16, 19, and 20). Therefore they contributed no data to the relative frequencies or to analyses.

The relative frequency of single, double, treble, quadruple and quintuple tunneled drill sites is 4%, 36%, 50%, 8% and 2% respectively (Table 7). Regarding just field-colonized bolts (248 drill sites dissected) the relative frequencies for the different architecture types were 6%, 36%, 44%, 11% and 3%, while for non-colonized bolts (321 drill sites dissected) they were 2%, 36%, 55%, 6% and 2% for single, double, treble, quadruple and quintuple tunneled drill sites respectively (Table 7). The proportion of the various architecture types differed significantly between field-colonized and non-colonized bolts (G-test: G = 14.8, d.f. = 4, $P = 0.0053$; Fisher's exact test: table probability = $2.4e^{-7}$, $P = 0.0049$; Figure 10).

There was no difference in the average number of tunnels per drill site between field-colonized and non-colonized bolts (Welch's test: $F_{1, 464.3} = 0.018$, $P = 0.89$; because Levene's test: $F_{1, 567} = 13.73$, $P = 0.0002$) when all 569 observations were included. The mean number of tunnels per drill site on the field-colonized bolts (Mean = 2.67, SE = 0.055, n = 248) was almost exactly the same as the mean number of tunnels per drill site created on the non-colonized bolts (Mean = 2.68, SE = 0.038, n = 321). However, six replications (Cages 7, 8, 13, 15, 17 and 18; Table 5) only had drill sites uncovered in the xylem on one of the bolts in a pair. When data was restricted to cages that had at least one drill site uncovered on each bolt in a pair and these six cages were excluded, the difference between the two means was significant (Welch's test: $F_{1, 383.3} = 7.36$, $P = 0.0069$; because Levene's test: $F_{1, 431} = 3.92$, $P = 0.048$…. ANOVA: $F_{1, 431} = 7.36$, P

= 0.0064). In this case the mean number of tunnels per drill site on the non-colonized bolts (Mean = 2.68, SE = 0.043, n = 247) was greater than the mean number of tunnels per drill site on the field-colonized bolts (Mean = 2.49, SE = 0.054, n = 186). One other cage (Cage 6, Table 5) had pair of bolts in which one bolt received only one drill site that penetrated into the xylem. Further restricting analysis to data that comes from cages in which both bolts in a pair received at least five drill sites yields a significant difference (ANOVA: $F_{1, 407} = 7.02$, $P = 0.0084$; Figure 11) between the average number of tunnels per drill site on non-colonized bolts (Mean = 2.68, SE = 0.047, n = 224) and field-colonized bolts (Mean = 2.50, SE = 0.052, n = 185).

Height on bolt: There was no difference (ANOVA: $F_{1, 567} = 0.20$, $P = 0.65$) found between the mean number of tunnels per drill site created on the top halves (Mean = 2.67, SE = 0.039, n = 383) of a pair of bolts compared to their bottom halves (Mean = 2.70, SE = 0.056, n = 186) when all 569 observations are included. Two cages (Cages 8 and 15; Table 5), contained bolt pairs that only received drilling which penetrated into the xylem on one half of the bolts (i.e. top or bottom); the other half contained no drill sites. Redoing the analysis with these two cages removed did not change the result (ANOVA: $F_{1, 560} = 0.12$, $P = 0.73$). Excluding two additional cages (Cages 6 and 7; Table 5) in which less than five total drill sites were counted penetrating into the xylem on the bottom half of the bolt pair (the total number of drill sites for the top half of bolt pairs was always greater than five) did not change the result either (ANOVA: $F_{1, 534} = 0.13$, $P = 0.72$).

Early stage offspring mortality

In total, 408 drill sites were dissected. Two-hundred and thirteen of these drill sites were from non-colonized bolts and 195 were from field-colonized bolts (Table 8). The estimated number of eggs laid, signs of oviposition, the number of eggs in oviposition tunnels, the number

of live and dead larvae in oviposition tunnels, the number of larval feeding galleries, the estimated number of first instar larvae and the number of live feeding larvae recovered for the different drill site architecture types and bolt types are summarized (Table 8).

There was no difference in the distribution of tunnel types dissected between non-colonized and field-colonized bolts (G-test: G = 3.78, d.f. = 4, P = 0.44; Fisher's exact test: table probability = 0.00015, P =0.46). This is important because drill sites of different architecture types are expected to have different numbers of eggs oviposited in them, but they may also have differences in egg hatch or larval survival and this could obscure inferences made on differences between the two bolt types (i.e. field-colonized and non-colonized). Having similar distribution of drill types dissected between field-colonized and non-colonized bolts safeguards against a false impression of differences in mortality that were to occur because the relative ratios of the different architecture types dissected were different.

Depending on the assumptions being made, estimates of early-stage offspring mortality vary for both the non-colonized and field-colonized bolts (Table 9). Regardless of the assumptions and offspring stage being investigated, the percent mortality estimate is always lower for the non-colonized bolt when compared to the field colonized bolt (Table 9).

Change in moisture content of field bolts

The ends of most bolts had a decrease in moisture content (MC) after being returned from the field, although some bolts gained moisture (Figure 12). Fortunately, the change in moisture content for both bolts in a pair was relatively similar (Figure 12). That is, if the field-colonized bolt gained moisture, so too did the non-colonized bolt. The moisture content on the ends of non-colonized bolts after three months was significantly different (t-test: t_{19} = 2.60, P = 0.018) from the original moisture content estimates taken immediately after they were returned from the

field. The moisture content estimates of field-colonized bolts after three months were also significantly different (*t*-test: $t_{19} = 3.78$, $P = 0.0013$) from the moisture content estimates taken immediately after they were returned from the field. Both the field-colonized bolts and non-colonized bolts had, on average, a reduction in moisture content when estimated at their ends. That said, the field-colonized bolts had a significantly larger reduction in moisture content when compared to non-colonized bolts (*t*-test: $t_{19} = 2.94$, $P = 0.0083$). The non-colonized bolts lost, on average, 5.9% (SE = 2.27%) moisture content, while the field-colonized bolts lost an average of 9.9% (SE = 2.62%) moisture content on their ends.

There was no difference detected (*t*-test: $t_{19} = 0.23$, $P = 0.82$; Figure 13) between the mean moisture content on the ends of field-colonized bolts (Mean = 31.06% MC, SE = 0.97%) and non-colonized bolts (Mean = 31.3% MC, SE = 1.04%) immediately after they were returned from the field. However, this difference was significant (*t*-test: $t_{19} = 2.47$, $P = 0.023$; Figure 14) after approximately three months had elapsed. The difference in moisture content at the center of these bolts was also significant after three months (*t*-test: $t_{19} = 2.58$, $P = 0.018$, Figure 15). The non-colonized bolts had, on average, higher moisture content estimates at their ends (Mean = 25.4% MC, SE = 2.38%) and centers (Mean = 42.6% MC, SE = 2.29%) compared to the ends (Mean = 21.2% MC, SE = 2.82%) and centers (Mean = 34.7% MC, SE = 3.47%) of field-colonized bolts.

DISCUSSION

The size of *Sirex nigricornis* reared from trap trees varied considerably. This is not surprising as other *Sirex* also have a wide size range (Spradbery 1977, Neumann and Minko 1981, Fukuda et al. 1993, Ryan and Hurley 2012). The variation in size of emerging adults is

attributed to conditions of the host, predominately moisture content (Madden 1981). There clearly are differences between trees or height on a tree as it pertains to the size of emerging adults, but even specimens oviposited at the same drill site and emerging concurrently can be of very different sizes due to microsite differences (Madden 1981).

The mean body length as measured from the tip of the ovipositor to the vertex of the head of *S. nigricornis* females measured here was 19.7 mm (SE =1.1) although this estimate was taken from only 17 specimens. The mean body length from the vertex of the head to the tip of the ovipositor, as estimated from the relationship between this body length and body length measured from the tip of the cornus to the vertex of the head, was 21.2 mm (SE = 0.6, n =72). This is shorter than the mean body length of both nematode infected (21.6 mm, SE = 0.4) and uninfected females (23.6 mm, SE = 0.3) calculated by Keeler (2012). Specimens measured by Keeler were caught in baited panel traps, while the specimens measured here were all reared from trees approximately 12.7 cm in dbh. Although the specimens measured here were not selected at random, specimens spanning the size range were measured and according to the Shapiro-Wilk W test, they were normally distributed (W=.981, P = .35; H_0: The data are from the normal distribution). The size difference between uninfected females emerging in the lab and uninfected females trapped by Keeler could be caused by a few factors. First, larger female *Sirex* are known to be better at dispersing and they live longer (Madden 1974, Bruzzone et al. 2009), both factors should increase the likelihood that, on average, larger females encounter traps than smaller ones. Another possibility pertains to the size of the host. Owing to Forest Service regulations, we were only allowed to fell trap trees under 12.7 cm in dbh and this restricts the size of material that the *Sirex* develop in to 12.7 cm in dbh or less. Smaller bolts appear less conducive to the development of *Sirex* than larger bolts (Coutts 1965). If this is the case, using

bolts less than 12.7 cm in dbh should reduce the average size of emerging females compared to a population of *Sirex* emerging from bolts of various sizes.

Similar to body measurements, there was considerably variation in the potential fecundity of *S. nigricornis*. No females who were dissected contained nematodes. The mean potential fecundity of *S. nigricornis* females was ~176 eggs, but this ranged from 37 eggs to 413 eggs and was generally dependant on the size of the female being measured. Potential fecundity (PF) can be predicted by the equation: $PF = 9.77(2.52^{PW})$, where PW = pronotal width measured in mm. The range of eggs found for *S. nigricornis* is similar to that of other *Sirex* studied, but particularly the other *Sirex* that prefer colonizing pine, *S. noctilio* and *S. nitobei* (Spradbery 1977, Spradbery and Kirk 1981, Fukuda et al. 1993).

The architecture of *S. nigricornis* drill sites appears strikingly similar to that of *S. noctilio* to a point where deciphering between the two is likely impossible (Coutts 1965, Spradbery 1977). Both woodwasps create 1 to 5 tunnels per drill site in the xylem where they oviposit (Coutts 1965, Madden 1974, Spradbery 1977). It has been shown for *S. noctilio* that as the number of tunnels per drill site increases, so too does the number of eggs laid (Madden 1974, Spradbery 1977). For *S. nigricornis* studied here, as the number of tunnels per drill site increased, so too did evidence that an egg was deposited. Assuming that the likelihood of uncovering vestiges of oviposition is similar among all architecture types, then as the number of tunnels per drill site increases, so too does the number of eggs oviposited.

One thing worth noting is that *S. nigricornis* occasionally oviposited eggs into bark. This has previously only been reported in *Sirex cyaneus.* When eggs were oviposited into bark, the bark was generally thicker although likely not so thick it kept ovipositing females from reaching

the xylem. Unlike tunnels that penetrate into the xylem and are perpendicular to the longitudinal axis of a tree or bolt, tunnels which ended in the bark were more parallel to the longitudinal axis.

Sirex nigricornis showed little preference for where it created probing drill sites in relation to other insects inhabiting host material. Drilling occurred with the same frequency on bolts previously colonized by other insects as it did on bolts not colonized by other insects. The lack of preference could arise for multiple reasons. First, is that the tests require more repetition. Take for instance, the study involving field-colonized bolts. The mean difference in drill sites created was approximately 11 drill sites with more drill sites counted on non-colonized bolt. Given the standard deviations on the number of drill sites counted on non-colonized and field-colonized bolts, 38 and 27 respectively, and assuming a power level of 0.80, it would take about 120 more replications for the difference of 11 drill sites to be significant at a significance level of 0.05. Another possibility is that the colonized bolts did not differ much from the non-colonized control bolts, so *S. nigricornis* females responded accordingly, drilling equally in both. The last possibility is that woodwasps, after being attracted to a host, generally will not decipher the oviposition quality of that host until they have assessed the host's suitability with their ovipositor. Anecdotal evidence supports this idea as woodwasps will drill into material unsuitable for the development of their offspring. Female *S. nigricornis* in our lab, on numerous occasions, have been seen drilling into plywood cages despite there being pine bolts for them to oviposit into. We have also seen specimens drill into matchbooks, pieces of paper and even into the plastic floor of a Sterelite® storage tub. Other siricids too have been documented drilling into sand (Blackman and Stage 1924) and weathered wooden tables (Anonymous 2012).

Sirex nigricornis may create drill sites with equal frequency as it pertains to the contents of potential host material, but two factors, shaved bark and height up a bolt, both

increased the likelihood drill sites were created. More drill sites were created on shaved areas of bolts than unshaved areas. *Sirex noctilio* also seems to show a drilling preference for bark which has been stripped down on live trees (Coutts and Dolezal 1966). Exactly why female *Sirex* show a preference for shaved bark is unknown. The fact that *S. nigricornis* created more drill sites on the top halves of bolts compared to their bottoms halves may relate to their behavior. The wasps appear to walk up the bolt as they search for drill sites and once they reach the top, they fly back down a ways to start over. If they do not fly all the way down to the base of the bolt every time, we would expect more drill sites on the top than bottom half of a bolt because the wasps spend more time in that area.

Sirex nigricornis females may be relatively liberal with what they drill into, but they appear to be more frugal once their ovipositor has penetrated a considerable distance into potential host material. If a female is indiscriminate about where she probes, then whether an area is suitable for oviposition will not be determined until after she has probed into the host material. Once she has probed into host material, sensory pits in her ovipositor, detecting various characteristics of that specific microsite, provide feedback that either stimulates drilling additional tunnels or to reject the area (Madden 1974).

The experiments conducted here provide evidence that the presence of subcortical beetles increases the likelihood a probed site is deemed unsuitable for oviposition. Comparatively more tunnels per drill site were created on non-colonized bolts over beetle colonized bolts (both *Ips*-colonized and field-colonized). There was, however, no difference in the mean number of tunnels per drill site between the tops and bottoms of bolts.

What inhibits *S. nigricornis* females from ovipositing on beetle colonized bolts was not investigated here, but work done on other *Sirex* species may provide some insight. The presence

of beetle vectored *Leptographium wingfieldii* inhibited the number of drill scars (tunnels) created by *Sirex noctilio* compared to areas of the host that were inoculated with *Ophiostoma minus* or left untreated as a control (Ryan et al. 2011c). There are also anecdotal accounts of *S. noctilio* and *S. nitobei* avoiding or reducing oviposition activity on areas of hosts that contain blue-stain and decay fungi (Hanson 1939, Spradbery and Kirk 1978, Fukuda and Hijii 1996) which are vectored and aided in establishment by subcortical beetles and their associated mites (Moser 1985, Kukor and Martin 1986, Wingfield 1987, Speight 1989, Paine et al. 1997, Siitonen 2001, Jankowiak and Rossa 2007, Hofstetter 2011).

Another factor that may inhibit oviposition in beetle colonized bolts pertains to physical and chemical changes in host material which is facilitated by beetles and their associated fungi (Speight 1989, Siitonen 2001). Madden (1974) reports that "perception of a stimulus from the phloem at the onset of drilling results in either no egg being released from the oviduct or the release of eggs at a rate related to the strength of the stimulus; thus double, treble and higher but less frequent drills are made." Osmotic pressure of the phloem is believed to be the chief stimulus sensed by the ovipositor, thus factors that affect the osmotic pressure of the phloem will also affect the egg laying rate of *Sirex* (Madden 1974). Mining of phloem by subcortical beetles changes the physical structure of phloem, possibly its chemical nature as well, which may augment the way sensory pits in the ovipositor are stimulated, thus affecting egg laying rate. If mining of phloem and creation of galleries into the xylem by subcortical beetles change the moisture content of the sapwood, this too may have an effect on the egg laying rate of *S. nigricornis*. *Sirex noctilio*, for example, creates the most tunnels per drill site and oviposits the most eggs in woods at intermediate moisture contents around 40% to 75% oven dry weight (Coutts 1965, Morgan and Stewart 1966b). Another *Sirex* species, *S. nitobei*, oviposits more

eggs per drill site in bolts that are 4 to 24 days old (intermediate bolts) compared to older bolts (Fukuda and Hijii 1996). It is believed the older logs were deemed suboptimal by *S. nitobei* because the bolts "were markedly different in physical and biochemical properties from intermediate or optimum logs," in addition to the older logs having been invaded by decay fungi and other boring insects.

The decrease in mean number of tunnels per drill site on bolts colonized by subcortical beetles compared to non-colonized bolts may be an adaptive response by *S. nigricornis* to avoid ovipositing eggs in areas of hosts in which they have comparatively high chances of mortality. A variety of fungi were uncovered while dissecting the field-colonized bolts, while very little fungus was revealed in the non-colonized bolts. Fungi were not identified, but some bore resemblance to and likely were ophiostomatoid (blue-stain), *Trichoderma*, *Beauvaria* and other fungi commonly associated with subcortical beetles and their galleries. These fungi have been implicated in causing *Sirex* offspring mortality (Hanson 1939, Rawlings 1953, Coutts 1965, Morgan and Stewart 1966a, Neumann and Minko 1981). It is generally thought these fungi cause an increase in mortality to *Sirex* by outcompeting the woodwasps' fungal symbiont, *Amylostereum* (Coutts 1965, Neumann and Minko 1981, Ryan et al. 2011b). Ophiostomatoid fungi capture more uncolonized resources than *Amylostereum* when the two are inoculated concurrently and ophiostomatoid fungi can capture pine substrate from living cultures of *Amylostereum*, but *Amylostereum* never colonizes space occupied by living cultures of ophiostomatoid fungi (Ryan et al. 2011b). Apparently ophiostomatoid fungi are well suited to outcompete decay fungi like *Amylostereum* (Brown and Webber 2009). The growth of *Amylostereum* is also reportedly hindered by other fungi commonly isolated from trees. *Trichoderma* instead of *Amylostereum* has been recovered in areas where care was taken to

inoculate only *Amylostereum* into host trees (Tabata and Abe 1999) and *Trichoderma* as well as *Sphaeropsis sapinea* (Fries) Dyko & B. Sutton supposedly cause death of *Amylostereum* hyphae on agar plates (King 1966). These results suggest that at least some fungi vectored or aided in establishment by subcortical beetles can impact the growth and spread of *Amylostereum.* This in turn may impact the successful development and survival of *Sirex* offspring because they are, at least during the early larval stages, dependent on *Amylostereum* for nutrition (Stillwell 1966, Coutts and Dolezal 1969, Madden and Coutts 1979, Martin 1992, Ryan et al. 2011b).

Moisture content of the wood also appears to be strongly related to the successful establishment and growth of *Amylostereum,* as well as the development of *Sirex* offspring (Coutts 1965, Coutts and Dolezal 1965, Madden and Coutts 1979). The range of preferred moisture content for *S. noctilio* oviposition (40 to 75 %), favors the growth of *Amylostereum* (Coutts 1965, Coutts and Dolezal 1965, Madden and Coutts 1979) and this moisture content likely favors eclosion and early offspeing development (Morgan and Stewart 1966b, Talbot 1977, Neumann and Minko 1981, Ryan and Hurley 2012). As is evident from the field study where moisture content was measured, the presence of subcortical beetles and associated organisms was accompanied by lower moisture content of the sapwood after 3 months.

According to performance-preference linkage theory, we would expect *S. nigricornis* females to behave, on average, in a way that maximizes the performance of their offspring and/or their own total fitness. *Sirex nigricornis* females used in this study drilled into bolts colonized by subcortical beetles with nearly the same frequency as they did bolts from which the subcortical beetles had been excluded. Although the evidence was far from conclusive, the fact that estimates of mortality were higher on field-colonized bolts over non-colonized bolts suggests that the field-colonized bolts were less conducive to the development of *S. nigricornis*

offspring. If this is the case, then females drilling with equal frequency on colonized bolts (both *Ips* and field) as they did on non-colonized bolts seems counterintuitive. However, probing into a host even with an increased chance of larval mortality or reduced offspring development may have arisen in response to females' maximizing their own fitness. Mating, locating a potential host, probing into a host to assess its suitability and then ovipositing eggs all require expenditures of energy that is of limited supply to *Sirex* females adults. Mating takes place close to where a female emerges (Morgan 1968, Madden 1988), while the location of pine material suitably stressed enough to oviposit into is unknown. Females *Sirex* are, however, guided to suitably stressed pine trees by volatiles emitted from them (Simpson 1976, Simpson and McQuilkin 1976), but the wasps are still relying on external factors to cause host material to be in a suitably stressed state. That said, completely rejecting host material because it has other insects in it likely reduces a females total fitness. Looking for a new host increases the probability a female dies with a lower realized fecundity due to her extended search exhausting more of her finite energy as well as increasing her exposure to aerially hunting birds. In addition, there is no guarantee that an alternate host is devoid of other subcortical insects or that it is more suitable. From her fitness's point of view and assuming all off her offspring will be equally fecund, it is better to lay 100 eggs of which only 30% survive than to lay 10 eggs in which 100% survive.

The fact that *S. nigricornis* female's created fewer tunnels per drill site on colonized bolts as opposed to non-colonized bolts demonstrates that they show some discernment of where they oviposit; more tunnels being associated with more eggs being laid (Madden 1974, Spradbery 1977). This is in accordance with performance-preference linkage theory as larval survival was reduced on field-colonized bolts compared to non-colonized bolts. However, differences in size

and fecundity of emerging females was not determined and these too are components of offspring performance (Thompson 1988).

CONCLUSION

The oviposition behavior of *Sirex nigricornis* is very similar to other *Sirex* studied although the preferred condition of host material may differ. *Sirex nigricornis* apparently drills into material colonized by subcortical beetles and other insects with the same frequency as bolts devoid of other organisms. However, they do not seem to lay as many eggs in bolts that contain other subcortical insects.

Subcortical beetles hasten the loss of sapwood moisture content and this may affect the survival of *Sirex nigricornis* offspring. Antagonistic fungi vectored by subcortical beetles may also affect the development and survival of *S. nigricornis* offspring.

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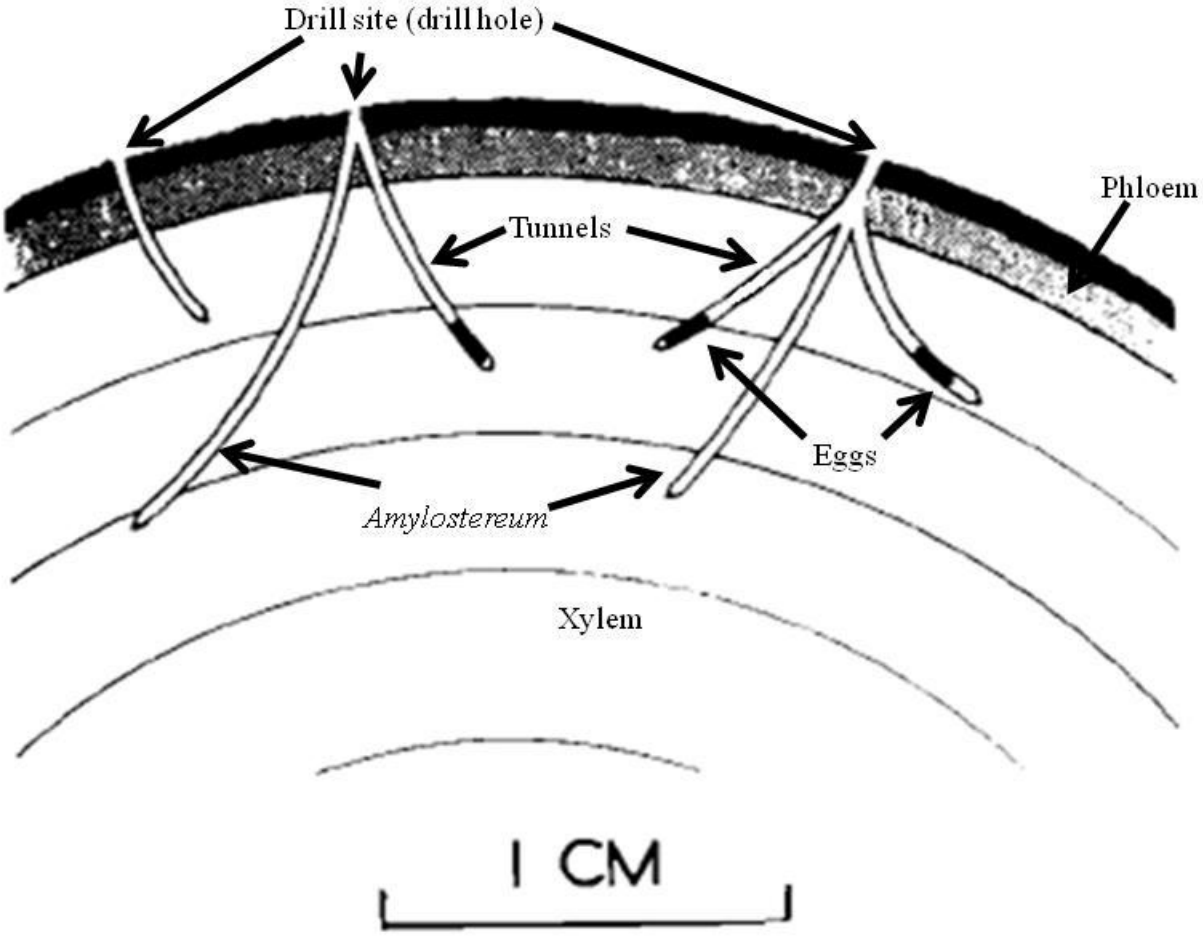
FIGURES

Figure 1. General structure of *Sirex* drill site.



Adapted from: Coutts & Dolezal. 1969. Forest Science 15(4):412-416

Figure 2. Shaving patterns for bolts used in bark study. Each bolt had 50% of its bark shaved (represented by black area). Shaving patterns were (from left to right): bottom shaved, top shaved, diagonal shave, top/bottom shaved, middle shaved.

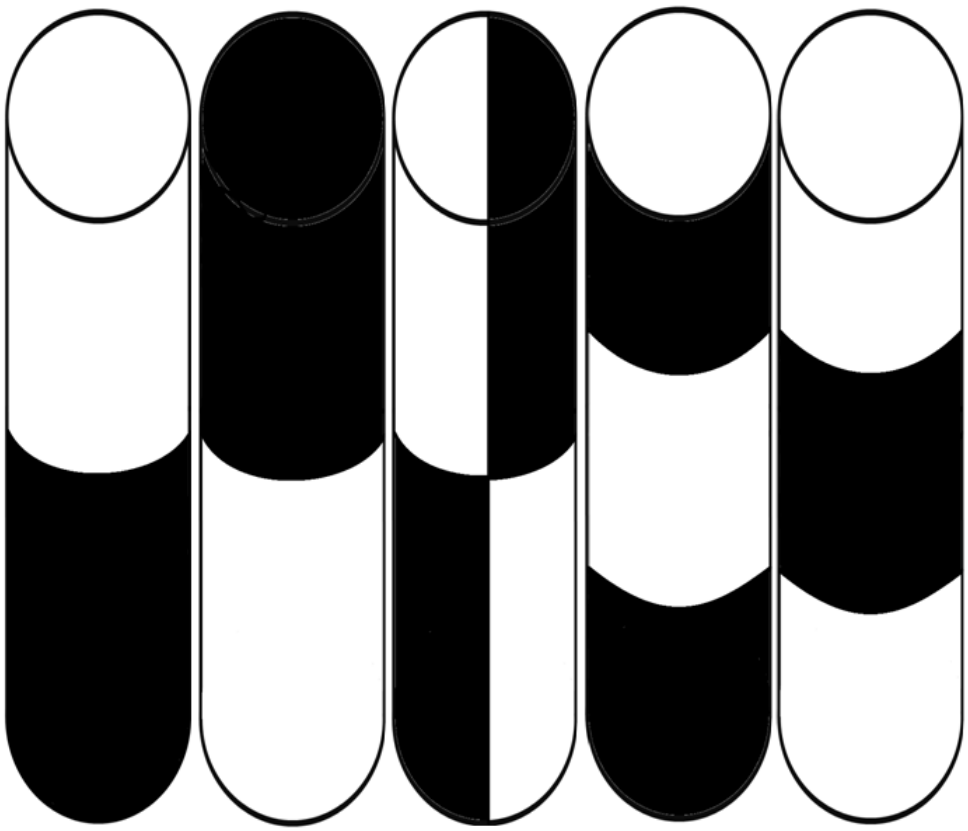


Figure 3. Relationship of potential fecundity (PF) to pronotal width (PW).

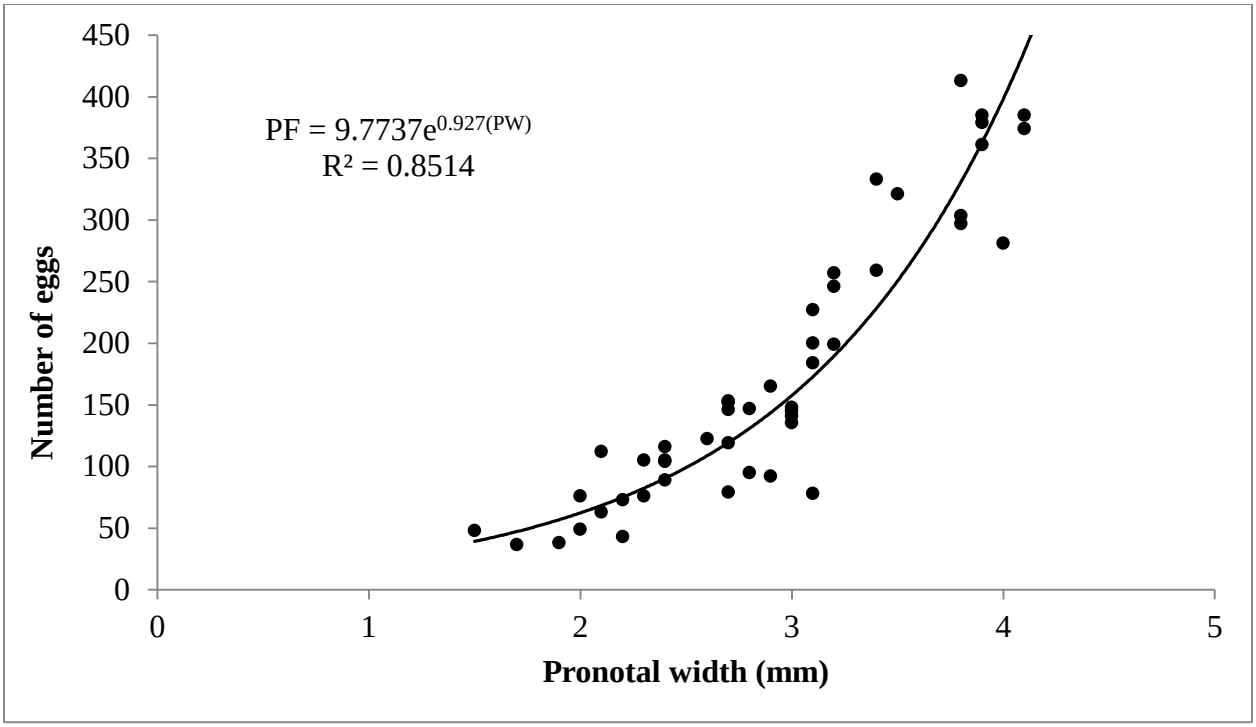


Figure 4. Difference in the number of drill sites between shaved and unshaved portions of a bolt's bark.

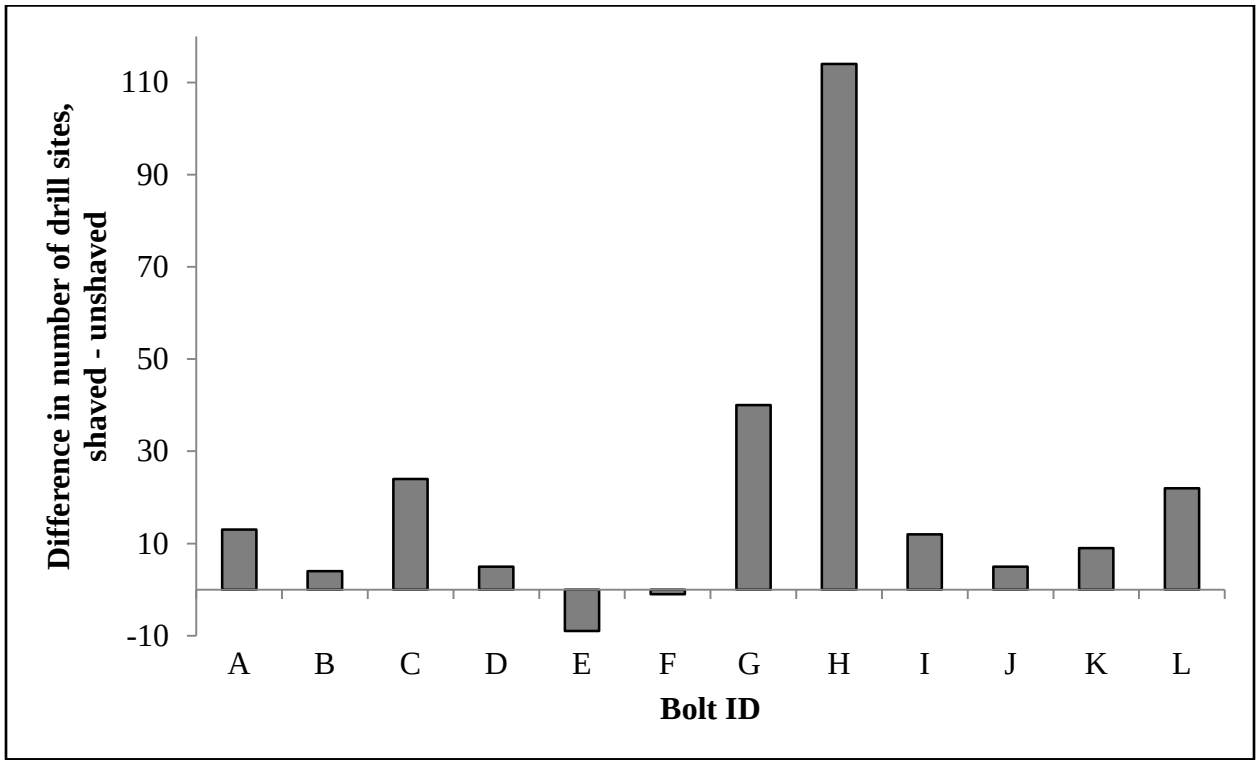


Figure 5. Difference in the number of drill sites created between paired *Ips*-colonized and non-colonized bolts.

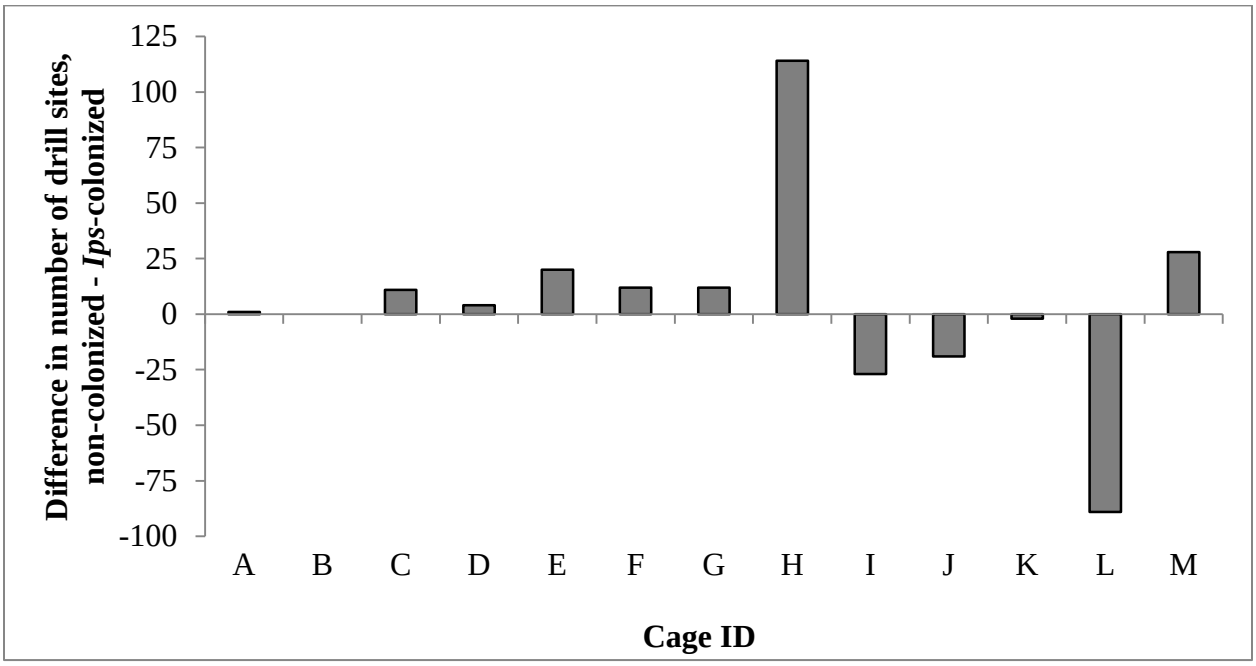


Figure 6. Difference in the number of drill sites created between paired field-colonized and non-colonized bolts.

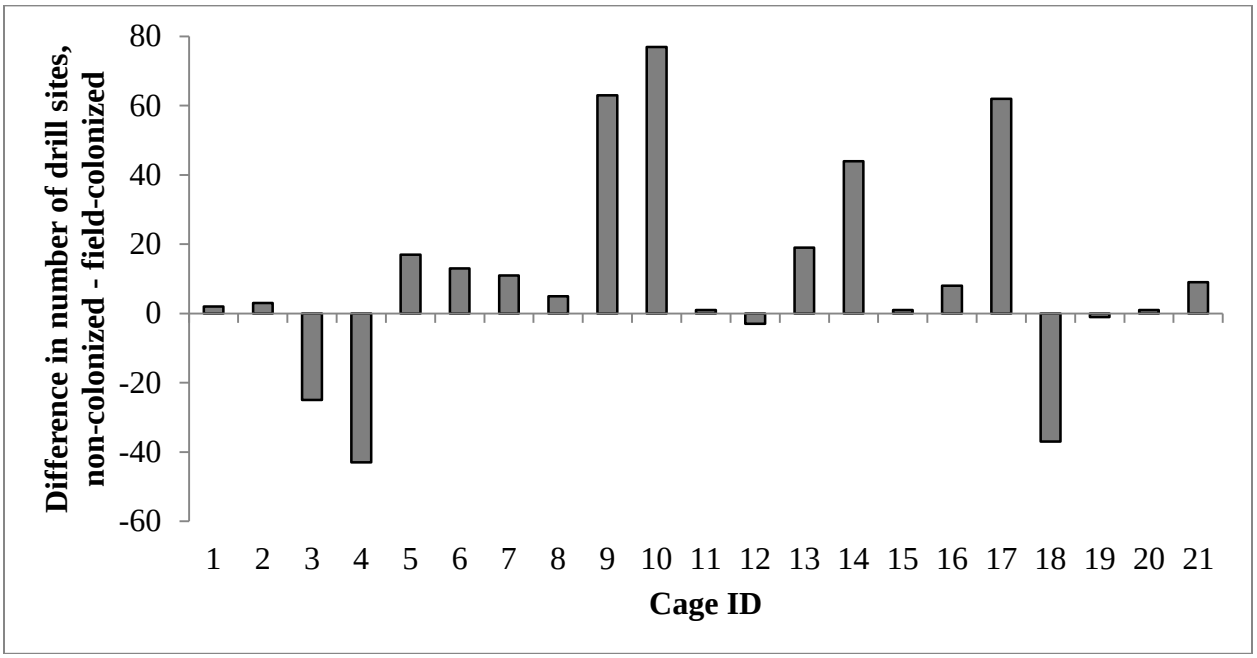


Figure 7. Difference in the number of drill sites created between tops and bottoms of bolts. Number in bolt ID corresponds to cage bolt came from. Bolts with same number came from same cage. An 'F' denotes field-colonized bolt. ID's without a letter denote non-colonized bolts. Bolts which received zero drill sites excluded from graph. Bolt 2F had an equal number of drill sites on its top and bottom.

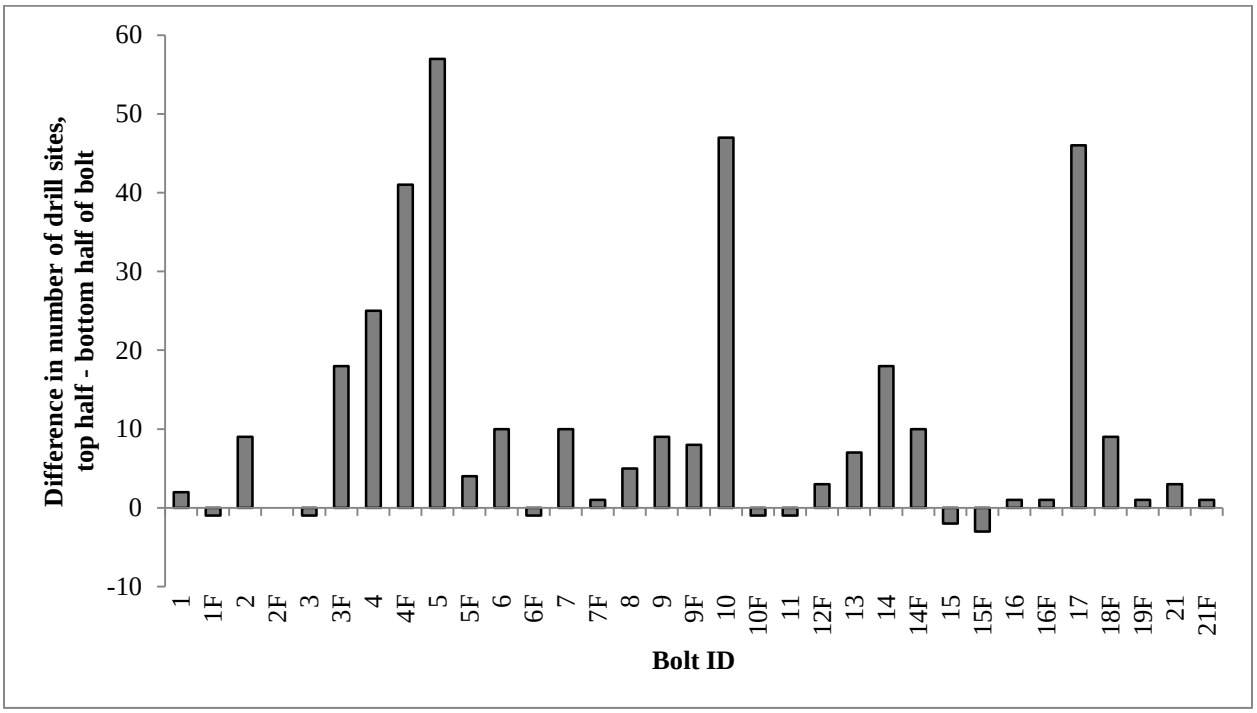


Figure 8. Proportional distribution of architecture types for *Ips*-colonized and non-colonized bolts.

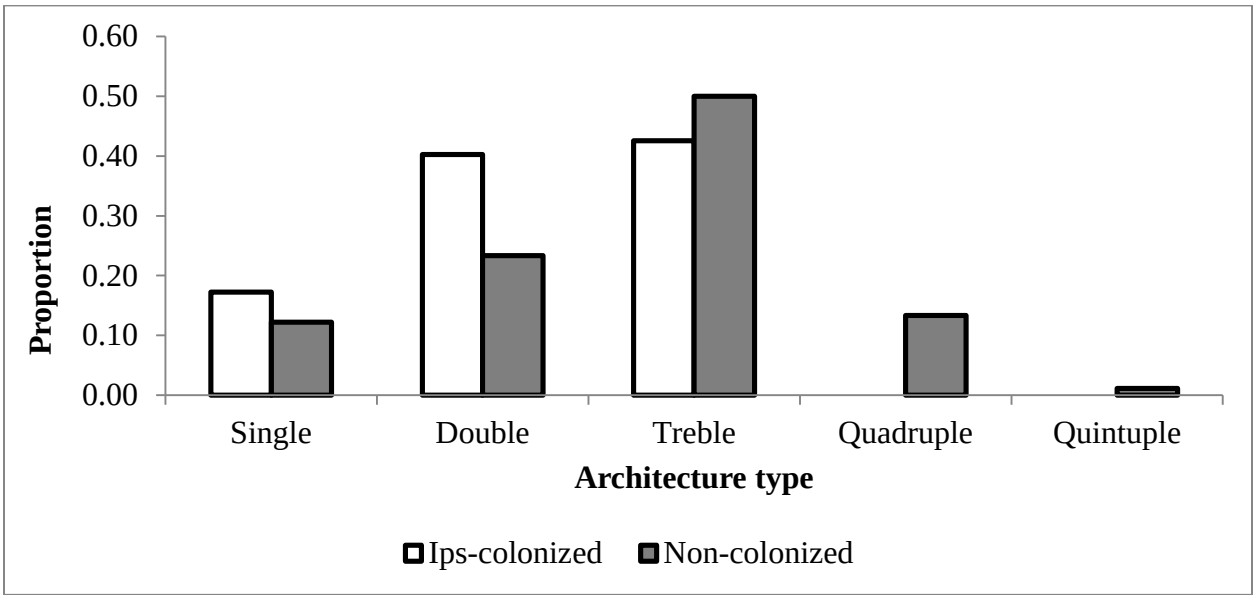


Figure 9. Average number of tunnels per drill site for *Ips* study bolts. Bars indicate 95% confidence interval.

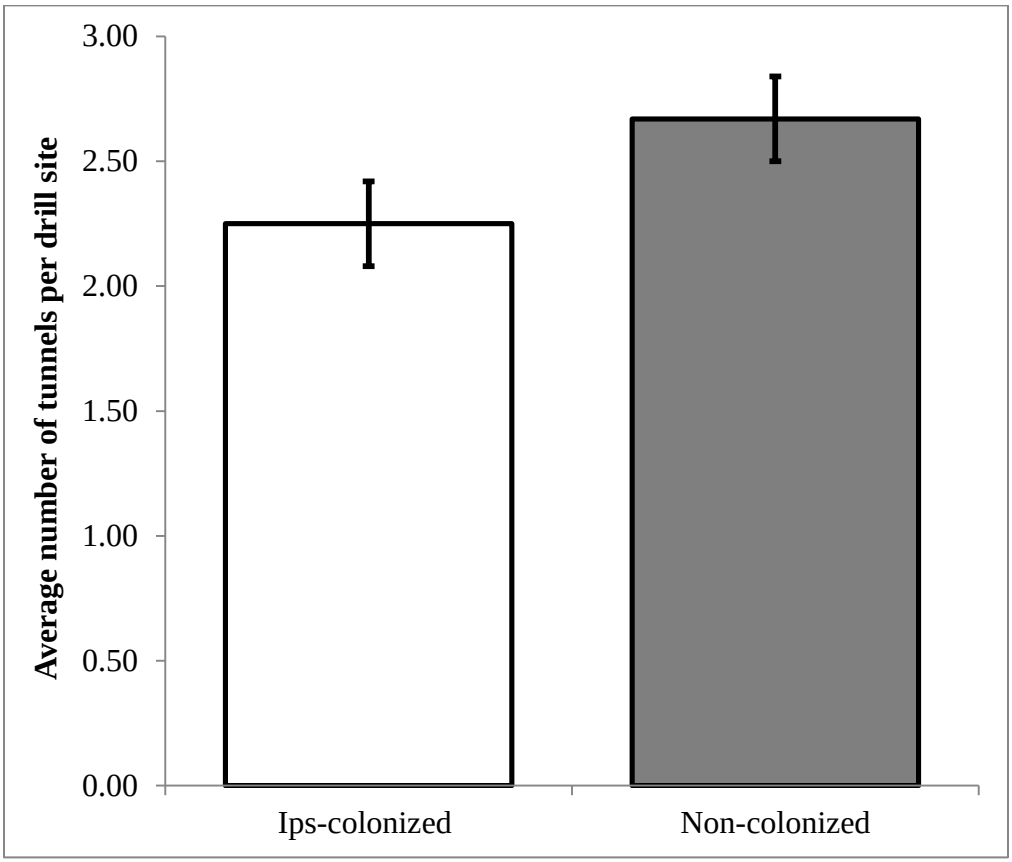


Figure 10. Proportional distribution of architecture types for field-colonized and non-colonized bolts.

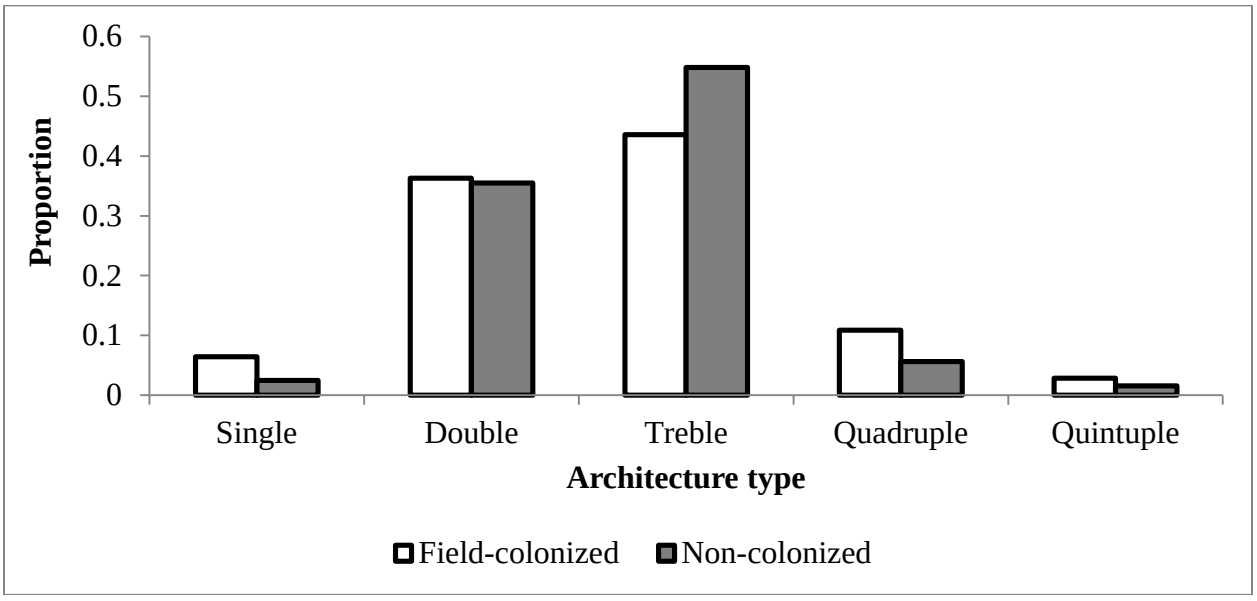


Figure 11. Average number of tunnels per drill site for field study bolts in which both bolts in a pair had more than five sample points. Bars indicate 95% confidence interval.

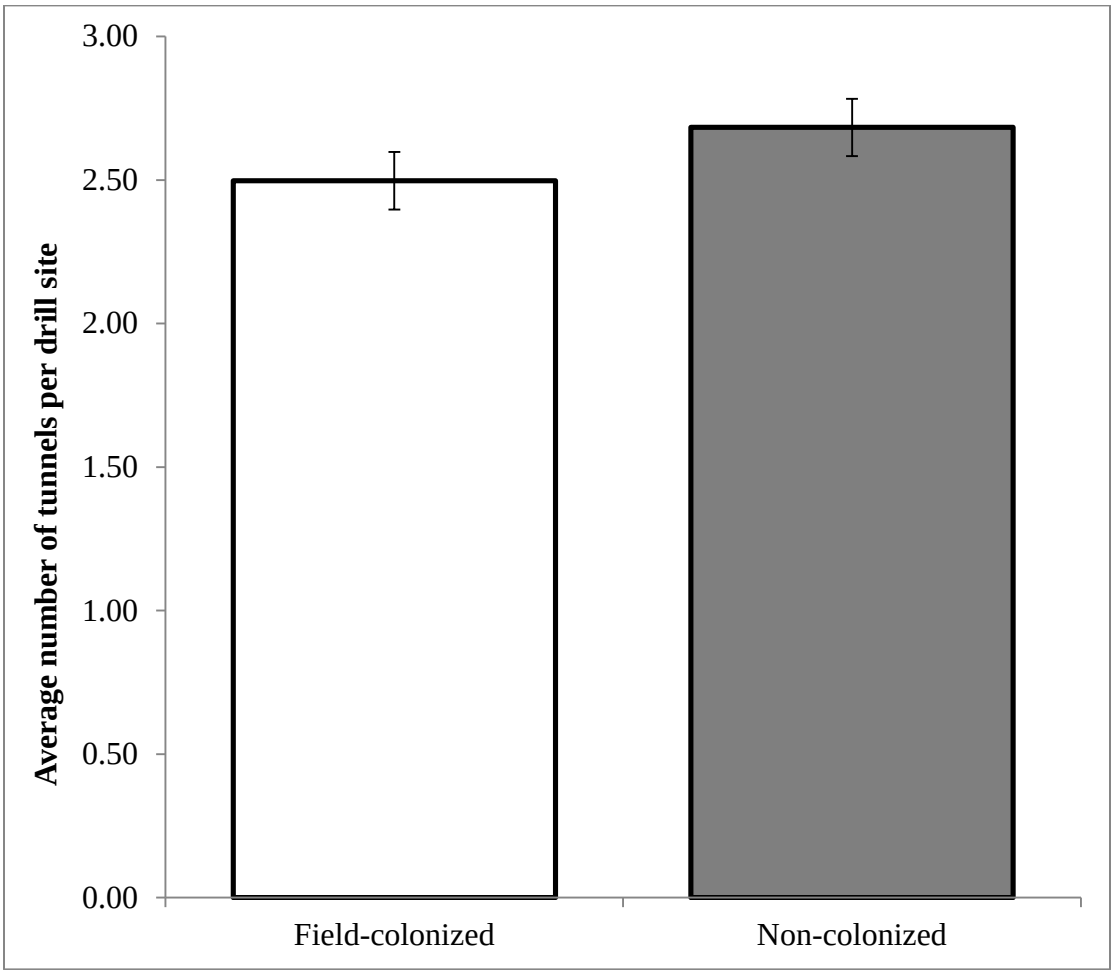
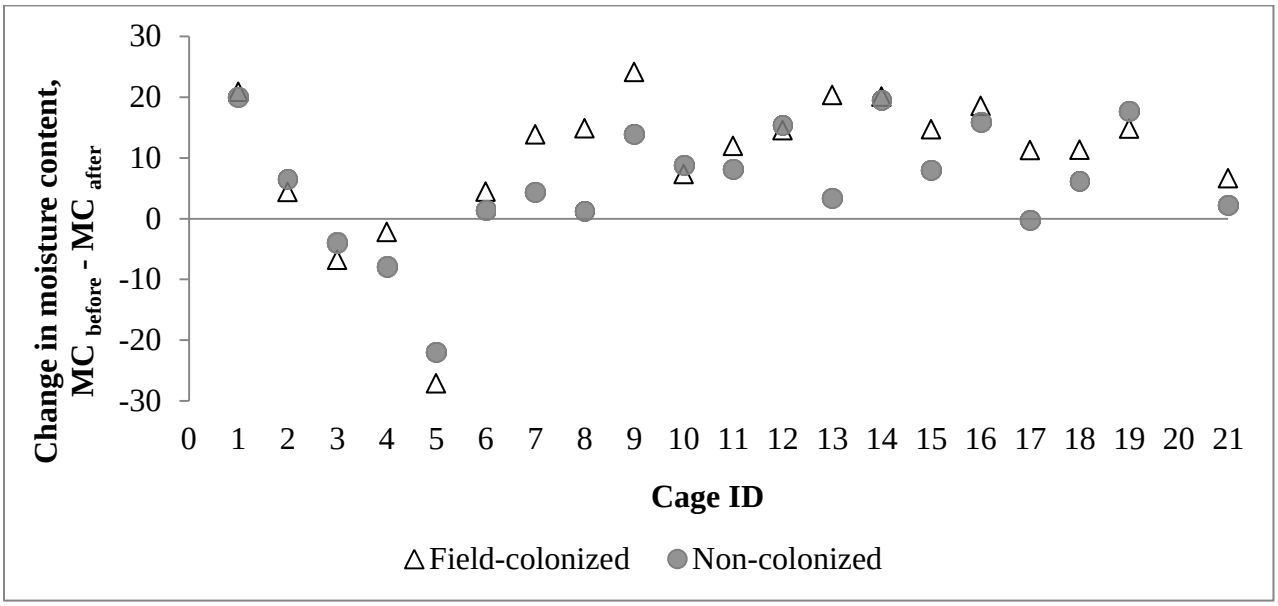


Figure 12. Change in moisture content (MC) on the ends of field study bolts after three months.



MC_{before} was taken after bolts were returned from traps. MC_{after} was taken three months later when bolts were cut in half.

Figure 13. Moisture content at the ends of field bolts immediately upon being returned from traps.

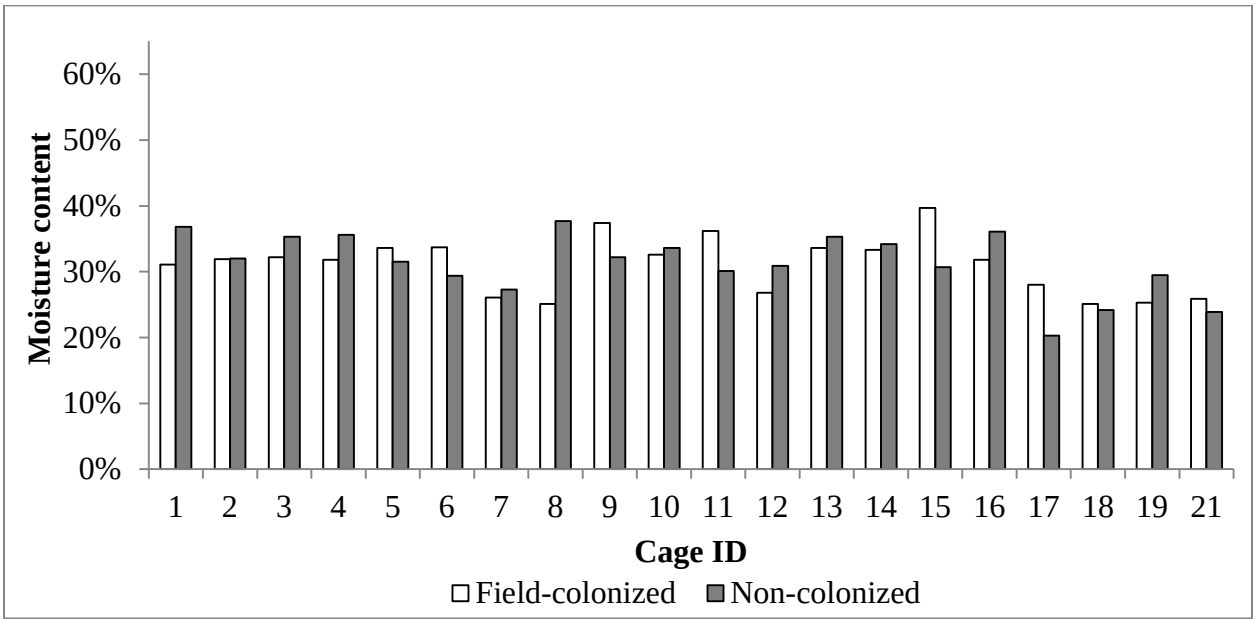


Figure 14. Moisture content at the ends of field bolts three months after being returned from traps.

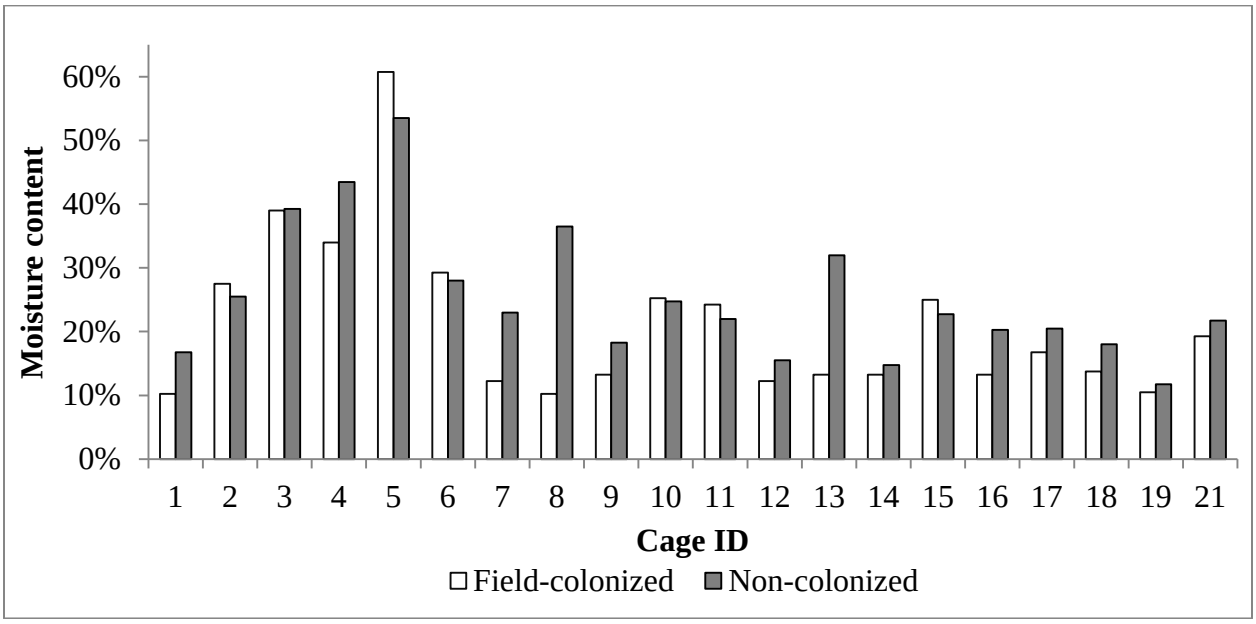
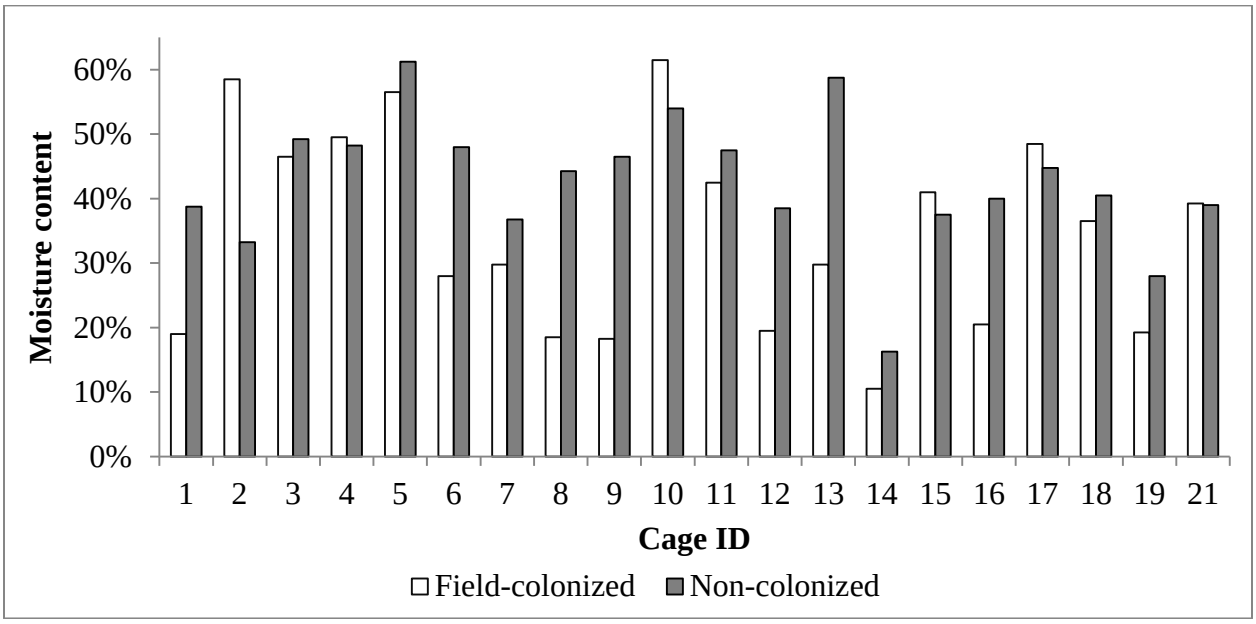


Figure 15. Moisture content at the center of field bolts three months after being returned from traps.



TABLES

Table 1. Mean, maximum and minimum values, standard error (SE) and number of specimens going into mean and SE calculations (N) of select body measurements and potential fecundity of *Sirex nigricornis*.

	Mean	Minimum	Maximum	SE	N
Pronotal width (mm)	3.0	1.5	4.5	0.10	72
Ovipositor sheath length (mm)	9.5	5.5	13.2	0.30	70
Body length to cornus tip (mm)	19.1	9.5	28.7	0.50	72
Body length to ovipositor tip (mm)	19.7	12.0	28.0	1.10	17
Predicted body length to ovipositor tip (mm)	21.2	10.8	31.7	0.59	72 (predicted)
Potential fecundity (# eggs)	176.2	36.5	413.0	16.00	48

Table 2. Correlation coefficients which summarize the linear relationship of select body measurements to each other and to a females potential fecundity.

	Pronotal width	Body length to cornus tip	Body length to ovipositor tip	Ovipositor sheath length
Pronotal width	1.00			
Body length to cornus tip	0.96	1.00		
Body length to ovipositor tip	0.95	0.99	1.00	
Ovipositor sheath length	0.95	0.94	0.96	1.00
Potential fecundity	0.92	0.91	0.89	0.91

Table 3. Summary of the number of drill sites created on shaved and unshaved portions of bark. Dashed lines separate cages.

Cage ID	Bolt ID	Part shaved	Number of drill sites		Number of *Sirex* in cage	Age of *Sirex*	Age of bolt(s)
			Shaved	**Unshaved**			
Big	A	Top/Bottom	**34**	**21**	18	Five *Sirex* were zero days old and the other 13 were 26 days old	7 days
	B	Middle	**5**	**1**			
	C	Top/Bottom	**26**	**2**			
	D	Middle	**11**	**6**			
	E	Bottom	**14**	**23**			
	F	Top	**0**	**1**			
1	G	Bottom	**61**	**21**	2	Zero days	15 days
2	H	Top	**225**	**111**	2	Zero days	15 days
3	I	Diagonal	**14**	**2**	5	One *Sirex* was 14 days old and four were 1 day old	21 days
	J	Diagonal	**16**	**11**	5		
4	K	Diagonal	**38**	**29**	3	2 days	22 days
5	L	Diagonal	**41**	**19**	3	2 days	22 days
6	M	Bottom	**0**	**0**	5	14 days	21 days
	N	Top	**0**	**0**			21 days

Total drill sites	
Shaved	**Unshaved**
485	**247**

Table 4. Number of drill sites created on paired *Ips*-colonized and non-colonized bolts.

	Number of drill sites			
Cage ID	**Non-colonized bolt**	***Ips*-colonized bolt**	**Group**	**Batch**
A	**1**	**0**	A	1
B	**5**	**5**	A	1
C	**36**	**25**	A	1
D	**5**	**1**	A	1
E	**20**	**0**	A	2
F	**32**	**20**	A	2
G	**27**	**15**	A	2
H	**122**	**8**	A	2
I	**0**	**27**	A	2
J	**14**	**33**	B	3
K	**14**	**16**	B	3
L	**23**	**112**	B	3
M	**28**	**0**	B	3

Total drill sites	
Non-colonized	***Ips*-colonized**
327	**262**

Table 5. Number of drill sites created on paired field-colonized and non-colonized bolts. Number of drill sites counted on both bolts in a cage before the bolts were cut in half (total sites pre halving). Combined number of drill sites counted on the top halves (bolt tops) and bottom halves (bolt bottoms) of both bolts in cage after they had been sawn in half. All drill site counts were taken with the bark still intact on the bolt.

	Number of drill sites		Total sites	Number of drill sites	
Cage ID	Non-colonized bolt	Field-colonized bolt	pre halving	Bolt tops	Bolt bottoms
1	**3**	**1**	4	**2**	**1**
2	**48**	**45**	93	**46**	**37**
3	**11**	**36**	47	**32**	**15**
4	**33**	**76**	109	**80**	**14**
5	**101**	**84**	185	**123**	**62**
6	**14**	**1**	15	**12**	**3**
7	**12**	**1**	13	**12**	**1**
8	**5**	**0**	5	**5**	**0**
9	**81**	**18**	99	**58**	**41**
10	**134**	**57**	191	**115**	**69**
11	**1**	**0**	1	**0**	**1**
12	**0**	**3**	3	**3**	**0**
13	**19**	**0**	19	**12**	**6**
14	**70**	**26**	96	**62**	**34**
15	**4**	**3**	7	**1**	**6**
16	**9**	**1**	10	**6**	**4**
17	**62**	**0**	62	**54**	**8**
18	**0**	**37**	37	**22**	**13**
19	**0**	**1**	1	**1**	**0**
20	**1**	**0**	1	**0**	**0**
21	**29**	**20**	49	**23**	**19**
22	**0**	**0**	0	**0**	**0**
23	**0**	**0**	0	**0**	**0**
24	**0**	**0**	0	**0**	**0**
25	**0**	**0**	0	**0**	**0**
26	**0**	**0**	0	**0**	**0**
27	**0**	**0**	0	**0**	**0**

Total drill sites		Total drill sites	
Non-colonized	Field-colonized	Tops	Bottoms
637	410	669	334

Table 6. Frequency of different architecture types for bolts used in *Ips* study and the total number dissected (# Dissected).

	Drill site architecture type					
	Single	Double	Treble	Quadruple	Quintuple	**# Dissected**
Non-colonized	11	21	45	12	1	**90**
Ips-colonized	15	35	37	0	0	**87**
Total	**26**	**56**	**82**	**12**	**1**	**177**

Table 7. Frequency of different architecture types for bolts used in field study and the total number dissected (# Dissected).

	Drill site architecture type					
	Single	Double	Treble	Quadruple	Quintuple	**# Dissected**
Non-colonized	8	114	176	18	5	**321**
Field-colonized	16	90	108	27	7	**248**
Total	**24**	**204**	**284**	**45**	**12**	**569**

Table 8. Number of tunnels dissected, estimated eggs laid and contents of the dissected tunnels for the various architecture types on bolts from the field study. One quintuple tunnel from field-colonized bolts and four from non-colonized bolts combined into quadruple drill counts.

		Drill site architecture				
Bolt type dissected		Single	Double	Treble	Quadruple & Quintuple	**Total**
Field-colonized	Number dissected	13	72	92	18	**195**
	Estimated eggs laid	1	50	147	41	**240**
	Sign of oviposition	2	18	68	18	**106**
	Eggs in oviposition tunnel	0	2	6	1	**9**
	Live larvae in oviposition tunnel	0	1	0	0	**1**
	Dead larvae in oviposition tunnel	1	0	0	0	**1**
	Larval feeding galleries	1	15	62	17	**95**
	Estimated first instar larvae	2	16	62	17	**97**
	Live feeding larvae	0	6	14	0	**20**
Non-colonized	Number dissected	9	79	110	15	**213**
	Estimated eggs laid	0	55	176	35	**266**
	Sign of oviposition	2	37	78	11	**128**
	Eggs in oviposition tunnel	0	0	0	0	**0**
	Live larvae in oviposition tunnel	0	4	8	0	**12**
	Dead larvae in oviposition tunnel	0	1	0	2	**3**
	Larval feeding galleries	2	33	69	9	**113**
	Estimated first instar larvae	2	37	78	11	**128**
	Live feeding larvae	1	10	20	1	**32**
Total	Number dissected	22	151	202	33	**408**
	Estimated eggs laid	1	106	323	76	**506**
	Sign of oviposition	3	56	146	29	**234**
	Eggs in oviposition tunnel	0	2	6	1	**9**
	Live larvae in oviposition tunnel	0	5	8	0	**13**
	Dead larvae in oviposition tunnel	1	1	0	2	**4**

Larval feeding galleries	3	48	131	26	**208**
Estimated first instar larvae	4	53	140	28	**225**
Live feeding larvae	1	16	34	1	**52**

Table 10. Mortality estimates of early stage *S. nigricornis* offspring in bolts from the field study.

	Bolt type		
	Non-colonized	Field-colonized	Total
Percent egg mortality	52%	58%	55%
Percent mortality before feeding	56%	59%	57%
Percent mortality before feeding including LLOT	53%	58%	56%
Percent mortality to feeding larvae at time of sampling	72%	79%	75%
Percent mortality to feeding larvae at time of sampling including LLOT and DLOT	66%	78%	71%
Percent total estimated mortality at time of sampling	83%	91%	87%

CHAPTER 4 – CONCLUSION

In Arkansas, *Sirex nigricornis* adults fly later in the season than a majority of associated subcortical insects, most of which are beetles (e.g. Cerambycidae and Scolytidae). These subcortical beetles alter the pine host substrate by inoculating fungi, feeding and mining phloem and increasing the rate of moisture loss. *Sirex nigricornis* females drilled into bolts colonized by subcortical beetles with the same frequency as they did bolts that were not colonized by beetles. However, females created fewer tunnels per drill site on beetle-colonized bolts compared to non-colonized control bolts. This suggests that *S. nigricornis* females do not detect the presence of subcortical beetles, or at least they are not deterred by their presence, when initially probing into host material. However, the fact that *S. nigricornis* females appeared to oviposit less on beetle-colonized bolts compared to non-colonized bolts suggests that they detect the presence of subcortical beetles once their ovipositor is in the host; possibly sensing changes in phloem characteristics, the presence of antagonistic fungi or change in moisture content associated with these beetles. *Sirex nigricornis* may avoid ovipositing into host material colonized by beetles because the presence of these beetles decreases larval survival and fitness. These results suggest that *Sirex* are negatively affected by the presence of associated insects. Therefore it is likely that the Eurasian woodwasp, *Sirex noctilio*, will be of little economic significance in North America, much like it is in its native range, because pressures from associated subcortical insects keep populations from reaching damaging levels.