ABSTRACT

GARDNER, MICAH JOHN. Development of Southern Pine Beetles (*Dendroctonous frontalis* Zimmerman) in White Pine (*Pinus strobus*), a Non-Traditional Host. (Under the direction of Fred Hain.)

The southern pine beetle (SPB, *Dendroctonous frontalis* Zimmerman) is a major pest of southern forests. Its outbreak population dynamics allow it to colonize and kill large areas of pine under the right conditions. The traditional hosts of SPB are loblolly pine (*Pinus taeda*) and shortleaf pine (*Pinus echinata*). Recent outbreaks indicate SPB's ability to cause mortality in the non-traditional host, white pine (*Pinus strobus*), as well. A number of developmental parameters including egg and larval gallery length, eclosion rate, and survival of several life stages were measured and compared between white and loblolly pine in order to gain an understanding of the suitability of white pine as a host for SPB. SPB were found to produce significantly shorter egg galleries with higher density of egg niches in white pine. SPB were also found to eclose successfully at a significantly higher rate in loblolly pine. The overall survival of SPB in both hosts and in our laboratory rearing colony was extremely low and indicated the weakness of the SPB collected from the field. Difficulty in locating, trapping, and obtaining enough SPB for experimentation spurred the development of a new SPB trap for use in laboratory research. This new-style trap was compared with an older SPB trap from the U.S. Forest service. The new-style trap provided fifteen times the surface area of the old-style traps and captured significantly more SPB for use in experimentation.

Development of Southern Pine Beetles (*Dendroctonous frontalis* Zimmerman) in White Pine (*Pinus strobus*), a Non-Traditional Host

by
Micah John Gardner

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APPROVED BY:

_____	_____
Fred Gould, PhD	Fred Hain, PhD Chair of Advisory Committee
_____	_____
John King, PhD	John Monahan, PhD

BIOGRAPHY

Micah John Gardner was born on July 1st, 1983 in Youngstown, Ohio to Elaine and Mickey Gardner. He was homeschooled until eighth grade and spent most of his youth in Marietta, Georgia while attending Fellowship Christian High School in Roswell, Georgia. His desire to study and understand the natural world was formed through numerous field trips, courses taught to homeschoolers by professional science, math, and English teachers, as well as self-study. In high school Micah took as many AP classes as were offered at his small school and was fortunate enough to have the opportunity to take organic chemistry and biochemistry courses prior to graduation. Micah attended the University of Georgia from 2002 to 2006 and graduated Cum Laude with separate degrees in Biology and Ecology. At UGA Micah first encountered entomology in a course taught by Dr. Ken Ross, and was immediately drawn to it. Micah worked as a lab technician in the crop and soil science department and then with Dr. Ross in entomology by rearing red imported fire ants and capturing live nests from the field. Knowing entomology was where he wanted to be, Micah took the advice of his UGA professors and applied to the entomology department at NC State. This thesis represents his work from that time till now.

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INTRODUCTION

The Southern Pine Beetle (*Dendroctonous frontalis* Zimmerman) is the most damaging pest to southern pine forests in the United States. Each year this pest causes millions of dollars in losses to landowners, national forests, and the timber industry. While the southern pine beetle (SPB) is currently tied to a small number of hosts, there is concern that rising temperatures due to global climate change may allow it's range to expand northward, threatening the forests and tree species that are found just above the upper limit of its current range (Ungerer and Ayres 1999, Ayres and Lombardero 2000, Williams and Liebhold 2002, Gan 2004). In order to adequately address this problem, it is important not only to understand the life cycle and behavior of the SPB, but also to foster a healthy respect for the incredible success that this species has achieved over evolutionary time.

Despite its minute size, the southern pine beetle is able to colonize and kill pine trees of varying ages, healthiness, and size (Thatcher *et al*. 1981). SPB accomplishes this feat through a mass-attack strategy driven by a complex system of visual, abiotic, and chemical cues (Thatcher *et al*. 1981). Beetles will often select weakened trees, trees in disturbed environments, and lightning-struck trees as hosts (Coulson *et al.* 1986). These types of hosts allow SPB to build up its population and radiate outward, killing trees in their path. Typically a host tree will respond to an attack by releasing resin into the galleries and holes created by SPB, often trapping and sealing them inside (Raffa and Berryman 1993). However, during a mass attack when there are many beetles

simultaneously boring into the tree's cambium, the tree is often unable to release enough resin to exterminate them (Wagner 1984). It is in this manner that SPB forces a tree to "pitch itself out," meaning rapid exudation of highly metabolically valuable resins, oleoresins, and terpenes to the wounded area and throughout the host leading to death (Raffa and Berryman 1993).

In this mass attack strategy, the target tree is attacked by female beetles that first land on the bark, and bore inside. They proceed to release a pheromone that causes other female beetles to aggregate and attack the tree (Thatcher *et al*. 1981). This pheromone also serves as an attractant to male beetles that join the females in the tree and aid in the construction of their signature "s-shaped" galleries through the host's vascular cambium. After mating the female beetle digs a number of small egg chambers along the gallery and lays a single egg in each terminus (Thatcher *et al*. 1981, Wagner 1982). Female beetles possess a mycangium on the anterior portion of their pronotum which serves as a carrier for different types of fungi. As the female lays her eggs she also leaves some fungi in the chamber. The fungi flourish inside the host tree and some are consumed by SPB, enriching the larval diet. This mutualistic interaction between the fungus and the beetle is required for successful beetle development. Adult beetles will often reemerge from the tree once they have mated and laid eggs, and fly to another tree to complete this processes again (Thatcher *et al*. 1981).

The southern pine beetle begins its life as an egg in the vascular cambium but soon emerges within its egg niche as a 1st instar larva. Three more instars follow as the

beetle slowly eats outward from its mother's gallery (Thatcher *et al*. 1981). SPB then pupate and become a teneral, or callow, adults that follow the galley back out to the bark, allowing its exoskeleton time to sclerotize, and finally emerging from the tree as an adult. The length of each of these stadia is influenced by a number of factors (Coulson 1978, Fargo *et al*. 1979, Wagner 1982, Flamm 1993). Humidity, time of year, and host quality all play a role in the development of SPB, but none of these factors are as important as temperature (Wagner 1984). Warmer temperatures are more conducive to rapid development, with a maximum developmental rate achieved at around 27 ºC, and a full developmental temperature range of 10-33 ºC (Wagner 1984). Because of the vital role temperature plays, it is important to consider what type of effect global climate change might have on SPB populations.

The traditional host for SPB is the loblolly pine (*Pinus taeda*). The geographic range of the southern pine beetle is closely tied to the natural range of the loblolly (Thatcher *et al.* 1981). There is a possibility, however, that rising global temperatures could facilitate a northward shift of the southern pine beetle outside of the natural range of the loblolly pine. This would put the beetle well within the lower range of the white pine (*Pinus strobus*). A host shift, at least in the northern part of its range, would constitute a significant ecological event in which SPB could affect greater economic and environmental damage than it does currently. There is a need to construct accurate, predictive infestation models as part of a suite of preparatory actions for this potential range expansion and host shift. Assessing the suitability of white pine as a host for SPB is

an integral part of the larger research effort to understand and predict the possible scenarios we may face in the coming years.

LITERATURE REVIEW

Southern Pine Beetle Development

The southern pine beetle begins its life as a small, ovoid, white egg about 1-1.5mm in length (Fronk 1947). Females deposit these eggs individually at the terminus of a brood chamber after mating and before reemergence. The female will typically begin laying eggs after constructing 3cm. or more of the brood chamber (Mizell and Nebeker 1979b). The egg stage typically lasts 3-10 days over a range of 15˚-30˚C (often considered the prime developmental temperature range), though the complete developmental range of SPB is about 4˚-33.3˚C (Thatcher and Pickard 1967, Mizell and Nebeker 1978, Wagner et al. 1984). The temperature at which gallery construction and oviposition are initiated has a profound effect on the size and number of each (Wagner *et al*. 1984). Using the developmental range of temperatures and under laboratory conditions it was found that the most eggs were laid and longest galleries constructed at mid to lower temperatures (Wagner *et al*. 1982). SPB laid the least number of eggs at 10˚C and created the shortest galleries at 30˚C (Wagner et al. 1982). Coulson (1978) and his colleagues also noted that the density of galleries and eggs decreased as the number of mated adult pair per dm^2 increased. The action of density-dependant forces on gallery length and oviposition is echoed throughout the literature (Feldman et al 1981, Flamm *et*

al. 1993, Thatcher *et al*. 1981, Wagner 1982, 1984). Eggs are always deposited inside the cambium of the tree, but can develop outside under laboratory conditions if proper procedure is followed (Wagner 1984, Thatcher *et al.* 1981). Under these laboratory conditions the average mortality of the egg stage was found to be slightly less than 22% over the prime developmental temperature range (Wagner *et al*. 1984).

Upon hatching SPB enters its larval stage that lasts from as little as 15 days at warmer ambient and phloem temperatures, to over 40 days at cooler temperatures (Gagne 1980, Wagner 1984). It is important to note that phloem temperature is almost always higher than ambient temperatures, especially in the summer (Logan and Powell 2001). One of the key features of the larval stage is the development of mandibles that can be seen during the last 24 hrs. of the egg stage (Fronk 1947). These mandibles along with a total of three thoracic and ten abdominal segments, a yellowish-white body color, and a reddish head color help to distinguish this life stage (Thatcher *et al*. 1981). Larvae begin their development at about 2mm in length and grow to between 4 and 5mm by the end (Fronk 1947). SPB develops through 4 stadia during its larval period (Fronk 1947, Mizell and Nebeker 1979a). Each of these instars performs special tasks and can be differentiated from one another in a variety of ways. Fronk (1947) was able to differentiate each instar by measuring the width of their head capsules. The widths he measured were 0.294mm to 0.336mm for the first instar, 0.378mm to 0.504mm for the second instar, 0.546mm to 0.672mm for the third instar, and 0.756mm to 0.960mm for the fourth instar. Head capsule measurement, however, may not be the most reliable

technique for differentiating instars because of the possibility that the width of the capsule may overlap from instar to instar (Fronk 1947).

First and second instars construct feeding galleries arising from their egg niche (Goldman and Franklin 1977). These feeding galleries are important because their length can be indicative of host quality and brood health. Elongated feeding galleries have been associated with a cessation of larval development, poor host quality, and larval mortality (Wagner *et al*. 1979, Feldman *et al*. 1981). The reason for this is that the beetle must eat a greater volume of the low quality host material than it would of high quality host material in order to pass through its developmental gate and proceed to the next stadium. Following the construction of the feeding gallery, third instar larvae build a feeding chamber while fourth instars migrate to the outer bark area where they pupate (Goldman and Franklin 1977, Thatcher *et al*. 1981). X-ray imaging has been used to study the characteristics of each instar and found to be as accurate as destructive counting methods (Fatzinger and Dixon 1965). Mizell and Nebeker (1979b) described each of the SPB instars by using radiographs, though they found first and second instars are difficult to distinguish by radiograph. Despite this it was observed that first instars often appear in a small, pin-sized galleries emanating from the terminus of the egg niche at a right angle. A widening of this same gallery differentiated second stage instars. They also noted that most larvae show up as small white spots near the end of these galleries with each instar being easier to see than the last. Third instar larvae were found to be about half the size of the adult and fourth instars about the size of a full adult (Mizell and Nebeker 1979b,

Thatcher *et al*. 1981). The larval stage is very important to the development of SPB because it is during this time that beetles eat and gain energy to pupate. The larval stage has also been found to be the most susceptible to mortality from drought or changes in the phloem and xylem moisture (Wagner *et al*. 1979).

The next stage of life for SPB is its pupal stage that can last from 5 to 17 days over its prime developmental temperature range of 15˚-30˚C (Gagne 1980). The pupal stage of SPB looks similar to the adult stage in many ways (Fronk 1947). The pupal stage is initiated following migration to the outer bark and construction of a smooth and compact cell in which the beetle will pupate. These cells are compact and smooth in appearance, and the orientation of the beetle within them allows for all of the abdominal segments to be exposed (Mizell and Nebeker 1979b). Mizell and Nebeker (1979b) also noted that wing pads were visible in the pupal stage. Pupae are about 3-4mm in length and the same color as larvae with their wings and legs folded under their bodies (Thatcher et al 1981). While rearing under laboratory conditions the average mortality of the pupal stage was found to be about 13% (Wagner *et al*. 1984).

Following the pupal stage the beetle becomes a callow, or teneral adult. This life stage lasts from about 6 to 14 days over the prime developmental temperature range (Gagne 1980). Beetles in this stage show adult features but maintain a lighter hue than fully developed adults (Mizell and Nebeker 1979b). Their bodies are elongate and cylindrical with their color change occurring in the following order: yellowish-brown to lighter-brown followed by a reddish-brown and ending with the dark-brown to black

color of a full adult (Thatcher *et al*. 1981). The teneral adult beetle spends all of its time near the outer bark in the same area as its pupal stage. Once the color change and full sclerotization have taken place the beetle is considered a full adult. Adult SPB are about 3-5cm in length and brownish to black in color (Fronk 1947). Thatcher (1981) gives an in-depth description of SPB wherein he observes that both sexes have seven-segmented clubbed antennae, chewing mouthparts, and a frontal groove on the head. Females differ from males in that they have a mycangium on their anterior pronotum. This structure will be covered in greater detail in a following section. Though both sexes have elevations and tubercles on their head, the males are more prominent. Both males and females produce pheromones, but the release rates, ratios, and exact type of pheromones differs (Dickens and Payne 1977). Once the beetle becomes an adult it will emerge from the tree forming what is referred to as a "shot hole" and fly away to mate and colonize another host.

Attack Strategy and Pheromone System

Survival for SPB is dependent on the execution of a successful mass attack on its host. Initially it may seem that this diminutive beetle is clearly outmatched by the size and defenses of its formidable host, but SPB are possessed of an array of advanced tools to aid them in their quest for a safe home. A highly complex pheromone system lends a competitive advantage to SPB and to bark beetles in general. Other members of the pine bark beetle guild such as *Dendroctonous terebrans* Oliver, *Ips calligraphus* Gramar, *Ips grandicollis*, and *Ips avulses* utilize similar methods in their own colonization efforts

(Coulson *et al*. 1986). In this same study, it was found that SPB consistently caused the largest infestations out of all members of the pine bark beetle guild. While their overarching strategy is not unique, the specific methods by which SPB communicate and attack are.

Perhaps the most important step in the mass-attack strategy is the selection of the initial host tree. The selection of a host tree is performed by female beetles and often involves both visual recognition and some sort of chemical attractant. SPB are innately attracted to vertical objects and will not attack trees that have been felled (Gara 1967). SPB have the ability to detect weakened or stressed trees in disturbed areas, and despite the fact that random landings do occur, they often select these as hosts (Coulson et al 1986). Among these weakened and stressed trees, lighting-struck trees are often the target of the initial or "pioneer" attack by both SPB and its close relative the mountain pine beetle (MPB, *Dendroctonous ponderosae* Hopkins) which inhabits the western regions of the United States (Gara *et al*. 1984, Raffa and Berryman 1993). MPB has shown the ability to detect fire-weakened trees as well as lighting struck trees (Gara *et al*.1984). Weakened trees often act as a population springboard, providing the colony with minimal host resistance and allowing their population to grow easily (Raffa and Berryman 1993). It was also noted by Raffa and Berryman (1993) that weakened trees aid in the initial colonization but are rarely chosen as hosts once the population grows larger. This is because of the evolutionary penalty inflicted on brood development and health if the mother selects poor quality host material. Weakened trees usually have thinner phloem

that cannot continuously support a robust population of bark beetles in the way a healthy tree can (Lorio 1968, Raffa and Berryman 1993). Research has shown, however, that lightning-struck trees often have about the same number of adult SPB as a healthy tree does at the peak of infestation (Flamm *et al*. 1993). SPB infestations tend to move in the same manner regardless of the quality of the initial host (Coulson *et al*. 1986). Flamm et al. (1993) noted that beetles typically select, attack, and begin aggregation during the same day which, they suggest, helps to minimize mortality by pheromone-sensitive predators. In addition to host selection, the pheromone system of SPB, driven by their powerful olfaction organs, is also used for intraspecific communication.

The antennae of SPB are the primary pheromone-sensing apparatus and are made up of many walls of sensilla (small protrusions about 30 microns long) and many tiny pores (Payne 1975). These olfactory receptors have the ability to sense multiple compounds at the same time (Dickens and Payne 1977). Males and females have the same sensing system but are known to show differential sensing capabilities, meaning that the nerves and sensors themselves are not physically different, but they can send different types of signals to the insect's brain (Payne 1975). Kinzer et al. (1969) noted that the chemical compound frontalin, a name they coined themselves, is the most important chemical in terms of bark beetle aggregation and that it originates in the hindgut of the beetle. Frontalin is typically released by the female upon entry into a host and serves as a powerful attractant to male and female beetles alike (Thatcher *et al*. 1981). Prior to this, Vite *et al*. (1964) noted that the primary attractant chemical of SPB

also attracted a variety of predators to infestations. Females are known to produce frontalin in much smaller quantities than males (Kinzer *et al*. 1969). Male and female SPB receptors are known to be maximally responsive to frontalin, but their reaction to frontalin can be very different depending on the concentration and presence of other compounds (Dickens and Payne 1977). Male beetles have been observed to respond by landing much closer to the pheromone site than females (Coster *et al*. 1977). The presence of host terpenes (especially α-pinene) can greatly increase the attractive effect of frontalin (Rudinsky 1974, Payne 1975). Though the primary pheromone produced and sensed by SPB is frontalin, they are also sensitive to other compounds including verbenone, trans-verbenol, *endo*-brevicomin, and α-pinene (Gara *et al*. 1965, Rudinsky 1973, Dickens and Payne 1977, Thatcher *et al*. 1981).

The SPB pheromone system is composed of many multifunctional chemicals such as frontalin and verbenone. These chemicals afford the beetles an adaptive advantage over their hosts and contribute to the overall effectiveness of SPB herbivory. In male beetles verbenone is a well-known inhibitor of aggregation, while in females it synergizes with frontalin to act as an inhibitor (Dickens and Payne 1977). SPB uses α-pinene to synthesize verbenone but in the absence of the terpene, trans-verbenol is used as a synergist instead (Vite 1975). Verbenone acts on SPB in a highly complex manner. In large amounts verbenone does inhibit male attraction, but in low doses it can elicit an attractant "chirp" from the male by means of stridulation (Rudinsky 1973). This same compound in medium amounts will elicit a rivalry "chirp" from males which serves to

ward off other males (Rudinsky 1973). Since verbenone is produced primarily by males, an abundance of it in the air indicates that the area is already occupied; yet at the same time a low amount tells the beetle to call out for mates (Rudinsky 1973, Rudinsky *et al*. 1974). Rudinsky *et al*. (1974) also noted that a compound called myrtenol acts in the same manner as verbenone in terms of attraction and inhibition. Verbenone appears to play a major role in regulating the sex ratio of landing beetles, typically resulting in a 1:1 ratio (Vite and Renwick 1971, Coulson *et al*. 1979). In addition to verbenone, both brevicomin and *endo*-brevicomin serve as inhibitors of frontalin (Vite and Renwick, 1971). Even though these compounds inhibit the response to frontalin in SPB, they do not do so in predators that are sensitive to the attractant (Vite and Renwick 1971).

Because of the nature of airborne pheromones, it is important to consider the abiotic effects on SPB attack strategy. Abiotic effects can differ depending on the time of day that an attack occurs. SPB is most active in the late afternoon around 3 to 5 pm but can be active at almost any time of day (Coster *et al*. 1977). During the summer the weather is prime for chemical attraction and females do not need to do as much work in selecting a host because their antennae guides them (Thatcher 1981). In the spring and fall, however, the weather is not as conducive to chemical attraction so the number of "pioneer" attacks increases (Gara 1965). Weather has been found to affect flight behavior as well as the pheromone system of SPB. Coster, Geer, and Johnson (1981) found that humidity, solar insolation, temperature, and precipitation had very little effect on flight patterns during the summer, whereas all the factors except precipitation had a pronounced

effect during the winter. The length of time it takes for gallery construction and oviposition is influenced by local weather and tends to be much shorter during warm temperatures and far longer in cold temperatures (Coulson *et al*. 1979, Coster *et al*. 1981). Weather also plays a major role in the synchrony of generations in SPB (Gara 1967).

After a female has selected a host, she attacks and releases frontalin (Kinzer *et al*. 1969, Thatcher 1981). The attack pattern of SPB is always the same, with females attacking first, releasing pheromone, and then males and other females responding and aggregating. Using frontalin to bait host trees, Coster et al. (1977) were able to attract only female SPB to their test trees. Within 24 hours these pioneer females stimulated a mass-attack on their hosts. This type of behavior has been observed repeatedly in SPB (Gara *et al*. 1965, Gara 1967, Coster *et al*. 1977, Coulson et al. 1979, Thatcher 1981). Host chemicals such as α-pinene can attract females and synergize with frontalin to increase its attractive qualities to both sexes (Vite, 1975). Host trees will continue to release α-pinene throughout the attack and subsequent infestation. Gara and colleagues (1965) observed an initial attack stage where beetles focus narrowly on the spot where females infested first, followed by a mass-aggregation of beetles on vertical objects around the tree, including non-host objects. After the initial attack has commenced, the number of SPB infesting grows over a period of time and peaks somewhere in the first three days, declining afterward but continuing for well over a week (Coster *et al*. 1977). This differs slightly from observations by Flamm *et al*. (1987) who noted that peak attack

seems to occur between five and eight days after the initial attack. Initially beetles attack the middle of the tree, spreading out toward either end of the bole over time, but tapering off sharply near the base or crown (Gara 1965, 1967, Coster *et al*. 1977, Flamm *et al*. 1987).

The impetus for females to release frontalin is the physical act of boring into the bark of the tree. The female will also release the frontalin synergist trans-verbenol upon entering the tree (Renwick and Vite 1969). Females bore into the bark and begin creating their galleries as males land, search for, and then enter these same holes. In order to attract female SPB, the make will often "chirp" through stridulation with their elytra upon entering the initial attack hole (Rudinsky 1973). Males are very protective of their holes emitting verbenone as well as utilizing a "rivalry-chirp" to discourage other males from entering (Rudinsky 1973). The combined effect of these behaviors is the segregation of mating pairs to their own galleries and an overall balance of the sex ratio of attacking beetles.

Males are known to help females in clearing galleries by removing frass and generally cleaning the areas (Thatcher *et al*. 1981). Towards the end of the gallery building process the female builds a nuptial chamber wherein mating occurs (Thatcher *et al*. 1981). Once mating takes place females begin building their unique "S-shaped" or serpentine egg galleries. These galleries are found in the vascular cambium of the tree and typically go against the grain of the wood (Thatcher *et al*. 1981, Wagner 1982). The length of these galleries and number of eggs laid per female is highly variable depending

on SPB density, host quality, weather, predation, pathogen-presence, and a host of other factors (Coulson 1978, Fargo *et al*. 1979, Wagner 1982, Flamm 1993). After 2-3cm of gallery is completed the female will begin building small egg niches along both sides of the gallery in which she oviposit her eggs singly. Flamm *et al*. (1987) recorded an average of 1.59 eggs laid per cm. of gallery, a number that agrees with other recorded averages (Thatcher et al. 1981, Wagner *et al*. 1982). Flamm *et al*. (1987) also observed that gallery density and mean gallery length decreased as overall attack density increased. This is an example of the density-dependant oviposition process in SPB. After a female is finished laying her eggs she will emerge from the tree much like her male partner does after mating. Galleries built through the vascular cambium effectively girdle the host tree and under these conditions host mortality is all but guaranteed. In restricted infestation tests, trees that were not girdled were found to survive whereas their girdled compatriots suffered swift mortality (Gara *et al*. 1965).

Beetles that have completed the gallery construction and mating process are not simply content to rest or die; instead most of them participate in a process called reemergence. Reemergence is very common in SPB with frequencies in field populations found to be as high as 97%, and laboratory frequencies of 90% (Coulson *et al*. 1978, Gagne *et al*. 1982, Wagner 1982). SPB adults leave their host tree with the ability to produce and respond to pheromones, build galleries, mate, and lay eggs still intact (Coulson 1978, Thatcher *et al*. 1981). Reemergence tends to peak soon after the initial attack (1-3 days) and continue for 16-20 days in the field (Coulson *et al*. 1978).

Temperature plays an important role in reemergence patterns as well. Laboratory tests of reemergence over the prime developmental temperature range found that the longest residence time occurred at 12.5˚C (46 days) and the shortest residence time occurred at 27˚C (12 days) (Gagne *et al*. 1982). Gagne and his team (1982) also noted that males reemerged about 1-2 days sooner than females in temperatures up to 30˚C with the opposite occurring over 30˚C. Coulson and colleagues (1978) identified several important results of the reemergence process. They noted that it allows SPB to meet the criteria for a successful infestation in that it allows for a sufficient number of beetles to initialize and sustain an infestation. Reemergence can also help in host identification and colonization, while maintaining local attractiveness to SPB. Coulson also proposed reemergence as a method of minimizing the impact of local weather disturbances on SPB populations (Coulson *et al*. 1978, 1979).

While adult beetles reemerge and build the local infestation, the eggs laid by females go on complete their development inside the tree in the manner discussed previously. The process of new adult emergence is similar to reemergence but differs in several important ways. Emergence of new adults occurs at a sex ratio of about 1:1, similar to reemergence ratios (Coulson *et al*. 1979). Gara (1967) observed that beetles tend to stay very near their brood tree as long as there is attractant pheromone present in the air. If there is little or no pheromone, as is common at colder temperatures, the likelihood of long distance flights increases (Gara 1967). Flights of up to 1 mile can occur if weather conditions permit, but if pheromone is present the flight distances tend to be closer to 8-12 meters (Gara 1967, Coster *et al*. 1977). Large infestations tend to

grow relatively slowly and move away from the initial site in a single direction (Price and Doggett 1978, Thatcher 1981). The spatial pattern of emergence appears to follow closely with the pattern of attack and reemergence (Coulson *et al*. 1979). The process of mass-attack and colonization is an awe-inspiring process to study but it does not occur without resistance from the host tree, pathogens, and predators.

Pathogens, Predators, Competitors, and Mutualists of SPB

Bark beetles and SPB in particular are not adept at defending themselves against the host of malevolent creatures that wish to make a meal of their host, or the beetles themselves. Instead their strength lies in numbers as evinced by the fact that no known predator, parasite, competitor, or pathogen can fully control a population in the midst of a full outbreak. This does not mean that SPB is immune to predation; on the contrary there are plenty of creatures that make their living off of these bark beetles. SPB are unique among the bark beetles in that they do not suffer from overcrowding a host tree like many other beetles in the pine bark beetle guild do (Aukema and Raffa 2004). This is due to their chemical communication system and allows them to avoid not only intraspecific competition, but the increased predation rates that often accompany overcrowding. Disease, however, is often a problem for SPB. Sikirowski et al. (1979) studied the prevalence of pathogens (primarily protozoans) in SPB populations throughout Mississippi. They found that about one fifth of the state's SPB population was infected with a fungus or disease. They observed that warmer months tended to see rising levels of

fungal infections whereas cooler months saw an increase in protozoa-related mortality (Sikirowski 1979).

One of the primary predators on SPB is the clerid beetle *Thanasimus dubius.* This beetle hunts day and night for bark beetles and has been found to significantly decrease SPB populations in laboratory studies (Dix and Franklin 1977, Reeve 1997). Thatcher and Pickard (1966) recorded an average daily consumption of 2.2 adult SPB per adult *T.* dubius in the laboratory over a period of 5 to 10 weeks. They also noted that developing larvae of *T. dubius* consumed about 100 larval SPB in the course of their lives (Thatcher and Pickard 1966). Reeve (1997) identified *T. dubius* as an important part of the initial colonization phase of SPB showing the possibility that *T. dubius* could severely damage a new population of SPB if it was already present in the selected host. Though a powerful predator, *T. dubius* is not a good candidate for biocontrol because of its long life cycle which has been observed to increase in the field (Thatcher and Pickard 1966, Reeve 1997). Moser (1975) studied the ability and readiness of 51 different mite species to attack and kill SPB. He found 32 to be predatory but was only able to identify four which showed any potential as a biological control agent (Moser 1975). All of the candidates were phoretic and attacked SPB at least 74% of the time when presented to it (Moser 1975). Mites are not always bad news for SPB however; Kinn (1980) identified the mite *Dendrolealaps neodietus* as a mutualist of SPB. This mite is phoretic and in its presence SPB was not infected with certain deleterious nematodes that were present in the

population at large (Kinn 1980). Aside from direct predation there are many other things that threaten SPB survival from parasitism to competition for resources.

One of SPB's primary competitors is the large cerambycid beetle *Monochamus titillator*. *M. titillator* lives in the inner bark of trees feeding on phloem and other resources in much the same manner as SPB (Coulson et al. 1976). SPB and *M. titillator* compete through both interference and exploitation with SPB suffering about 70% mortality in areas occupied by the beetle (Coulson et al. 1980). The presence of *M. titillator* and its activities are highly variable depending on weather and spatial location, so much so that they cannot be relied upon to control an SPB outbreak (Coulson et al 1980). There are a host of parasitoids that attack insects within the bark of a tree, but while few are specialized on SPB, many will parasitize them as generalist parasitoids. Among the parasitoid wasp families, members of Brachonidae, Pteromalidae, Euritomidae, and Eupelmidae are known to parasitize SPB (Dix and Franklin 1978, 1982, 1983). Dix and Franklin (1982, 1983) noted that species from the genus Eurytoma (F. Euritomidae) tend to parasitize early larval stages and arrive at an infestation around 25 days after the initial attack. Most Brachonid parasitoids were observed arriving about 16-30 days after initial infestation while the Pteromalid's arrival was noted at around 30 days (Dix and Franklin 1982, 1983). Parasitoid activity was observed to be the greatest in the late afternoon which follows closely with SPB peak activity (Dix and Franklin 1978). Dix and Franklin (1978, 1982) also noted that some species are nocturnal and that their search efficiency and behavior appeared uniform regardless of tree height. While the extent of

the impact parasitoids have on SPB populations is hard to judge due to the complex competitive and non-competitive interaction inherent to the system, there is little chance in theory that any parasitoid or parasitoid complex could exert enough pressure on an SPB colony to quell an outbreak or even prevent initial infestation.

Despite the many predators, competitors, and pathogens that assail the SPB, they appear to have found a friend in fungi. Female SPB have a specialized compartment on the anterior of their pronotum called a mycangia which serves as a spore repository. Females leave behind spores of several fungul species in the niches where their eggs are laid (Thatcher et al 1981). Fungal species *Ceratocystiopsis ranaculosus,* and *Entomocorticum sp.* are well known mutualists of SPB. SPB face a problem as phloem-consumers in that the nitrogen and phosphorous content of pine phloem is often too low to promote proper growth and development (Ayres et al. 2000). Most mycangial fungi aid SPB by raising the nitrogen and phosphorous content of the phloem surrounding the developing beetles (Paine and Stephen 1987, Ayres et al. 2000). Larvae that do not get enough nitrogen, often because of a lack of mutualistic fungi, have to eat more of their host in order to develop. This leads to elongated larval feeding tunnels that are often indicative of poor host quality and of an absence of mutualistic fungi (Thatcher et al. 1981, Ayres et al. 2000). With the help of mutualistic fungi SPB are able to meet their Nitrogen requirement, which, as Ayres et al. (2000) pointed out, means they are always able to fulfill their phosphorous requirements as well. They also noted that trees with more nitrogen tended to produce larger beetles and that Nitrogen levels within the tree

tended to double over the course of an infestation primarily due to the presence of SPB's mycangial fungi. Not all mycangial fungi are helpful, in fact the "blue-stain" fungus *Ophiostoma minus* is a well known antagonist of SPB. *O. minus* does not raise phloem nitrogen levels enough to supplement the diet of SPB and thus if the immediate area around a SPB larvae becomes infected it will not be able to support proper SPB development (Paine and Stephen 1987, Klepzig and Wilkens 1997, Ayres et al. 2000). Paine and Stephen (1987) also noted that the blue-stain fungi are one of the only fungi associated with SPB to produce a defensive reaction in loblolly pine and play a major role in the infection and death of its host. Klepzig and Wilkens (1997) studied the competition between *O. minus* and various mutualistic fungi finding that *O. minus* greatly out competes all other fungus species even going so far as to invade and destroy its competitors. Fungi play an important role in the life cycle of SPB, but Dwyer et al. (2004) indicated that the effect of fungi, predators, and pathogens is much more powerful when combined and examined as a complex. Though no single deleterious force has the power to control or exterminate SPB, the enemy complex along with weather conditions and host resistance are often enough to stem the tide of an outbreak.

Host Biology and Resistance

Observing a pine stand in the midst of a full SPB outbreak is akin to watching Gulliver toppled and subdued by the Lilliputians. Though unlike the aforementioned

literary character, pine trees can fight back with a variety of powerful defensive systems. Understanding the biology, physiology, resistance, and defensive mechanisms of pine trees sheds new light on the life cycle of SPB and gives insight into the ongoing battle for survival common to all living things. Franceschi et al. (2004) describes the general strategy of pine defense as a combination of induced and constitutive mechanical and chemical mechanisms. Most tree species follow some variation of this model, but few do in the same manner as the pine. The goal of resistance and defense is to protect the tissues of the tree necessary for growth and life. Franceschi et al. (2004) identified the phloem, transpiration stream, and vascular cambium as the most susceptible structures to SPB attack and some of the most important in the life cycle of the pine. This assessment is echoed throughout the scientific literature (Bannan, 1936, Mergen 1963, Lorio 1968, Feldman et al 1981b, Raffa and Berryman 1983, Lombardero et al. 2000). SPB infest the vascular cambium and feed off the phloem which, if enough beetles are present, will result in rapid death by girdling. Raffa and Berryman (1983) reported the threshold of tree mortality is crossed once the infestation reaches the level of about 40 galleries per square meter (g/m^2). They also noted that too many galleries (over $90/m^2$) would result in decreased efficiency for SPB and that the optimal host-killing density exists at approximately 62 g/m^2. Since SPB are able to infest almost any pine, it is important to assess the susceptibility of each of the common host species.

Among the four most common hosts for SPB (loblolly, longleaf, slash, and shortleaf pine), Hodges et al. (1979) found loblolly and shortleaf to be more susceptible

to infestation than the others. Hodges and his team used four major oleoresin characteristics from one of their earlier papers (1977) to conduct this assessment. After analyzing a suite of chemical and physical characteristics the most important including, flow rate, viscosity, total flow, and time to crystallize were identified (Hodges et al. 1977, 1979). Another important conclusion of Hodges et al. (1977) was that the physical characteristics of oleoresin are more important to host resistance than the chemical characteristics. They also noted that the morphological features of a tree are not strongly related to the oleoresin characteristics (Hodges et al. 1977). Since the antagonist blue-stain fungus carried by SPB is one of the major components of tree mortality, it is important to understand the response of pines to inoculation. Pine trees respond to fungal contamination by forming hypersensitive lesions containing less sugar, more monoterpenes, and spatially located around the infected area (Cook and Hain 1985). After inoculation lesions grow quickly, but eventually table off in much the same way as the resin levels within the trees rise initially and then lower. Cook and Hain (1985, 1987) observed differential susceptibility to *O. minus* between shortleaf and loblolly pine. Even though loblolly pine has longer lesions, stronger bark, and more monoterpenes, Cook and Hain (1985, 1987) identified it as more susceptible than shortleaf to *O. minus*. While differences in host species and reaction to fungal infestations are an integral part of the biology of pines, there are other factors that are more important to a host tree's defense of a SPB attack.

One of the primary means of defense against SPB is the release of host chemicals (typically terpenes) into the air both prior to an infestation and in response to an attack.

Raffa and Berryman (1983) noted that different variations of host terpenes could interfere with the chemical communication systems of SPB. One of the main reasons trees release terpenes in the first place is that they are toxic compounds capable of killing various herbivores (Mergen 1963, Franceschi 2005). SPB have evolved the ability to take these and use them to enhance their chemical communications, but this does not mean they are immune to the toxic effects of monoterpenes. Pine trees are known to utilize high concentrations of monoterpenes to battle fungal infestations (Cook and Hain 1985). A much more powerful weapon in the pine tree's arsenal is its oleoresin (often referred to simply as 'resin'). Oleoresin in pines refers to the viscous secretion of hydrocarbons and various volatiles from resin cells, ducts, radial rays, axial rays, and blisters (Hodges et al. 1979, Thatcher et al 1981, Franceschi et al. 2005). This resin is stored in special pressurized cells that release their contents upon wounding (Franceschi et al. 2005). Not all conifers have resin-producing structures, but all the members of the Pinacae do (Franceschi et al. 2005). Oleoresin is a danger to SPB because of the toxic terpenoids and phenolics it contains, and the physical harm the sticky liquid can cause by closing off galleries, trapping, and crystallizing around beetles. Because trees, like all plants, require carbohydrates for energy, growth, and survival, resin is a very costly substance for a tree to simply release. The induced release of resin, especially in reaction to a large infestation, is one of the primary causes of mortality during a SPB attack (Lombardero et al. 2000, Franceschi et al.2005). Resin duct structure and location differ from species to species but in general they are more scattered around the xylem in young trees than in adults (Bannan 1936). The shape of a resin duct is similar to a canal with several points

coming off of the end according to Bannan (1936). The number of resin ducts in white pine (*Pinus strobus*) was found to vary over a large latitudinal range with more being observed in northerly trees (Mergen 1963). This is important to note because white pine may become a more common host for SPB in the future and the variation in resin duct prevalence could play an important role in its suitability to SPB infestation and development. White pine's biology, physiology, and susceptibility will be discussed in greater detail later in this paper.

Knowledge of the physical properties and physiology behind resin release is of critical importance to the effort to better the SPB/pine system. Though the chemical component of resin is important, Hodges et al (1979) indicated that the physical properties such as resin flow rate, total flow, and crystallization time are the most important factors to consider in terms of a tree's ability to resist SPB colonization. Blanche et al. (1992) correlated resin flow to both environmental factors and developmental progress. Vertical resin duct density, soil water levels, growth factors, and air temperature were all part of a complex which, according to Blanche et al. (1992) explains about 73% of the variation of resin flow in loblolly pine. This is one example the importance of host quality and ontogeny in the development of defense systems against SPB. Lorio (1968) observed different levels of oleoresin exudation pressure (OEP) in loblolly pine depending on the flatness of their site. Trees on flatter sites were less capable of maintaining a high OEP in drought conditions (Lorio 1968). This is an example of the impact of environmental factors on a tree's defense system.

Lombardero et al. (2000) completed a comprehensive analysis of environmental impact on the resin system of the loblolly pine. Since carbohydrates are the most important component of growth in trees, proper allocation is pivotal to the its success. Trees cannot achieve maximum growth all year long, instead they usually only grow for about 2/3 of the year (Blanche et al. 1992). Lombardero et al. (2000) noted that trees tend to keep resin flow down during periods of growth and thus must up-regulate it after the initial attack of SPB. They also indicated that resin flow is highest when there is an excess of water and nutrients in the tree. In pines excess nutrient and water levels only occur under drought conditions. This indicates that the tree may better able to use resin as a defense against SPB when in a weakened state. The tree will still increase resin flow and cease growth when under attack regardless of its initial condition (Lombardero et al 2000). This is part of the initial step in a defensive process outlined by Franceschi et al (2005) in which the tree first seeks to repel or inhibit its attackers. Lombardero et al. (2000) observed a doubling in resin flow after about seven days of SPB attack. This is a problem for pines because some infestations have already peaked by 3 days (Gara 1965, Lombardero et al. 2000). As the infestation progresses trees can even form new resin ducts, referred to as "traumatic ducts," that help to increase overall resin flow (Franceschi et al 2005). As mentioned before the tree can induce greater resin flow after an attack, and this induced resin is often chemically different than the constitutive resin (Franceschi et al. 2005). Usually this means that the resin contains more terpenoids and phenolics than are typically found in the constitutive resin. All of these induced defenses are meant to inflict higher mortality on SPB but can serve another purpose. The second part of the

defense strategy for loblolly pine outlined by Franceschi et al. (2005) is a compartmentalization of infestation followed by a sealing and healing of wounds. Resin is integral in this process because of its ability to crystallize to a very hard substance. This causes it to not simply kill the SPB, but aid the host in the healing process. Together all of these defenses work to protect pine trees from infestation, but outbreak-sized populations of SPB can render them ineffective.

Climate Change and the SPB

Global temperatures have been on the rise recently and almost every climate change model predicts that this will continue for the foreseeable future. The most recent report from the International Panel on Climate Change predicts a rise of 1.4˚ to 5.8˚ C over the period of 1990-2100 (IPCC 2003). Because of the integral role temperature plays in the life cycle of SPB, any sort of global climate change is sure to affect SPB populations. Powell et al. (2000) were able to show experimentally that temperature alone can synchronize the life cycles of SPB. This is important because understanding the voltinism of SPB will help build stronger predictive models. Temperature helps to control synchrony by way of setting the developmental speed for a given generation of SPB. Since the mass-attack strategy is necessary for the survival of the species, it follows that synchronous emergence would help to improve the chances of a successful, coordinated attack. Climate change may act directly on SPB, a scenario which will be discussed later, but it also acts on the environment as a whole. Peterson et al. (2001) examined the consequences of global climate change on forest disturbances. They indicated that

climate change might lead to rapid alterations in the intensity, frequency, and timing of forest disturbances (Peterson et al. 2001). This is important to SPB because it is forest disturbances like lightning or fire that weaken trees enough for a pioneer beetle to successfully initialize an infestation. A change in any of those factors of forest disturbance would be felt throughout the local SPB populations and may indeed lead to larger-scale changes in SPB range and damage. Peterson et al. (2001) also noted that climate change could result in a shift in the behavior and intensity of herbivores and possibly cause changes in the suitability of native host species to invasion by introduced species. There are many other general environmental impacts of global climate change but they will not be discussed any further, instead I will turn my attention to models and predictions of SPB range.

There is a host of scientific research indicating that SPB's natural range would expand northward under rising global temperatures (Ungerer and Ayres 1999, Ayres and Lombardero 2000, Williams and Liebhold 2002, Gan 2004). Range expansion due to temperature has already been observed in the SPB's close cousin the mountain pine beetle. Logan and Powell (2001) described how MPB were able to infest five-needle pines that typically grow at a much higher altitude than MPB usually inhabit. In this scenario several years of slightly higher than normal average annual temperatures allowed MPB to make the jump in altitude and infest new conifers. In this paper they note that the hundreds of feet in altitudinal relief could comfortably translate into hundreds of miles in horizontal space (Logan and Powell 2001). This is important

because it is an example of a range shift by a bark beetle and could be considered a prototype for the range expansion expected of SPB. To this effect, Ayres and Lombardero (2000) applied general global climate change models to 32 different pests and pathogen species including SPB. They predicted that a rise in global temperatures would have a direct effect on the development of SPB as well as an expansion in their range. They also cautioned against potential predator and parasitoid behavior shifts in response to temperature changes as well as possible changes in tree defense systems. Ungerer and Ayres (1999) built their model around the assumption that the most important factor in bark beetle range is the lower lethal limit of temperatures in a given area. This means that the colder an area gets is the most important to SPB because the lower end of their prime developmental temperature range is around 4˚C (Ungerer and Ayres 1999). In all of their scenarios SPB moved northward, even under the mildest temperature change. They noted, however, that a change in temperature would not guarantee a northward movement, but that it was the only foreseeable barrier to such a shift. In the worst case scenario used by Ungerer and Ayres (1999) SPB could become more common in Pennsylvania and Ohio with occasional forays into Wisconsin, Michigan, Massachusetts and New York with as little as a 3˚C rise in average annual temperature.

Williams and Liebhold (2002) also predicted a northward movement of SPB under even the mildest rise in temperatures. They also questioned whether or not the northward movement would occur in a "block." In other words they wanted to know if

the lower distribution limits would move north as well. They concluded that there was a possibility for such movement despite the fact that it is rarely observed in other species (Williams and Liebhold 2002). Gan (2004) completed another very detailed ecological and economical assessment of potential range shifts in SPB. Under all of the scenarios he ran there was an increase in the damage caused by SPB with a doubling of atmospheric CO_2 leading to a 2-2.5X increase in SPB outbreaks and a 4-7.5X increase in monetary cost (Gan 2004). He also noted that warmer winters and springs would lead to an increase in SPB outbreaks with an increase in fall temperatures would lead to a decrease in SPB attacks in the summer (Gan 2004). In his paper Gan (2004) also indicated that higher CO_2 levels may result in an increase in tree size which, when added to the model, results in even more monetary damage by SPB. The effect of global climate change on the host plants of SPB is another area of particular interest. Iverson et al. (2004) built models to predict the migration of several tree species, including loblolly pine, in response to warming. Out of all the trees tested it was determined that loblolly pine has the greatest potential to move and to move the farthest when it does (Iverson et al. 2004). Even so the furthest northward their models predicted loblolly pine to move was 10 to 20km with a slight possibility for localized populations further than 100km north (Iverson et al. 2004). According to Iverson et al. (2004), the reason that loblolly is the most potentially mobile tree in the tested cohort is probably because it is a border tree in many forests. Regardless this information indicates that SPB's traditional host may be moving northward as well, which would surely aid SPB in its own expansion.

If the SPB does move northward there is a possibility that it may move outside of the natural range of loblolly pine. If this does happen there are many potential new hosts for SPB including white pine.

A New Southern Pine Beetle Trap for Research and Laboratory Rearing

Micah Gardner
M.S. Thesis Chapter

ABSTRACT

Southern Pine Beetle field trapping methods are well known and employed in yearly monitoring surveys across the southeastern Unites States. Trapping methods meant to keep Southern Pine Beetle alive for laboratory rearing and research have not developed beyond the large funnel traps of the 1970's. A set of newly designed Southern Pine Beetle traps were compared with old funnel traps to see which was more effective at capturing live Southern Pine Beetle. Traps were monitored at both 12 and 24 hr rates for 15 days. The new-style traps captured significantly more live beetles ($t=11.15$, $p<0.0001$, 14 df) and the 24 hr monitoring rate was found to capture significantly more live beetles as well ($t=5.\ 22$, $p<0.0001$, 14 df).

INTRODUCTION

Monitoring techniques for Southern Pine Beetle (SPB) field populations have long employed pheromone-baited Lindgren funnel traps. While these type of traps attract, capture, and kill SPB they do not work well as a means to preserve live individuals for experimental use. There are no commercially available SPB traps designed to preserve live individuals emerging from a lab-reared colony. The best trap option at the time of this research was a design from the US Forest Service (Figure 1.1) produced in the 1970's. It consisted of a rectangular wooden frame housing a pair of cylindrical, metal

drums about 2 feet long and 1.5 feet in diameter which were connected at the base to a thin-gauge wire-mesh funnel. This funnel emptied into a sealed mason jar filled with shredded paper. This trap would prove to be far from effective, even revealing itself to be the cause of many of our rearing troubles. In light of this, the development and maintenance of a successfully lab-reared SPB colony proved to be extremely difficult. However, the paucity of effective traps is only part of the problem.

The SPB is a cyclical outbreak pest whose populations have risen and fallen many times over the recorded portion of their history (Thatcher et al. 1981). Recently the size and virulence of the SPB population across its entire range has paled in comparison to the robust populations of the mid-1970's (Coulson and Stephen 2009). Researchers across the Southeastern US, home range of the SPB, have struggled for the last several years to find active SPB spots and populations (Coulson and Stephen 2009). Despite this, we were able to obtain at least a dozen infested trees per year for use in our rearing colony and in the production of individuals fit for experimentation. Further obstacles to a successful colony presented immediately as close observation of dead and living beetles in the rearing colony revealed evidence of conspecific mutilation and even cannibalism. Beetles were often observed missing several to all of their tarsi, with punctured elytra, and in many cases damaged to the point of death but still clinging to one another and attacking. Colony mortality was between 85%-95% throughout the first two years of rearing attempts prompting a new approach to SPB capture within the laboratory.

Conspecific mutilation behavior was hypothesized to arise from crowding and contact between individual SPB in the capture area. To remedy this a new trap was designed which provided an increase in total surface area and refugia with the goal of higher SPB capture rates and ultimately a reduction of the colony mortality rate in the laboratory. The new-style trap (NST) offers 15 times more surface area (22,410 cm^2 compared to 1535 cm^2 in the original design) and 4 new types of refugia, spread throughout the trap (Figure 1.2). The new trap design was tested against the old-style trap (OST) design by comparing live capture rates, mortality rates, and spatial location within the trap at time of capture over a set period of time. The frequency of trap monitoring and removal was also tested by monitoring some traps every 12 hours, and the rest every 24 hours. Due to the apparent importance of surface area, we hypothesized that the new trap design would significantly outperform the old design in its ability to capture and preserve healthy SPB from field-infested material.

MATERIALS AND METHODS

Trap Construction. This newly designed trap was built by attaching three discrete sections together: the frame, the containment portion, and the capture portion. The trap was primarily constructed of several long, 5 cm by 5 cm (2x2 inch) pieces of pine. Four of these were cut to 101cm lengths, four at 91cm lengths, and eight at 48 cm lengths. Additionally one-half inch thick plywood was cut into 4 sizes including four, 50 cm by 40 cm square sections, four, 50 cm by 38 cm inch square sections, two, 50 cm by

13 cm parallelograms used as side braces, and one, 97 cm by 13 cm parallelogram used to brace the lower length of the trap.

Pieces of wood were pre-cut in our woodshop and assembled using screws and an electric drill. Some fitting of plywood required pounding and shaping with a large rubber mallet. Construction of the trap frame was completed as follows: the 101 cm sections stand vertically and form the "legs" of the trap as well as the four corners of the upper rectangle, while the 91 cm and 48 cm 2x2's form the length and width, respectively, of the upper and lower rectangles that enclose the four legs. Two of these lateral rectangles are to be formed, the upper at the top of the legs which represent the top of the trap, and the lower rectangle 38 cm below. The rectangle is then split in half, transversely, by the attachment of a square formed of 48 cm long 2x2s and a single 50 cm by 13 cm piece of 1 cm thick plywood. This produces two "trap boxes" within the main trap which are to be enclosed using the remaining plywood, 48 cm by 38 cm sections along the width and 48 cm by 40 cm sections along the length (Figure 1.2). The short parallelograms are then placed across the width and positioned very low on the legs to provide stability. The long parallelogram section was attached across the length and positioned low on the legs as well.

The containment portion of the trap consisted of two, 1.52 m by 1.22 m sections of heavy screen door material that were cut and attached, one at a time by Gorilla® tape, to top of the trap frame and surrounding one trap box each. When the ends are overlapped

and clipped closed using five clothespins per trap box, this screen door material forms a strong barrier which is too fine a gauge to allow any SPB escape. Next eight holes were drilled in the lengthwise plywood panels of each trap box, four per front panel and four per back panel. Holes were one inch above the lower 91 cm long 2x2 on each side, evenly spaced, and large enough to accommodate a 1 cm thick by 54 cm long piece of rebar. The rebar formed a sturdy base on which we rested our field-infested pine bolts.

In order to guide falling beetles to the capture portion of the trap a funnel was installed. The funnel was constructed out of plastic sheeting cut into 163 cm by 40 cm strips. With a ruler and a marker the strip was separated into four, 40 cm by 40 cm, connected rectangles. A mark was placed 7.5 cm from the corner on both sides of each top, lengthwise portion for 8 total marks. Using a ruler a line was drawn from each mark to the corresponding corner below it. This formed eight individual 7.5 cm by 20 cm by 21.5 cm triangles which were colored in lightly as well as four, uncolored trapezoids. The strip was then folded so as to connect the trapezoids and form a 4 sided trapezoidal funnel. The funnels were then attached, facing downward, to the bottom of each trap box (Figure 1.3). Sections of organza fabric 2.44 m by 61 cm were cut and attached to the funnels using gorilla® tape on both sides of the attachment portion. This formed a sort of drape that could be easily attached to the actual removable trap portion of the design using Velcro® (Figure 1.3).

The removable receptacle was composed of a Rubbermaid® container 81 cm long, 45 cm wide and 15 cm tall. Three pieces of verti-cel® (Figure 1.4) were placed in

each container with their open compartments facing upward and arranged side by side so as to cover most of the bottom of each container (Figure 1.5). Shredded paper was then placed on top of the verti-cel® until the container was filled near to its top (Figure 1.6).

Live Capture and Trapping Experiment. Live SPB-infested loblolly pine was cut into 1 meter bolts and removed from the Paulding County Forest near Dallas, GA in December of 2009. Most SPB activity in the field can be found between three and eight meters from the ground and all bolts used in this experiment came from the aforementioned section of a single host tree (Coster 1977). Additional trees were cut and removed during this trip and used to maintain our continuous lab colony following the techniques of Bridges and Moser (1984). The ends of every bolt harvested was coated in paraffin wax to prevent desiccation. Bolts were housed in a basement room at Grinnells laboratory with a floor-to-ceiling tarp demarcating the side used for colony rearing and the side for trapping and collection of live individuals. The trap room was kept at 24º-26 ºC which is consistent with the optimal temperature range for SPB development (Wagner 1984).

Upon arrival from the field, eight bolts were removed from a host tree and four were selected at random and immediately placed into our SPB traps. One bolt was placed in each trap of both OST and NST traps. Traps were monitored over the course of 15 days, which mirrors SPB's normal peak emergence times in the field (Thatcher et al.1981). One of each style was monitored every 12 hours and the other every 24 hours.

All SPB found in the trap were recorded according to their location within the trap and condition as either alive or dead. Both live and dead SPB were removed from the trap after their information was recorded. Potential locations included the shredded paper, the side, bottom, or inside of the verti-cel®, the center or corner of the receptacle bottom, and other locations such as the rebar, organza, or Velcro.

Statistical Analysis. All data was analyzed using SAS 9.2 (SAS Institute, 2008). The count data of captured SPB per trap was fitted using a square-root transformation. The number of captured SPB was then analyzed with a three-way ANOVA taking day as a fixed factor (PROC GLM, SAS 9.2). The main effects of trap type (OST versus NST) and treatment (12 hour versus 24 hour monitoring period) as well any possible interactions were estimated. LSMeans estimates for main effects and each treatment combination were generated.

The ratio of live to dead beetles was estimated without any transformation of the data. The ratio data was analyzed with a three-way ANOVA taking day as a fixed factor (PROC GLM, SAS 9.2). Main effects of trap type and monitoring period, possible interactions, and LSMeans for all main and treatment effect combinations were also estimated.

RESULTS

The NST captured significantly higher numbers of SPB than did the OST ($F=124.31$, $df=59$, $p<0.0001$). With the increase in surface area of the NST it was expected that they would capture a higher raw number of beetles than the OST. This was

borne out in our results showing that the NST captured 440 SPB over the 15 day period whereas the OST captured 125 total SPB. The NST catches were 268 and 172 SPB for the 12 and 24 hour treatments respectively (Table 1.1). The OST catches were 88 and 43 SPB for the 12 and 24 hour treatments respectively (Table 1.1). The average number of SPB captured per day differed significantly between the NST and OST ($t=-11.15$, 59df, $p<0.0001$). The NST averaged 3.741 SPB per day whereas the OST averaged 1.982 (Table 1.2). Analysis of the type by treatment interaction revealed no significant effect ($F=.20$, 59df, $p=.6600$).

The 24 hour monitoring treatment captured significantly higher numbers of SPB than did the 12 hour monitoring treatment ($F=27.20$, 59df, $p<.0001$). The average number of SPB captured per day differed significantly between the 12 and 24 hour treatments ($t=-5.22$, 59df, $p<.0001$). An average of 3.272 SPB per day were captured at the 24 hour rate whereas an average of 2.450 SPB per day were captured at the 12 hour rate (Table 1.3). When comparing treatment combinations the highest average SPB catch per day belonged to the NST 24 hour treatment with 4.188 SPB (Figure 1.7). The NST 12 hour treatment averaged 3.295 SPB per day, OST 12 hour treatment averaged 2.359 SPB, and OST 24 hour treatment averaged 1.606 SPB (Figure 1.7). There was no significant effect observed from the type by treatment interaction ($F=0.21$, 58df, $p=.6464$).

There were no significant effect of trap type on live to dead SPB ratio ($F=3.19$, 58df, $p=0.216$). The estimated average ratio of live to dead SPB in the NST was 0.400

and 0.497 in the OST (Table 1.4). There was no significant difference between the 12 and 24 hour treatments in terms of live to dead SPB ratio either (F=1.46, 58df, p=.2337). The estimated average ratio of live to dead SPB in the 12 hour treatment was 0.485 and 0.411 in the 24 hour treatment (Table 1.4). A comparison of treatment combinations showed no significant differences (Figure 1.8).

The NST's ability to capture more live beetles than our older traps can also be seen in the total colony counts over the last 3 research seasons. Prior to the implementation of the NST in 2009, a maximum average of 3.3 SPB per field infested bolt were able to be captured and utilized (Table 1.5). After the new traps were constructed the total number of healthy beetles captured and used for experiments rose to 2,030 SPB compared to 495 SPB during the 2008 season and only 24 SPB in the 2007 season (Table 1.5). The average number of healthy beetles captured from any given bolt rose from 0.3 SPB in 2007 to 3.3 SPB in 2008 and reaching 16.916 SPB in 2009 which represents a fivefold increase over the previous season and an eighty-six fold increase over the first research season.

DISCUSSION

The NST appear to be significantly more effective than the OST at capturing beetles and keeping them alive within the trap so they may be removed and used in experiments. Informal observation of conspecific mutilation during the initial research season sparked interest in understanding the prevalence of this behavior in the lab colony.

When it was discovered that dead beetles on the floor of the rearing colony were often found clumped together, some latched on to another even in death, it became a primary concern to spatially separate captured beetles so as to avoid the inevitable violent endings that plagued our colony. This kind of conspecific mutilation reveals the importance of the SPB pheromone system and normal attack sequence in maintaining the SPB's natural population dynamics (Thatcher *et al.* 1981). Typically the SPB is confined to its tunnel within the vascular cambium and releases pheromones responsible for aggregation and disaggregation. The result of this is a natural spatial separation between conspecific SPB that is compromised in laboratory rearing and trapping situations.

The majority of dead beetles found in these traps were located on the bottom/bottom corner of the capture receptacle. This was true of the old-style mason jars as well as the new-style plastic containers. This observation was the inspiration for the addition of several layers of refugia material between the funnel portion of the trap and the bottom on the new traps. The reason for the high mortality rate on the bottom of the traps might be explained by the fact that a flat surface without any barriers does nothing to prevent contact and attacks from conspecifics in the same area. Prior to the use of shredded paper in the old-style traps, almost all SPB recovered from these traps showed signs of conspecific mutilation and cannibalism. After the introduction of the shredded paper there were some unharmed SPB found in the old-style traps. This fact coupled with the higher survival rate in the new-style traps, with their vastly increased surface area, indicates the potential importance of spatial separation in terms of preserving the health of captured beetles in both traps.

Removing SPB-infested material from the field and placing it in a laboratory setting carries with it many variables that can be difficult to control. For this experiment only 4 m of bolts from the same tree were used out of a total useable area of about 8 m. The choice of which sections to use was made at random, but there is always concern since the exact internal density of SPB could not be determined in a non-destructive manner. Another concern was the chance that the emergence time in the field would not correspond with the emergence time in the lab. Based on the number captured and the fact that after the experiment the occasional beetle was still found in the traps for up to two months, it appears that the duration of the experiment closely matched the emergence peak for this individual host tree. Armed with the knowledge that SPB is a cyclical outbreak pest it might seem that our recent success could be explained by an uptick in the natural population of SPB. Even a localized outbreak produces many more beetles than an endemic population of SPB, which leads more chances for the population to increase in both numbers and overall health. The fact that a higher raw number of beetles was caught coupled with the fourfold increase in the number of healthy beetles at our disposal for experiments and capturing a higher percentage of healthy beetles from any given bolt, indicates that the NST design could be responsible, in part, for our recent success.

Despite the success of this new trap, there are still many barriers to a continuous and healthy laboratory-reared SPB colony. The lowest mortality rate experienced among all the traps and monitoring rates was around 45%. This reveals the need for further improvement of SPB trap designs with the hopes that the established colony mortality

rate of 30% may someday be eclipsed. The addition of small amounts of disruptive pheromones to the verti-cel® portions of the traps may help to corral the SPB and keep them from moving to the bottom of the containers. Along the same vein it would be interesting to measure and codify the pheromone mixtures within a trap over the course of emergence time. Coupled with emergence and trap experiments over a range of temperatures it may be possible to determine if the masses of SPB found dead in our labs aggregated as such due to their own communication system. We did experienced some problems with the attachment of the organza drape to the capture receptacle, and while we solved it with strong tape, a more permanent and elegant solution could present itself with more research. This study has thus far lent strong support to the correlation between surface area in an SPB trap and overall SPB survival and should be extended to include alternative designs and options so as to better outfit researchers with the tools to study live SPB.

Figure 1.1 Old-style SPB traps in their original frame with fluorescent light below

Figure 1.2 The new-style trap.

Figure 1.3 Plastic funnel portion of new-style trap with organza drape attached

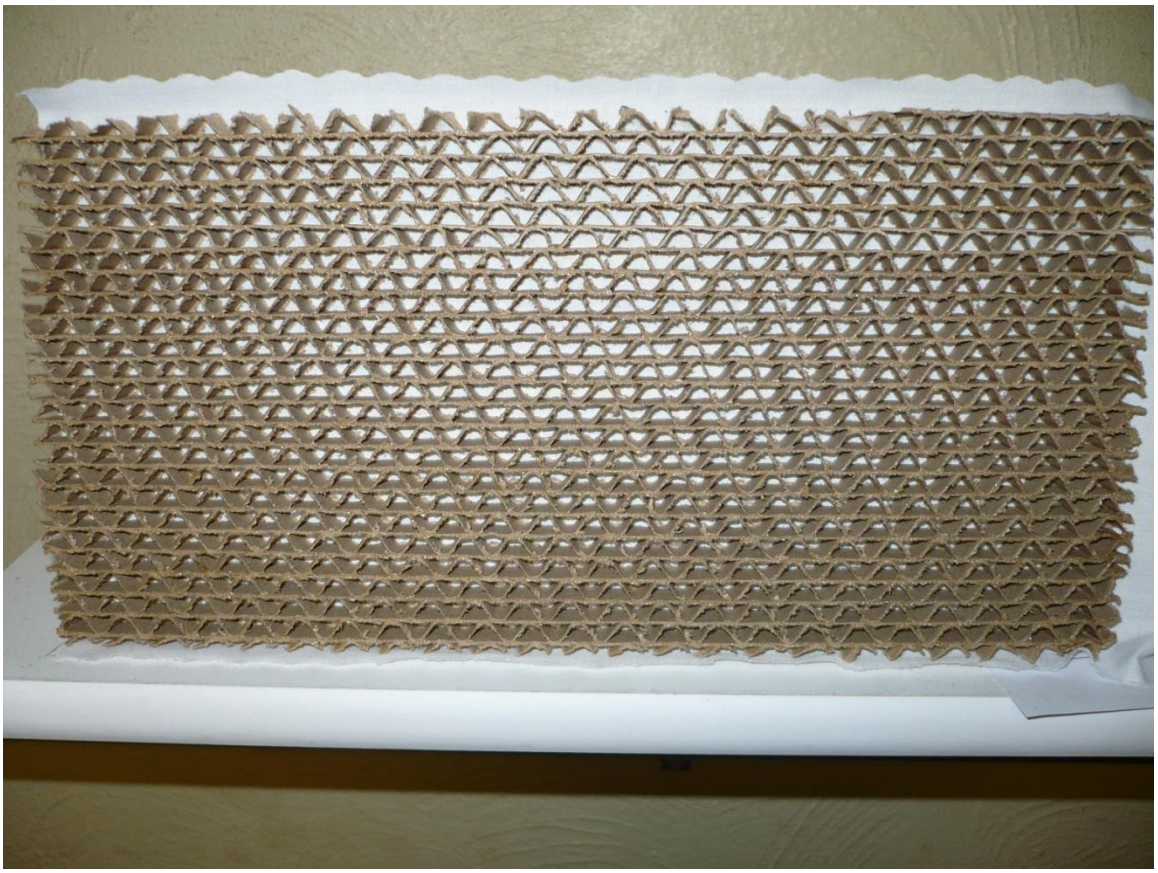

Figure 1.4 Verti-cel© packing material

Figure 1.5 Removable SPB receptacle with verti-cel© lining the bottom and shredded paper to the side

Figure 1.6 Removable SPB receptacle with shredded paper on top, this configuration was used during the experiment

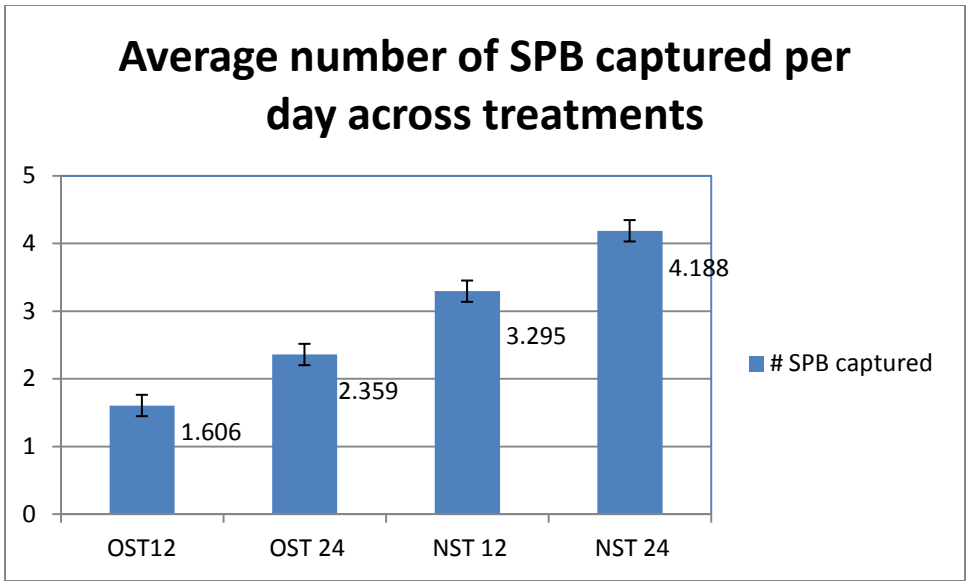

Figure 1.7 Average number of SPB captured per day across treatments

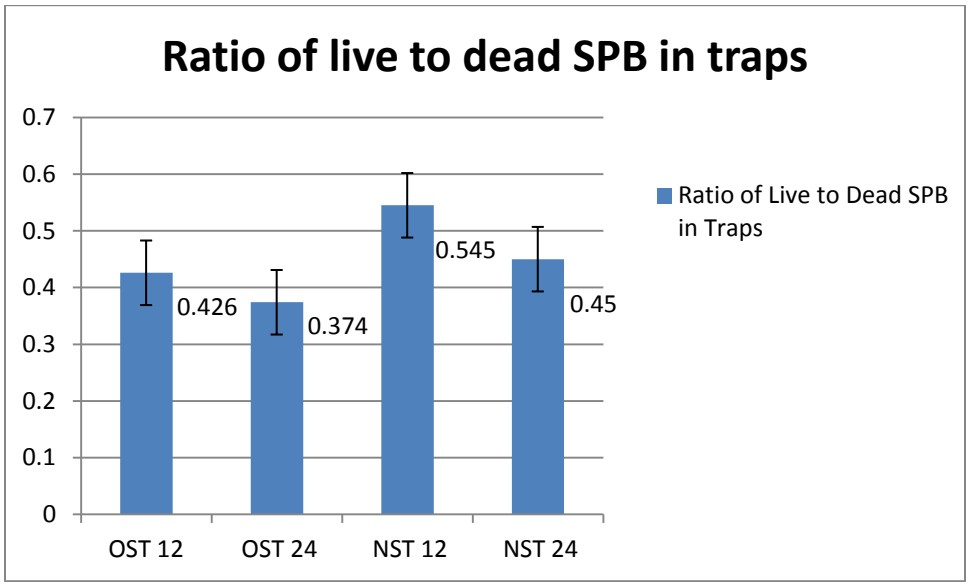

Figure 1.8 Ratio of live to dead SPB in traps

Table 1.1 Total SPB found alive or dead within the traps

	OST12	OST24	NST12	NST24
Total Captured Alive	34	16	120	89
Total Found Dead	54	27	148	83
Total	88	43	268	172

Table 1.2 Pair-wise comparison of trap styles monitored at the same rates for the average number of live SPB removed from a given trap on a given day

	OST	NST		12 hr.	24 hr.
Average # Live SPB Removed	1.982	3.742	**Average # Live SPB Removed**	2.450	3.273
T stat	-11.15		**T stat**	-5.22	
p-value (α=0.05)	< 0.0001		**p-value (α=0.05)**	<0.0001	
df	14		**df**	14	

Table 1.3 Pair-wise comparison of trap styles monitored at the same rates for the percent mortality of SPB removed from a given trap on a given day

	OST	NST		12 hr.	24 hr.
Ratio of Live to Dead SPB	0.400	0.497	**Ratio of Live to Dead SPB**	0.485	0.412
T stat	-1.69		**T stat**	1.28	
p-value (α=0.05)	0.0991		**p-value (α=0.05)**	0.2088	
df	14		**df**	14	

Table 1.4 SPB colony data from our insectary.

	# Total SPB Emergence (Estimated)	# Bolts Collected	# Healthy SPB used for Experimentation	Average # SPB / Bolt	Average # Healthy SPB / Bolt
2007	~500	80	24	6.25	0.3
2008	~3500	150	495	23.3	3.3
Implementation of New-Style Traps					
2009	~5100	120	2030	42.5	16.916

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Development of Southern Pine Beetles (*Dendroctonous frontalis* Zimmerman) in White Pine (*Pinus strobus*), a Non-Traditional Host

Micah Gardner
M.S. Thesis Chapter

ABSTRACT

Field-caught southern pine beetle (SPB) were allowed to attack and develop in either loblolly pine or white pine bolts for 30 days under laboratory conditions. Some SPB treatments were surface-sterilized before introduction to their host. After the developmental period was complete, biological parameters were recorded including the number of attacks, number of egg niches, length of egg and larval galleries, life stages present, proportion of successful egg hatch, and proportion of successful adult development. SPB were found to make significantly longer egg galleries, lay more eggs in, and hatch almost twice as often in loblolly pine than in white pine. Larval gallery lengths did not differ between host types. Surface sterilization and temperature treatments were not found to have a significant effect. Low proportional hatch, less egg niches, and more crowded galleries in white pine might indicate that it is a less suitable host for SPB than loblolly pine.

INTRODUCTION

The southern pine beetle (SPB) is a destructive pest of southern pine forests whose populations can sometimes rise to extraordinary levels over large areas. These outbreaks are cyclical in nature and cause a much higher degree of damage than at endemic levels where SPB populations are small and more stable, even serving a useful

purpose in the removal of weakened and injured pine trees from stands (Coster et al. 1977, Coulson 1986, Flamm et al. 1993, Thatcher et al. 1980, Wagner 1984). Historically the primary hosts of SPB are loblolly pine (*Pinus taeda*) and shortleaf pine (*Pinus echinata*), which has been found to be more susceptible to SPB than slash pine (*Pinus eliotti*), shortleaf pine (*Pinus echinata*), or longleaf pine (*Pinus palustris*). However SPB will attack other pine species as well(Hodges et al. 1977, 1979, Thatcher et al. 1980). The geographic range of SPB closely tracks that of loblolly and shortleaf pines and stretches east from Texas across the southeast and up to Virginia and the southern Appalachians (Burns et al. 1990).

Around the turn of the century SPB were observed attacking and destroying large areas of white pine (*Pinus strobus)* in the southern Appalachians. It is well known that SPB is able to attack and kill almost any coniferous species it encounters if its population is large enough (Flamm et al. 1993, Thatcher et al. 1980). This particular case is of interest in that the attacks occurred in areas typically thought too cold for successful SPB activity. One reason for this is that the natural range of white pine begins around the upper limit of the range of loblolly pine and runs north to Canada through the Appalachians (Burns et al. 1990). One theory is that global climate change could cause a rise in temperatures leading to a potential northward shift in the range of SPB (Iverson et al. 2004, Ungerer et al. 1999, Williams and Liebhold 2002). It is known that climate change can exacerbate forest disturbances by altering their timing, frequency, or intensity (Ayres and Lombardero 2000, Peterson et al. 2001). The literature is not unified, however, in predicting what will happen to populations of SPB and loblolly pine under

the effects of global climate change. Some northward movement of SPB is expected under most models with a northward shift by loblolly pine being likely (Gan 2004, Williams and Liebhold 2002). Though these theories help in explaining SPB activity on white pine, the work of Logan et al (1999) provides evidence for an altitudinal shift in pine beetle populations as temperatures rise which can help explain the SPB presence in the southern Appalachians.

The success of SPB in natural stands of white pine was unexpected and alarming, raising questions about potential host shifts for SPB, new areas of susceptible forest, and damage to white pine stands in the eastern US. To answer some of these questions the overall susceptibility of white pine, the development of SPB in white pine, and the degree of successful reproduction need to be determined. In order to explore these questions a colony of field-collected SPB was established and utilized in attack and developmental experiments in SPB's traditional host loblolly pine as well as in white pine. As part of this system it is important to consider the fungal associates of SPB, especially considering that fungi serves as a critical substitute to the diet of developing SPB (Ayres 2000). The importance of fungi, both beneficial and detrimental, to SPB diet and development is well established in the literature (Ayres 2000, Cook and Hain 1987, Dwyer et al. 2004, Klepzig 1997, Paine 1987). SPB females maintain beneficial fungi including *Ceratocystiopsis ranaculosus* and *Entomocorticum sp.* in a specialized structure called the mycangia which is located on the anterior pronotum (Thatcher et al. 1980). The most important detrimental fungi is known as blue-stain fungi (*Ophiostoma minus*) and can travel with SPB populations on the backs of phoretic mites as well as

through contamination of the mycangia or other body parts (Moser 1975, Thatcher et al. 1980). Blue-stain fungi inhibits SPB development by outcompeting the beneficial mycangial fungi and offering SPB larvae a much lower degree of nutrition (Klepzig 1997, Ayres 2000).

Due to a lack of information about the biology and effects of blue-stain fungi in white pine it was determined that some treatments were to undergo surface-sterilization procedures prior to experimentation. The goal of this was to attempt to remove as much of the blue-stain inoculum from the exterior of the SPB while maintaining the mycangial fungi. Coupled with fungi, temperature can be characterized as the most influential factor in the development of SPB (Powell et al. 2000, Wagner, 1984). For this reason it was determined that SPB development would be measured over a range of temperatures from the established lower developmental limits of 10°C to the optimal developmental temperature of 30°C (Wagner 1982, 1984). Due to difficulty in locating and rearing SPB, there were many less experimentally useful individuals than were desired and this led to a reduction in the experimental temperature range resulting in only 25°C and 30°C temperature treatments being utilized.

Understanding the behavior of SPB in loblolly pine proved to be integral in developing a trapping system capable of catching and maintaining SPB from field-infested bolts. The development of successful SPB capture is the foundation of all other aspects of this research and an appropriate example of the utility of a deep understanding of insect behavior in their hosts. The lack of such specific information concerning SPB behavior in white pine has proven to be a roadblock to fully understanding and combating

this pest. While attempting to control for environmental, predatory, and fungal factors SPB were allowed to attack and develop in both loblolly and white pine bolts. Using both these species is designed to give provide a better understanding of the susceptibility of white pine to attack and colonization by SPB. Based on prior success in natural white pine stands it was hypothesized that SPB attack rates, gallery construction behavior, oviposition, development, and success would not differ significantly between loblolly pine and white pine.

MATERIALS AND METHODS

SPB Rearing. SPB-infested loblolly pine was cut into one-meter bolts and removed from the Paulding County Forest near Dallas, GA in December of 2009. Trees removed during this trip were used to maintain a continuous lab colony following the techniques of Bridges and Moser (1984). The ends of each bolt harvested was coated in paraffin wax to prevent desiccation. Bolts were housed in a basement room with a floor-to-ceiling tarp demarcating the line between the colony-rearing side and the trapping and collecting side (Figure 2.1, 2.2). All lights were turned off or removed from the room during rearing and collection except for three, four-foot long fluorescent light fixtures. These were used to attract SPB to fresh bolts on the rearing side or to the collection portions of our traps on the other side. The rearing and trapping room was held at 24º-26º C, which is consistent with the optimal temperature range for SPB development (Wagner 1984).

SPB Development Experiment. One-meter bolts of uninfested loblolly pine were collected from the Schenck forest in Raleigh, North Carolina in December of 2009. Trees were felled and left on the ground for two days to allow time for excess resin to dry. Once removed from the forest the bolts were brought back to the laboratory and immediately covered on both ends with paraffin. Fresh bolts of uninfested white pine were collected from Ashe County, North Carolina in December of 2009. Due to time and travel constraints, the white pine material was removed from the site immediately after felling. White pine bolts were not immediately sealed with paraffin and instead were given 48 hours of drying time in the lab. Fresh bolts of loblolly pine were used in experiments within 48 hours after removal from the forest and white pine bolts were used within 96 hours after removal from their stand. Live beetles used in this experiment were obtained from several SPB traps located in the basement of the lab, and were utilized within fifteen minutes of their removal. SPB undergoing surface-sterilization were placed, as sets of five to ten, inside glass Pasteur pipettes. A solution of 4:1:95 ethanol, bleach, and water was drawn into the pipettes and passed over the SPB ten times followed by five minutes of drying time on filter paper. This sterilization solution was tested on SPB in our lab and no significant negative effects on individual SPB mortality, attack rate, or oviposition rate were observed (unpublished data).

The experimental design included 10 total pine bolts as experimental units, arranged by host type, temperature, and sterilization application or lack thereof. Five bolts used were loblolly pine and five were white pine. Temperature in the incubators

was either set at 25°C or 30°C with three of each of the host type developing at 30°C and two at 25°C. Of the sterilization treatments, one was applied to each of the temperature treatments with the remaining three bolts undergoing no sterilization. This made for eight unique treatment combinations with one replicate of the unsterilized, 30°C temperature treatment.

When the time came to infest, bolts were placed inside rectangular, 91 cm x 38 cm x 38 cm mesh butterfly cages with a plastic bottom portion. Cages used in both surface-sterilized and unsterilized treatments were sprayed down with the same sterilization solution for SPB as part of the initial cleaning of the equipment. All cages were given one hour to air dry before any plant or animal material was placed inside. Cages were situated inside a trio of incubators set at 25º or 30º C. Experiments were completed over the course of December 2009 to February 2010. Incubators were designated as surface-sterilized or unsterilized and housed only those respective treatments so as to avoid cross-contamination of our samples. 30 cm x 22 cm bolts were cut from fresh, uninfested loblolly with white pine bolts measuring 30 cm x 18 cm. Bolts were covered on the ends with melted paraffin wax to prevent desiccation and placed inside the cages. Fifty unsexed SPB, surface-sterilized or unsterilized depending on treatments, were placed in each cage and given 24 hours to attack. After 24 hours an additional 25 beetles were added to each cage. This second pulse of beetles was intended to mimic aggregation somewhat as well as provide additional mating pairs and combat natural losses occurring in the cages. For the first 72 hours of each infestation, a packet

containing the SPB aggregation pheromone mixture Frontalure® was attached to the upper-middle portion of each bolt with a pushpin. Bolts remained in the incubators and were observed daily for a period of 35 days at which point they were removed and destructively sampled for relevant biological parameters.

The number of attacks, number of galleries per attack, length of the main gallery, number of egg niches along each main gallery, proportional hatch rate, life stage present, and length of the larval gallery were recorded for each sample. An attack was defined by the presence of a "shothole," or small hole in the outer bark consistent with SPB gallery boring. Parameters that required measurement in terms of distance were obtained using small, metric, measuring wheel in combination with a small measuring tape. Each gallery was identified and marked initially then viewed under a stereoscope to confirm gallery identity. Measurements on number of egg niches, larval gallery length, and number attacks were made using the stereoscope as well. Life stage categories observed included egg, larval, pupal and adult.

Statistical Analysis. Results were split into three categories, egg gallery data, larval gallery data, and hatch data. All data was analyzed using SAS 9.2 (SAS Institute, 2008). Egg gallery data was square-root transformed and analyzed using a three-way ANOVA with the PROC MIXED procedure (SAS Institute, 2008). LSMEANS estimates were generated for all treatment combinations and were used to look for significant differences. Larval gallery length data was square-root transformed and analyzed using a three-way ANOVA with the PROC MIXED procedure (SAS Institute, 2008). LSMEANS

estimates were generated and used to analyze main effects and significant interactions. Proportional hatch data were left untransformed and analyzed as a binary/Bernoulli model looking for responses on the logit scale with the PROC LOGISTIC procedure (SAS Institute, 2008). Results from this procedure illuminated the effect of the three-way interaction of host, temperature, and sterilization treatment that we termed the "random beetle effect." The random beetle effect refers specifically to the impact of a the aforementioned factors on the attack initiation, gallery construction, and oviposition of a single female SPB. In this manner it helps to account for some of the randomness of an individual beetle's choices. Once the magnitude of the random beetle effect was determined it was integrated into the PROC MIXED procedure in terms of probabilities (SAS Institute, 2008). LSMEANS estimates for all main effects and treatment combinations were generated and used to look for significant differences.

RESULTS

Approximately 1,000 SPB adults were utilized in this experiment and only 53 adult exit holes were recorded across all treatments. Survival of SPB was very low across all treatment combinations, with an average of less than one SPB adult successfully developing and emerging from any given egg gallery (Table 2.1). It was consistently found that there were more egg niches in a given egg gallery than larval galleries (Table 2.1). On the whole, more larvae were found in a given egg gallery than pupae and slightly more pupae were found in a given egg gallery than adults (Table 2.1). In some cases the number of attacks and the number of egg galleries on a bolt differed.

This indicates either that some SPB used the same attack holes to build their egg galleries, or that the same SPB built galleries in different directions from the same attack (Table 2.1). It is impossible to tell which occurred in these instances. The average number of eggs per millimeter of egg gallery varied from 0.083 to 0.255 (Table 2.1).

SPB egg galleries were significantly longer in loblolly pine than white pine ($F=41.25$ $df=117$, $p<0.0001$). SPB galleries in loblolly pine were almost twice as long, on average, than in white pine (Figure 2.3). Egg gallery length differed significantly between sterilization treatments ($F=6.38$ $df=117$, $p=0.0129$) with longer galleries being excavated in the surface sterilized treatment than the unsterilized treatment (Figure 2.4). There was no significant difference observed between the 25°C treatment and the 30°C treatment ($F=0.34$, $df=117$, $p=.5605$) though gallery length was slightly longer in the 30°C treatment (Figure 2.5). At 225.50mm, the longest average egg galleries were found in the surface sterilized loblolly pine treatment (Table 2.1). Egg galleries in unsterilized loblolly pine averaged 160.85mm compared to 94.33mm for unsterilized white pine and 99.87mm for surface sterilized white pine (Table 2.1). All treatment combinations were found to be significantly different from each other with the lone exception of unsterilized versus sterilized white pine (Table 2.2). The largest differences were observed between sterilized loblolly pine and both sterilized and unsterilized white pine (Table 2.2, Figure 2.6).

In analyzing the larval gallery data, the length of the gallery after hatch as well as the number of galleries successfully ending in an exit hole are the most important factors. In terms of larval gallery length there was a significant interaction between host type and

treatment but no other interactions were significant (Table 2.3). Thus it is most important to compare treatment combinations. The sterilization treatment appeared to only affect SPB in loblolly pine, resulting in significantly longer galleries for the sterilized treatment compared to the unsterilized treatment ($t=-3.26$ $df=86$, $p=0.0016$, Figure 2.7). There was no significant difference between sterilization treatments in white pine ($t=1.16$ $df=86$, $p=0.2503$, Figure 2.7). A key difference in larval gallery length arises when comparing the length of larval galleries based on the success or failure of SPB development. A larval gallery that ultimately ended in successful SPB development was an average of 6mm, or 85%, shorter than an unsuccessful gallery ($F=57.02$ $df=1032$, $p<0.0001$, Figure 2.8).

Host type had the largest effect on the ratio of successful egg hatch to unsuccessful, or aborted, SPB eggs ($F=74.17$ $df=98$, $p<0.0001$, Figure 2.9). An SPB egg laid in loblolly pine was 34.32% more likely to successfully hatch than in white pine ($t=8.61$ $df=98$, $p<0.0001$). The effect of the sterilization treatment was smaller but still significant at $\alpha=0.05$ ($t=4.80$ $df=98$, $p=0.0309$, Figure 2.10). Though the results are counterintuitive, the unsterilized treatments were 8.72% more likely to successfully hatch than the sterilized treatments ($t=2.19$ $df=98$, $p=0.0309$). The 5 °C difference in temperature between treatments had the smallest, yet still significant, effect on successful proportional hatch ($F=4.08$ $df=98$, $p=0.0461$, Figure 2.11). An SPB egg laid at 25°C was 8.05% less likely to hatch than an egg laid at 30°C ($t=-2.02$ $df=98$, $p=0.0461$). A total of 144 SPB egg galleries were observed along with 2683 total egg niches and 1127 total larval galleries. Loblolly pine accounted for 89 total attacks and 1752 egg niches with 44 attacks and 931egg niches found in white pine.

DISCUSSION

There were significant differences between white pine and loblolly pine on several important parameters of SPB development. The hypothesis that SPB develop similarly in both hosts is at least partially rejected based on evidence from this experiment. The overall survival of SPB was very low across all treatment combinations so it is difficult to say if these differences represent a difference in the suitability of a particular host tree. The low survival of SPB might be attributable in part to the very small populations found in the field over the research period. High levels of parasitism, predation, and conspecific mutilation in the laboratory colony most likely contributed to the low quality of our experimental SPB population. Yet looking more closely at the chosen developmental parameters reveals that the life and fate of an individual SPB does depend, in part, on the host it chooses.

The most difficult part of this experiment was attaining enough SPB to infest a significant portion of host material. Despite many setbacks, enough field-infested SPB material was found in 2009-2010 to allow for this experiment. Additional SPB developmental experiments were attempted using a phloem-sandwich system for SPB development, gel-capsule based infestation of small, intact bark islands, and infestation of one-meter bolts using an analogue to the lard-can method from Wagner (1984). Insufficient amounts of healthy SPB prevented each of these methods from producing viable results, though important, tactile information about SPB behavior was gained that eventually led to an improved trapping and capture method (Gardner Thesis ch. 1).

Ultimately this experiment considers the effects of temperature, surface sterilization, and host type on the development of SPB. In terms of magnitude of these effects it seems that temperature is the smallest. This is not unexpected due to the wide developmental temperature range of SPB (5 °C to 31.5 °C) compared to the restricted range chosen in this experiment (25 °C or 30 °C). This range was chosen because it represents the ideal developmental temperature range for SPB in the field that helped insure useful results despite a limited supply of SPB (Wagner 1984). The 5 °C difference in temperature treatment did not have a significant effect on main gallery lengths in either host, but did show a slight effect on proportional hatch. At the higher temperature treatment an SPB egg can be expected to eclose about 8% more often than at 5°C lower. The SPB developmental rate over a range of temperatures from 5 °C to 31.5 °C shows the rate rise with temperature up to 30 °C were it begins to drop. Larger differences in developmental rate and other parameters are expected between a wider range of temperatures, but the difference between 25 °C and 30 °C is much smaller.

The sterilization treatment resulted in longer average main galleries for sterilized SPB versus unsterilized. A sterilized beetle enjoyed an 8% higher success rate in terms of proportional hatch. It is possible that this effect arises due to the removal or delay of blue-stain fungi activity in the host. The effect of the sterilization treatment was slightly larger than that of temperature but still quite small overall and compared to host effects. Blue-stain fungus was observed in every bolt used in this experiment but was often only just beginning to grow at the time of sampling. At the same time, all of the SPB egg and most of the larval galleries were lined with other (presumably mycangial) fungi as well.

The blue-stain fungus was not prevalent enough at the time of sampling, even in the unsterilized treatments, to warrant the cataloguing of its location and potential difference in SPB developmental parameters within these areas. Blue-stain's mere presence, however, implies that the surface sterilization technique does not provide complete fungal control. Yet the treatment ostensibly did not remove the mycangial fungi, making SPB development in this system, in terms of fungal interactions, not dissimilar from SPB in the field. Future research in this area should include the plating and identification of all fungal species and a comparison between host types and temperatures in order to clarify the fungal interactions.

Difference in host type had the largest effect on all SPB development parameters measured across the board. In terms of average egg gallery length, an SPB adult excavated almost twice as long a gallery in loblolly pine than in white pine. The visual result of this was that larval galleries in white pine appeared closer together than in loblolly pine. Gallery appearance in the loblolly pine treatments all appeared similar to the field collected loblolly pine, with long, s-shaped main galleries punctuated by shorter, larval galleries arising perpendicularly to the main gallery (Figure 2.12). In white pine main galleries were often much straighter and shorter with a crowded appearance and larval galleries arising in very close proximity to each other (Figure 2.13). Further analysis of larval gallery shape and surface area would be useful to tease out the structural difference in galleries between loblolly and white pine. Main gallery length is important in that it provides larvae with adequate food in the form of phloem surface area and safety in distance from conspecific larvae. Beyond the question of food volume is the

problem of conspecific mutilation in SPB. In several years of capture and research, mutilation of SPB by conspecifics in trapping and experimental situations proved to be one of the toughest obstacles to overcome. Shorter, more crowded galleries could lead to more interaction between conspecifics, increasing the risk of mutilation.

In terms of the effect of host type on larval gallery length, the results are more complex. At the $\alpha=0.05$ level there was a significant interaction between host type and treatment which made it difficult to isolate the magnitude of either. There was a significant difference in larval gallery length between unsterilized and sterilized loblolly pine. Larval galleries were longer in the sterilized treatment than in the unsterilized. The larvae of a sterilized SPB in loblolly pine excavated about 3mm more gallery for an average of 11.92 mm larval gallery length. This could indicate the potentially lower nutritional value of host material in the sterilized treatments, some degree of loss in mycangial fungi of the sterilized adult SPB, or perhaps some latent, anti-fungal activity of surface residue from the sterilization solution. In white pine the larval galleries were slightly, but not significantly shorter in the sterilized treatments.

Unsuccessful larval gallery lengths remained within a 3mm range of each other across all treatment combinations. The average unsuccessful larval gallery length across treatment combinations were between 1.36mm and 4.31mm longer than the average successful larval gallery (Figure 2.7, 2.8). This supports the hypothesis that unsuccessful SPB tend to excavate longer larval galleries (Thatcher *et al.* 1981). Published average larval gallery lengths for successful SPB are around 6 mm to 7 mm (Wagner 1984). In this study the average length of a successful SPB larval gallery was 7.6 mm while

unsuccessful galleries averages over 13 mm in length. The length of an average larval gallery indicates that all SPB had difficulty developing across all treatment combinations.

SPB for these experiments were very difficult to find and often involved travel to multiple states and locations within them. The difficulty in obtaining SPB for research was highlighted at a recent forest entomology conference (Coulson and Stephen 2009). Because there were no major outbreaks during any of our research seasons, the SPB we obtained were not at their full outbreak population density and could be considered weaker-looking and smaller in size than typical SPB (Fred Hain, personal communication). Despite the overall condition of the SPB population when we made our field material collection, all our SPB came from the same tree in a localized outbreak affecting several acres. Weaker SPB could be responsible for the low survivorship in our experiments. It is difficult to account for the effect of a smaller and weaker population of SPB without information on average SPB size and weight across generations. This kind of information would be integral to further research because it could help to explain the characteristics of the current field SPB population and could be compared between hosts and across temperature ranges.

The presence of significant differences in successful proportional hatch, wherein an SPB egg hatches successfully more than a 1/3 of the time in white pine compared to slightly more than 2/3 of the time in loblolly pine, show that host type has a major impact on SPB life stages from egg to larvae. The impact of host type on adult SPB can be seen in the shorter egg galleries with less overall eggs in white pine. Even though the shorter galleries are coupled with an decrease in egg density, the lower number of eggs overall

could represent a lower chance of an SPB population reaching outbreak density in white pine (Figure 2.6, Table 2.1). Populations of SPB are known to experience an alee effect in terms of attacking and overcoming a host tree (Coulson 1977). This highlights the importance, to SPB, of reaching the necessary population density to release from the control of host plant resistance. Though further research is needed to confirm the shorter main galleries and fewer egg niches in white pine, adult SPB appear to perform worse in white pine than in loblolly pine.

In the loblolly pine treatments the successful proportional hatch was over 70%, egg galleries were almost twice as long on average, and more eggs were laid overall. This relative level of success can be explained by the fact that loblolly pine is one of the two preferred hosts in the natural range of SPB. This differential success between hosts could point to some form of host resistance on the part of white pine. The low successful proportional hatch could indicate some sort of natural resistance to SPB, or the bark beetle guild in general, in white pine. This should be studied further to determine the potential mechanisms be they chemical, physiological, induced, constitutive, present, or absent. Shorter average main galleries in white pine reveals a profound impact on the adult SPB activity. From a near abandonment of classic SPB gallery shape to a lower egg density within, main galleries in white pine appear markedly different with these differences borne out through this experiment. This indicates the potential for host resistance to the adult stage of SPB in white pine. Since there was no obvious difference in survivorship, the suitability of white pine as a host for SPB becomes a difficult question to answer.

The overall trend in SPB development parameters in white pine is a downward one when compared to loblolly pine. In every category the average for each parameter was lower for white pine than loblolly pine, and significantly so for all parameters but larval gallery length. In all the treatment combinations, the host effect was always strongest and carried the most explanatory power. From this experiment it appears as if SPB perform differently, and typically worse in white pine than in loblolly pine, but this should be examined through more replication and controlled infestation on different host types. In future experiments it would be useful to determine the reemergence ratio in this particular laboratory system so that it could be compared to known reemergence rates in the field and other systems. Better understanding this parameter could give a clearer picture of the differences between field populations and this laboratory system, which could aid in illuminating the results of this experiment.

Though there is still much to learn about the development of SPB in white pine, this experiment reveals some key differences in SPB behavior between it and the traditional host loblolly pine. The impetus for this experiment came from field observations of SPB attacking and killing white pine. Thus the question was not whether or not SPB could survive in white pine, but rather how well do they survive and how likely are they to reach outbreak density? This experiment has shown that SPB does not survive at the same rate or in the same way in white pine as it does in loblolly, though more exhaustive experimentation is needed to highlight and understand these differences.

Figure 2.1 An example of loblolly pine bolts in a rearing room, note the fluorescent light behind the bolts and the SPB pheromone lure attached to the center bolt

Figure 2.2 An example of field-infested bolts (right side of image) in a rearing room across from uninfested bolts (left side of image)

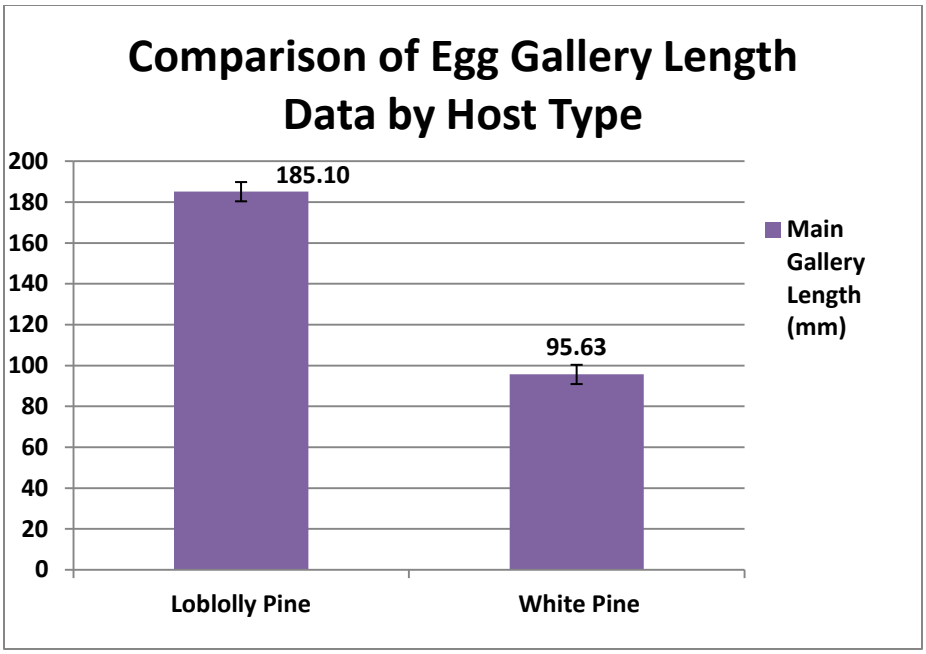

Figure 2.3 SPB egg gallery length, averaged across treatments combinations (n=9), between loblolly pine and white pine. (F=41.25 df=117, p<0.0001)

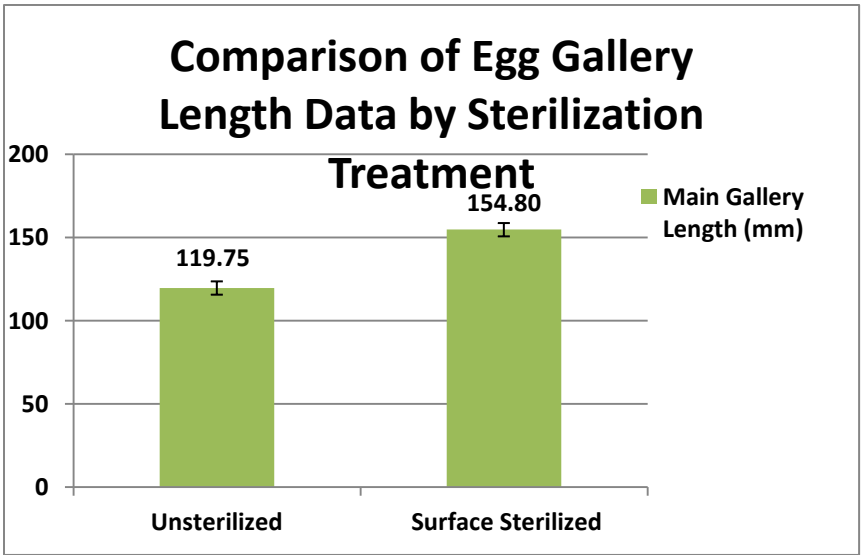

Figure 2.4 Average SPB egg gallery length, averaged across treatments combinations (n=9), between surface sterilized and unsterilized treatments. Results are significant at α=0.5 (F=6.38 df=117, p=0.0129)

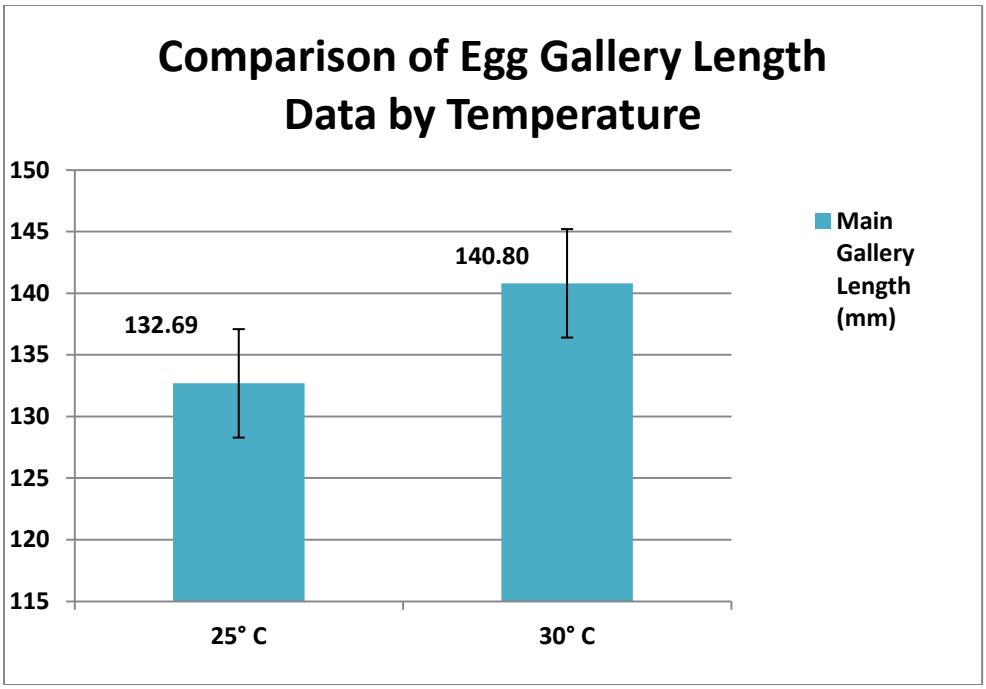

Figure 2.5 Average SPB egg gallery lengths, averaged across treatments combinations (n=9), between temperature treatments. Results were not significant (F=0.34, df=117, p=.5605)

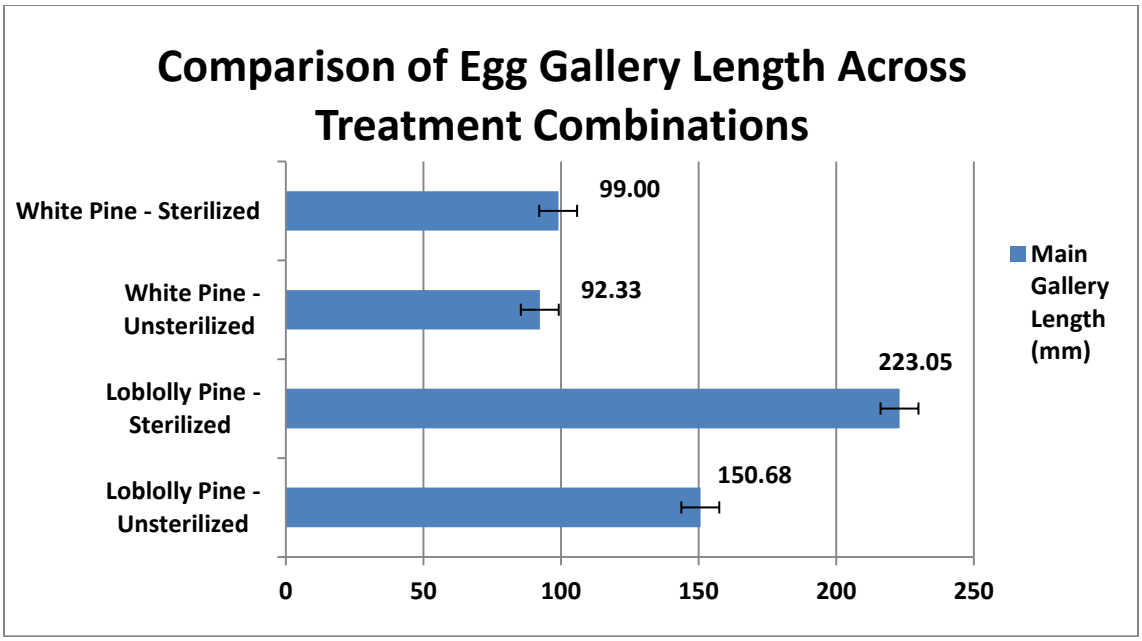

Figure 2.6 Average SPB egg gallery length between host and sterilization treatment combinations (Statistical differences for these combinations are found in Table 2.3). Temperature was left out of the combination due to its insignificant effect.

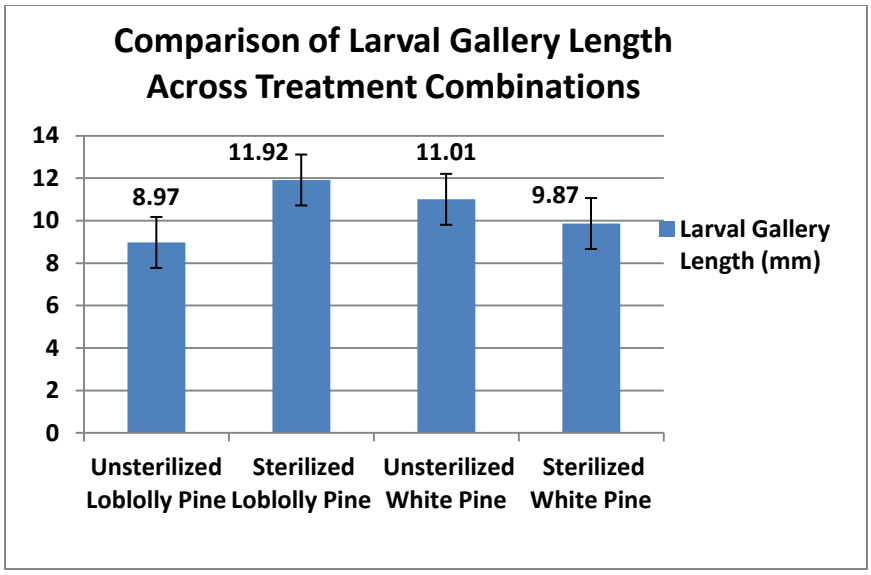

Figure 2.7 Average SPB larval gallery length between host and sterilization treatment combinations. The only significant difference in average length is found between the sterilized and unsterilized loblolly pine combinations (t=-3.26 df=86, p=0.0016)

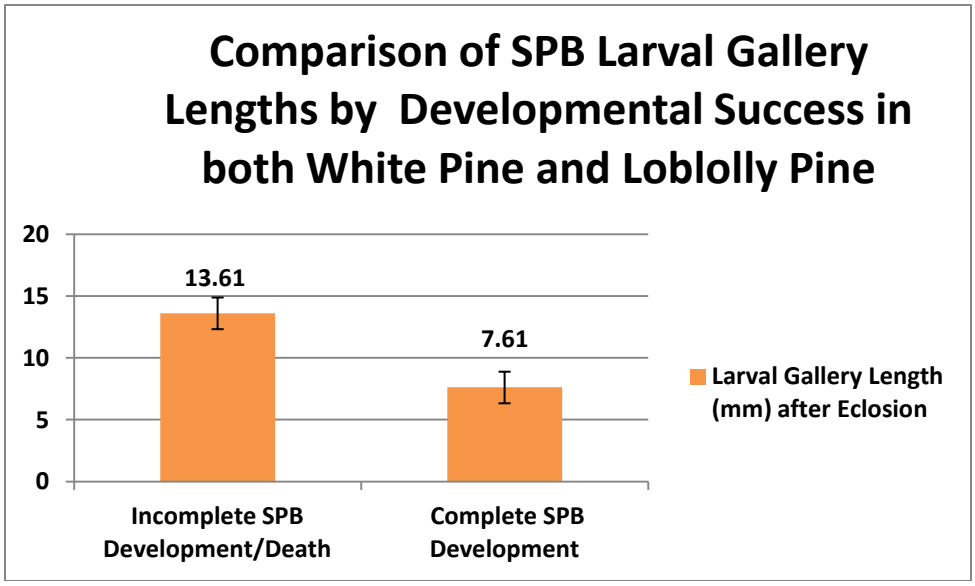

Figure 2.8 Average SPB larval gallery lengths, averaged across treatments combinations (n=9), between a successful and unsuccessful SPB (F=57.02 df=1032, p<0.0001)

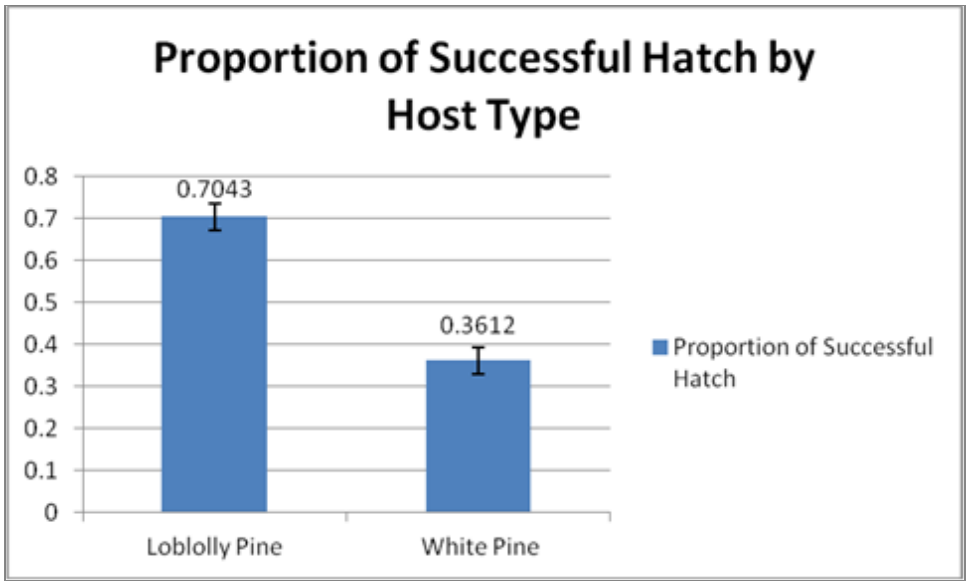

Figure 2.9 Proportion of successful, averaged across all species, between loblolly and white pine (t=8.61 df=98, p<0.0001)

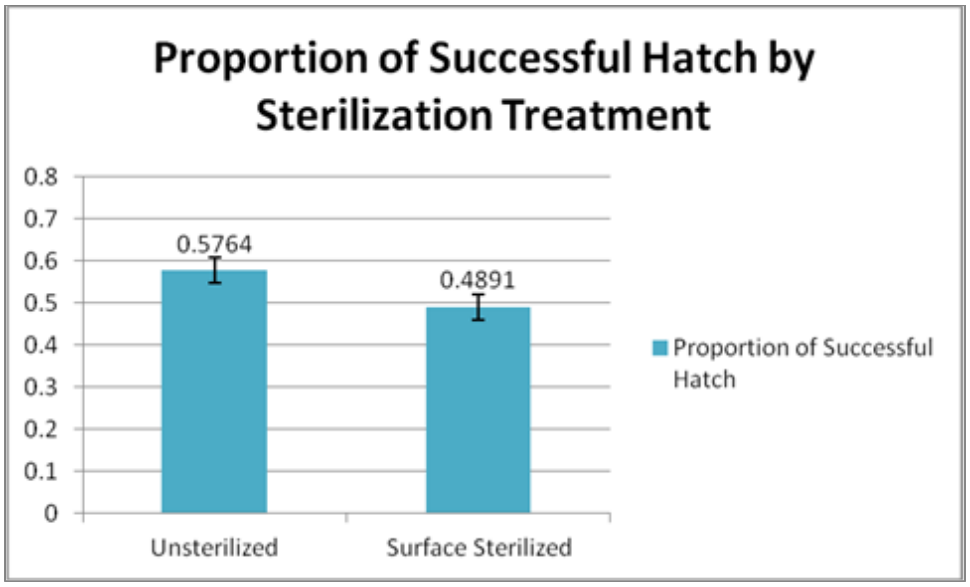

Figure 2.10 Proportion of successful hatch, averaged across all species, between unsterilized and sterilized treatments (t=4.80 df=98, p=0.309)

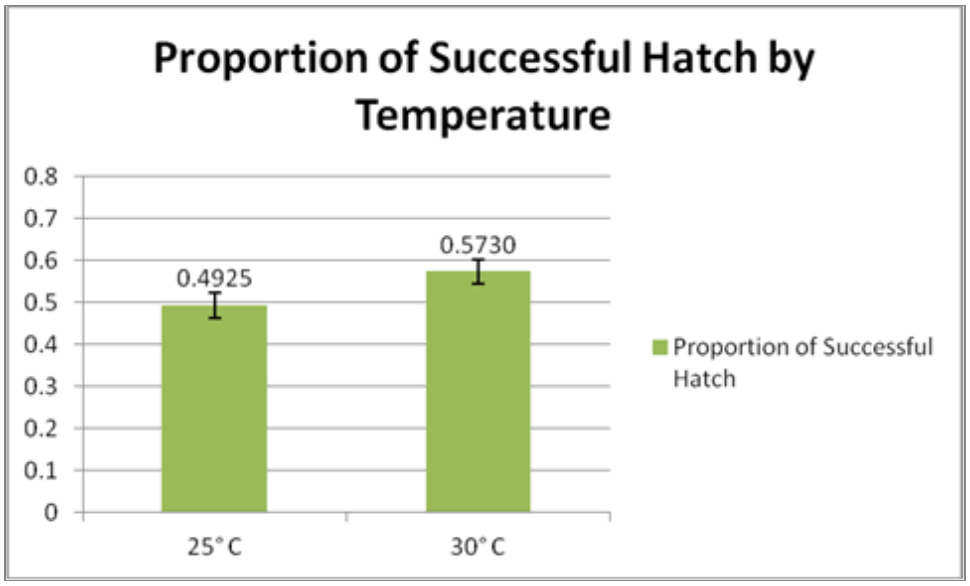

Figure 2.11 Proportion of successful, averaged across all species, between temperature treatments (t=4.80 df=98, p=0.309)

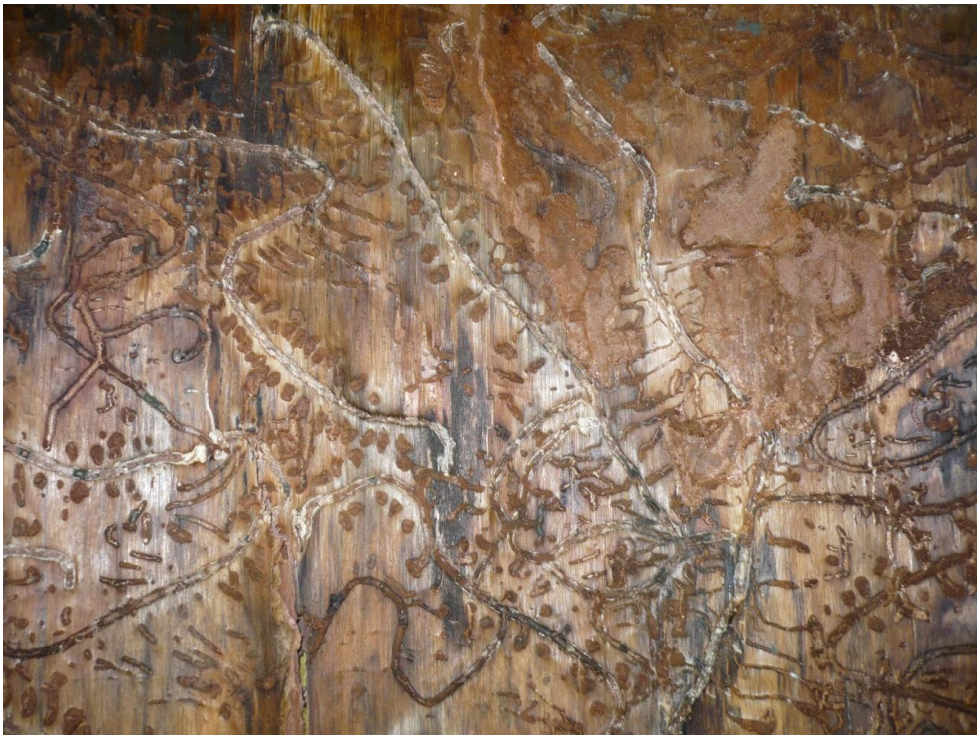

Figure 2.12 Typical SPB egg galleries in loblolly pine

Figure 2.13 Typical SPB egg galleries in white pine

Table 2.1 Attack, egg gallery, and data on survival of each life stage across all treatments

	Loblolly, Unsterilized 25°C	Loblolly, Unsterilized 30°C - A	Loblolly, Unsterilized 30°C - B	Loblolly, Sterilized 25°C	Loblolly, Sterilized 30°C	White, Unsterilized 25°C	White, Unsterilized 30°C - A	White, Unsterilized 30°C - B	White, Sterilized 25°C	White, Sterilized 30°C
# Successful Attacks	26	23	14	12	8	5	6	19	7	7
# Egg Galleries	27	24	14	12	8	5	12	20	9	7
Avg. Egg Gallery Length (mm)	144.5 +/- 60.9	164.3 +/- 74.1	184.1 +/- 59.1	221.7 +/- 112.7	231.3 +/- 69.4	106.8 +/- 39.63	113.8 +/- 39.9	80.9 +/- 49.0	93.1 +/- 26.6	108.6 +/- 38.4
Avg. # Eggs/Egg Gallery	14.66	26.29	15.29	27.16	23.13	21.8	11.58	14.15	22.77	27.71
Avg. # Eggs/mm of Egg Gallery	0.101	0.16	0.083	0.148	0.1	0.204	0.102	0.175	0.245	0.255
# Larvae/Egg Gallery	3.48	5.58	5.58	12.67	9.75	14.8	5.16	9.7	17.1	18.86
# Pupae/Egg Gallery	0.52	0.79	0.64	0.83	1	1	1.08	0.65	0.88	0.85
# Adults/Egg Gallery	0.48	0.79	0.64	0.67	1	1	1	0.65	0.77	0.85

Table 2.2 Average SPB egg gallery lengths in each of the major treatment combinations. Temperature effect discarded due to insignificance.

	N	Length in (mm)
Loblolly Pine - Unsterilized	**69**	**160.855**
Loblolly Pine - Sterilized	**20**	**225.5**
White Pine - Unsterilized	**39**	**94.333**
White Pine - Sterilized	**16**	**99.875**

Table 2.3 Average SPB Egg gallery lengths between host and sterilization treatment combinations.

	Difference (mm)	df	t value	Pr > |t|
Loblolly Unsterilized v. Loblolly Sterilized	**-7.07**	**117**	**-3.68**	**p=0.0004**
Loblolly Unsterilized v. White Pine Unsterilized	**7.11**	**117**	**3.68**	**p=0.0004**
Loblolly Unsterilized v. White Pine Sterilized	**5.41**	**117**	**3.04**	**p=0.0030**
Loblolly Sterilized v. White Pine Unsterilized	**28.36**	**117**	**5.87**	**p<0.0001**
Loblolly Sterilized v. White Pine Sterilized	**24.85**	**117**	**5.3**	**p<0.0001**
White Pine Unsterilized v. White Pine Sterilized	**0.16**	**117**	**-0.36**	**p=0.7186**

Table 2.4 Host x Sterilization Treatment interaction in terms of explaining the differences in average SPB larval gallery lengths. Other factors and combinations listed are not significant.

	F value	**df**	**Pr > F**
Host x Sterilization Treatment	**8.94**	**86**	**p=0.0036**
Temperature	**2.45**	**86**	**p=0.1213**
Sterilization Treatment x Temperature	**1.13**	**86**	**p=0.2918**
Host x Temperature	**1.21**	**86**	**p=0.2742**

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DISCUSSION

The primary difficulty with this project lies in the fact that it was contingent upon locating, capturing, and rearing large numbers of SPB. It is expected that there will always be a certain amount of trials and tribulations in dealing with live animals as research subjects. Insects often have very strict temperature, humidity, dietary, and habitat requirements. SPB is no exception in that it is only active during the warmer months of the year and lives and feeds within the vascular cambium of its host. It requires that the vascular cambium be moist enough to support the growth of beneficial mycangial fungi, prevent the desiccation of SPB eggs, and maintain high enough temperatures to support SPB growth and development. These requirements are difficult to meet outside of a host tree and are the major reason that there is no known artificial diet for SPB. The lack of an artificial diet means that the SPB must be retrieved from the field by extracting whole bolts of an infested host tree. This leaves the SPB researcher with little direct control over the internal conditions of the bolts, and thus over the rearing system of SPB. There are many measures taken throughout these experiments which attempt to compensate for this lack of control, yet the difficulty remained and will be detailed later in this section. Initially it was expected that these rearing system difficulties would be the toughest to overcome but, in time, the location and capture of SPB from the field proved to be the most vexing problem.

SPB Location and Capture

During the initial research season of 2007 a scant 400-500 total SPB was all that emerged from field-collected bolts. These bolts were extracted from a site in the eastern Georgia piedmont that had suffered a multiple hectare outbreak of SPB in the previous year. Despite the large-scale damage to the site by SPB in the past summer, the removal of infested material had left most of the site barren with only a few disparate trees supporting SPB by the time we arrived. SPB-infested loblolly pines were removed on two occasions with the first removal producing the lion's share of SPB captured that season. Contacts throughout the southeastern U.S. including state and USFS employees, the Army Corps of Engineers, and numerous other scouts and foresters were utilized in order to locate any SPB spot known to exist at the time. The first research season would prove to be a model for the following two seasons with many more calls and contacts made than actual SPB spots located.

In the summer of 2008 there were more SPB spots located, but still relatively few useable individuals captured. In total around 1000 useable SPB were extracted from three different sites. The first site surveyed was across the Virginia border near Kerr Lake. A small SPB spot was located on a slope near the water and served to supply five SPB-infested host trees which were removed over the course of two visits. Unfortunately the majority of these trees had clearly been infested for quite awhile as evinced by the extreme chlorosis (nearly red in some) of their foliage. This means that the cambium was drier due to the prolonged infestation, and very few SPB emerged as a result. A single

trip to the Francis Marion national forest in South Carolina was also completed during the summer of 2008. Despite torrential downpour and one of the most physically demanding removal processes to date, almost zero useable SPB emerged from the seven extracted host trees. This could be due to the fact that foresters in the Francis Marion had already (despite encouragement not to) cut and stacked the entire spot by the time we arrived.

Later in the summer of 2008 an additional SPB spot was located near Eatonton, Georgia. This spot was actually composed of several smaller spots spread throughout a large stand of mostly pulpwood sized loblolly pines. More than twenty five infested trees were removed over the course of three visits. Those trees provided the majority of SPB utilized in our failed phloem sandwich, bark-island, and gel-cap infestation experiments which will be detailed later in this section.

The third research season in the summer of 2009 yielded the most useable SPB, largest active spots, and resulted in the generation of the data detailed in the previous section of this thesis. An active SPB spot was located in the Paulding forest in Paulding County, Georgia during the later portion of the research season. Prior to the location of this spot there was a very real possibility of attempting these experiments on *Ips* bark beetles instead of SPB. A trip was made to Hyde County, North Carolina and about ten *Ips*-infested loblolly pines were removed. Thankfully the SPB spot was found shortly thereafter and before any major experiments with *Ips* were undertaken. Initially the Paulding County spot consisted of 50-100 very large and heavily infested loblolly pines.

The first of three extractions included two trees, each of which was nearly 100 years in age. By the second trip to the Paulding forest the first spot had been cut and removed, but an additional spot had developed nearby which included trees of various ages. Parts of five different trees were removed on the second trip and parts of seven different trees on the third and final trip. Throughout all three research seasons there were no SPB spots found in North Carolina.

SPB Rearing

During the first research season all rearing of SPB took place in the Insectary at the Biological Resources building at NCSU. The rearing room was held at 24°-26° C with humidity set at 70% and a 24 hr. dark cycle. The reasoning behind the 24 hr. dark cycle was twofold. Firstly since SPB develop in the darkness of the vascular cambium of their host there was no need to provide them with any light during development. SPB is known to exhibit phototaxis and thus the installation of several fluorescent light fixtures behind the uninfested bolts served to draw emerging SPB to a designated area. This made the task of locating emerging beetles much simpler. Unfortunately the use of fluorescent bulbs as SPB attractants led to some unforeseen problems. SPB congregated closely to the lights, and when emergence was at its peak they were observed to engage in conspecific mutilation and cannibalism. When SPB get in close quarters with one another they tend to attack each other and chew off tarsi. The upshot of this is that many SPB were found to be missing several to all of their tarsi which rendered them useless for

experimentation. It was very difficult to counteract this behavior, and often the only way to deal with it was to remove SPB as quickly as possible.

The rearing room in the insectary was composed of several large metal shelving units placed along the wall to the right and left of the entrance. On the left wall the field-infested bolts were placed vertically against the wall. Often times there were over 20 bolts on the infested side. Whenever new infested material was brought in to the insectary, the older material was moved to the back of the metal shelving unit with the newer bolts placed in front. Infested bolts were kept in the rearing room for a maximum of three months in order to allow for complete emergence. This method was employed in an effort to extract the maximum number of SPB from the field. If SPB had been easier to find the rotation of infested material would have occurred on a monthly basis. Keeping infested material in the rearing room for three months could have been responsible for some of the difficulties encountered in the rearing process. It is possible that the older infested material served as a reservoir for SPB predators, competitors, and parasitoids that were already present in the field. It is also possible that they served as a reservoir for Blue-stain fungus within the rearing room. Future SPB rearing attempts should limit the length of time infested material is stored in the rearing room.

On the right wall of the rearing room the uninfested bolts of loblolly pine were housed. Upon arrival the end of each bolt was painted with melted paraffin wax mixed with water. The goal of this was to prevent desiccation. Typically 10-15 bolts were

harvested from the Schenck forest and arranged in a line on the metal shelves. A small tube of the SPB aggregation pheromone Frontalure® was placed on the middle bolt using a thumb tack. SPB readily attacked the uninfested bolts throughout all three research seasons.

During the second and third research seasons an additional rearing room was established in the basement of Grinnells Laboratories at NCSU. All overhead fluorescent lights were removed and the entrance to the room was covered in thick construction paper to block out as much light as possible. This was done in an attempt to simulate the conditions in the insectary rearing room. There was no way of maintaining consistent humidity in the rearing room, but the temperature was held at 22°-26°C throughout the rearing period. The arrangement of infested and uninfested bolts mirrored that of the insectary and included fluorescent lighting behind the uninfested bolts as well as Frontalure® packets attached in the middle of the line of uninfested bolts. The rearing room in Grinnells was split down the middle by a large tarp which covered floor to ceiling. The rearing portion was located on the right side of the room with SPB traps located on the left side. The goal of the tarp was not to prevent migration of SPB or predators from one side to the other (which surely occurred), but to block light from the trapping side from the rearing side. The design and use of the SPB traps will be covered later.

The ultimate goal or our rearing methods was to collect field-infested specimens once per season and simply rotate the field-infested bolts out as the uninfested bolts became infested. In this manner we had hoped to maintain a continuous SPB colony that would provide individuals for experimentation throughout the year. This was never accomplished as levels of infestation on new material from the Schenck never reached the levels found in field infested material. There was no choice but to continue to harvest infested material from the field and attempt to capture and utilize as many SPB as we could from there. The reasons for the failure of the rearing system are myriad and complex but worth exploring.

One of the main problems encountered in the rearing rooms was the presence of Clerid beetles (*Thanasimus dubius*), numerous Brachonid parasitoids, and the presence of the Southern Pine Sawyer (*Monochamous titilator*). All of these insects represent a threat to the existence of the SPB colony. Throughout all three research seasons there was a consistent population of *T. dubius* across the Southeastern U.S. In speaking with the foresters responsible for SPB monitoring and trapping from South Carolina, Tennessee, Georgia, Texas, North Carolina, Alabama, and Mississippi, a trend emerged. Each season the number of *T. dubius* captured in their monitoring traps consistently outnumbered SPB trap catches (Ron Billings, personal communication). In 2008 and 2009 there wasn't a single SPB found in the monitoring traps in North Carolina, Tennessee, or Texas. The presence of large numbers of *T. dubius* represents the largest threat to SPB populations as an adult Clerid is known to consume over 100 SPB in the course of its lifetime. In an

attempt to deal with this problem, the rearing rooms were monitored daily for the presence of *T. dubius* and any individuals found were removed and destroyed. The fact remains that these predators also live in the vascular cambium and despite our best efforts to eradicate them, they were an ever-present problem in both rearing rooms. During this daily monitoring period an aspirator was utilized in the removal of any visible Brachonid parasitoids. As with the Clerids they were ever-present despite our efforts. Locating *M. titilator* was much easier than locating the Clerids or Brachonids because their movement and audible feeding . Unfortunately they also represent the least viable threat to SPB populations and problems with the colony continued despite the removal of any *M. titilator* individuals heard to be feeding.

Another rearing issue that bears mention is the presence of Blue-stain fungi from field-infested material. Blue-stain was found to be present in every bolt that was extracted and brought back to the lab. Since mycangial fungi are very important to the growth and development of SPB, any attempt to sterilize field-infested or uninfested bolts would result in the removal of both beneficial and detrimental fungi. This is to say nothing for the fact that complete sterilization of field-infested bolts is impossible to accomplish without disturbing or killing the SPB inside. It is quite possible that spores of Blue-stain pervaded each rearing room and some of the field-infested bolts became inoculated after being retrieved. It is also possible that the SPB itself, or any of their phoretic mites, transferred Blue-stain spores around the rooms and between infested and uninfested bolts. Regardless of the method of transmission, Blue-stain was an ever-present problem in the

rearing rooms and in every iteration of our experiments. This is part of the reason that the surface sterilization of SPB was attempted during the developmental experiments. A major shortcoming in these experiments was the lack of isolation and identification of fungi within the rearing rooms, experimental units, and on the SPB themselves. Any future work on SPB rearing should include a thorough analysis of any fungal or bacterial growth found within the colony or experimental units. Obtaining this information could have provided additional insights into the failure of our rearing methods.

An additional concern with these experiments was the quality of the field SPB population as a whole. Firstly since SPB were collected from three different states, and multiple sites within Georgia, it is likely that they differ somewhat in terms of genetics. Since no population genetics experiments were undertaken, it is impossible to know what, if any, genetic differences there were between populations and what, if any, effect they had on the success of rearing and experimental attempts. Secondly many SPB that were collected appeared exceedingly small in size compared to the classic SPB-size metric of a grain of rice. It was noted during each season that the SPB captured appeared smaller and weaker than SPB collected in the past (Fred Hain, personal communication). Unfortunately a major shortcoming of this project is that no systematic study of captured SPB size and weight was undertaken and thus no information is available on this subject. Any future research on SPB development should include data on SPB weight and length as this may help to determine if the current SPB population across the Southeast is

smaller and/or weaker than the SPB of the 1970's-2005 as well as highlighting any differences between local SPB populations.

Failed SPB Development Experiments

The initial research plan for this project included three different types of developmental experiments over five different temperatures from 10°-30°C. The aforementioned lack of SPB across all research seasons coupled with the failure of the rearing system resulted in a shift to smaller scale experiments that used less SPB. This occurred out of a desire to maximize the potential data collection while minimizing the number of required SPB. One of the first types of experiments attempted is referred to as the "bark island" experiments. The method for these experiments involved cutting wafers of loblolly and white pine with an average thickness of three inches (Figure 3.1, 3.2). Five wafers of both loblolly and white pine were utilized in the first round of experiments with an additional five of each species in the second, and final, round. Uninfested bolts selected for these experiments were matched according to circumference (range of diameters from 7 in. to 9 in.) so as to control for difference in total surface area of the vascular cambium. Each wafer included between 10 and 14 vertical cuts made with a hacksaw which penetrated from the outer bark down to the xylem. These vertical cuts were spaced approximately two inches apart around the entirety of the wafers. The result of these cuts was the formation of several "islands" of bark and vascular cambium (Figure 3.3, 3.4, 3.5). These wafers were placed in plastic containers into which twenty unsexed SPB were added. These containers were placed into incubators which were held

at 25°C or 30°C respectively. The wafers were monitored daily for 30 days upon which the bark islands were removed and examined for attacks. After one week of monitoring any adult SPB found outside the wafers were removed in order to distinguish between the initial attacking SPB and any potential second generation SPB.

Across the entire experiment there was no successful SPB development with zero SPB surviving from egg to adult stage. At the time of analysis there were eggs found in 25% of the white pine bark islands and in 30% of the loblolly pine bark islands. All of these eggs were desiccated, ruptured, or failed to undergo development. There were no larval galleries observed. Blue-stain fungi was present in all of the pine wafers as well. The reason that no development was observed is not known but the quality of SPB, presence of Blue-stain fungi, or some unknown factors may be responsible. Regardless these experiments served as valuable practice for later developmental experiments.

During the third research season the bark island experiment was revisited with a change in the attack method. Fresh wafers of the same size and shape as in the previous experiment were obtained. Infestation was attempted using clear gel caps that were approximately 15mm in diameter. The goal of this was to give the SPB no choice as to where to go within the pine wafer and, hopefully, increase the attack and oviposition rate. A small hole was made in the end of each gel cap using an insect pin so as to allow for air exchange. SPB were collected from traps in the rearing room and sexed. Holes were made in each bark island using a circular cork borer whose punch size approximated the

size of the gel caps. Female SPB were placed in each gel cap which was, in turn, placed in the holes on each bark island (Figure 3.6, 3.7). Females were given three hours to attack the wafers. If no attack was observed in that timeframe, another female SPB replaced the initial one. If an attack was observed within the timeframe, a male SPB was added to the gel cap. If the male did not enter the female's gallery within one hour, another male SPB replaced it. Unfortunately no oviposition was observed across this experiment and almost all the females failed to initiate an attack. Due to the failure of this experiment coupled with the concurrent success of the developmental experiment covered in the previous section of this thesis, all bark island experiments were abandoned.

Another failed experiment took place during the first research season and was based upon a design found in Wagner's (1984) article on SPB development across a range of temperatures. This experiment involved one foot long bolts of both loblolly and white pine as the host for SPB. Wagner's experiment involved the use of lard cans as the container for the bolts and small fans at the base to blow out any toxic terpenes inside. Our container design differed due to the unavailability of lard cans. Instead sections of metal ducting were sealed at the top with a thin sheet of metal attached by duct tape (Figure 3.8). A square hole was cut into the top of the container and covered with mesh (Figure 3.8). The bottom of the upper portion of the apparatus was covered in 1/2 in. thick weather stripping so as to create an airtight seal. The base of the container was composed of a plastic five gallon bucket lid with a square hole cut into its center. The lid

was propped up by three pieces of 2 in. tall PVC pipe attached to the bottom in a triangle shape (Figure 3.9). Inside the square hole on the base a CPU fan was attached using rubber cement. The CPU fan was hooked up to an AC adapter with adjustable amperage settings so as to afford some control over the speed of the fan (Figure 3.9, 3.10). The goal of this apparatus (I'm having a hard time visualizing this apparatus—maybe a diagram would help) was to house 1 ft. tall bolts of pine which would be attacked by SPB. Despite the design, none of our attempts to get SPB to attack the bolts once they were placed within the container succeeded. Almost all of the 400-500 SPB collected during the first research season were used in these experiments. No oviposition or development was observed. This experimental design was abandoned in favor of the more successful experiment covered in the previous section of this thesis.

The final failed developmental experiment involved the use of phloem sandwiches and took place during the second and third research seasons. This technique is well established in the bark beetle literature and was based on experiments by Taylor et al. (1992). The goal of a phloem sandwich is to couple an infested piece of pine bark and vascular cambium with a clear substrate so as to allow for direct observation of SPB development over time. In this case the clear substrate was 1/2 in. thick pieces of Plexiglas measuring 4 in. x 9 in. Pieces of bark approximately the same size as the Plexiglas and with vascular cambium intact were carefully removed using a hammer and sharpened chisel. The bark was attached to the Plexiglas, with the inner bark in contact with the Plexiglas, using four large binder clips with one on each side of the rectangle.

The edges of the sandwich were sealed using plumber's putty dispensed from a caulk gun. The goal of the binder clips and sealant was to prevent both desiccation and inoculation by airborne bacteria and fungi.

During the second research season twenty phloem sandwiches of both white and loblolly pine were constructed. They were placed in groups of 10 into large, open plastic containers. One of these containers of each species was placed in an incubator set to either 25°C or 30°C. Approximately 100 unsexed SPB were placed in each container and allowed 30 days to attack and develop. The initial round of attacks yielded zero successfully developed SPB and only 8 attacks observed in white pine and 7 in loblolly pine. Out of these attacks only a few examples of oviposition were observed.

The third research season saw an exact repeat of the aforementioned experiment with a slight change in attack strategy. The second round of phloem sandwiches were lightly scored using a Dremmel tool so as to create several small wounds on the bark portion of each sandwich. The goal of this was to release host volatiles and attract the beetles into attacking the sandwiches. The second time around 18 attacks were observed on white pine sandwiches and 14 on loblolly pine. A total of 36 eggs were observed in white pine and 16 in loblolly pine. Overall there were only 4 successfully developed SPB in white pine and 2 in loblolly pine. 100% of galleries appeared to contain Blue-stain fungi. These results show the difficulty in getting SPB to attack and successfully develop inside our phloem sandwiches. The presence of Blue-stain fungi could be responsible for

the low success rate. All fungi in the phloem sandwiches should have been isolated and identified and any future research utilizing this technique would benefit from such analysis. Almost 1000 SPB were utilized in the phloem sandwich experiments which netted practically no valuable data. The extremely low attack rate and anemic survival numbers caused this experimental technique to be abandoned in favor of others.

SPB Trapping and the New-Style Trap

In addition to the rearing system described above, field-infested bolts were placed in specialized SPB traps in order to capture and maintain emerging SPB for experimentation. For the first two research seasons the type of traps used were an old design from the forest service that were originally used from the 1960's onward. The capture portion of the trap was a mason jar and the bolt containment portion was a large metal drum. When the capture portions were removed and checked it was consistently found that more SPB were dead than alive. Knowing that conspecific mutilation and cannibalism is a problem in any SPB colony, the mason jars were filled with shredded paper in order to increase the total surface area available to the insects. This change was made late in the second research season. Almost immediately it was observed that SPB were found alive on the shredded paper and dead on the bottom of the jar. This observation served as the inspiration to design a new SPB trap that would counter the problem of conspecific mutilation and cannibalism by providing more surface area to the beetles.

The new-style trap design was developed in the spring of 2009 and constructed in the early summer. It features a sturdier frame, more space in the bolt containment portion, and over 15 times the surface area of the old-style traps with shredded paper. Observations on the capture portion of the old-style traps indicated that SPB come into contact with each other most often on the bottom of the mason jars. The new-style trap design did away with the small mason jars and introduced a large, square plastic container complete with three pieces of verti-cel®. Each piece of verti-cel® contains over a thousand individual compartments separated by cardboard. Many SPB were found and removed from these cells during experimentation indicating the usefulness of spatial separation in discouraging cannibalism. Shredded paper was placed atop the verti-cel® and served the same purpose as in the old-style traps.

Another key ingredient in the new design was the removal of the restrictive and rusted metal funnels that shunted SPB into the capture portion. A much wider funnel was constructed out of plastic sheeting and was noticeably smoother in texture than the metal funnel. The goal of this was to give SPB less chance of stopping on the funnel portion during their post-emergence drop. This ties in with one of the main concerns about the new-style traps; the seal on the bolt containment portion. (Again a diagram might be helpful)The seal was created using very thin-gauge screen door material taped around the bolt containment area and held together using multiple folds pinned shut by clothespins. While this design kept the bolts from moving, it was certainly not tight enough to guarantee SPB containment. Any SPB lucky or determined enough to fly to the areas in-

between the clothespins could potentially escape. This portion of the trap needs improvement in order to guarantee SPB containment. Prior to the implementation of the clothespin technique, velcro® strips were used as a sealant. While they created a much tighter seal, they were found to disengage from the screen door material after only a few uses. The same problem arose with the use of duct tape as a sealant. Future changes to this trap design could include hot-gluing the Velcro® to the screen door material, folding and stapling it shut, or some sort of zipper to aid in sealing off the bolt containment portion.

Another area of the trap that could be improved is the seal between the plastic capture portion and the organza material (this is the first time you mention this in the design of the trap) that extends from the end of the funnel to the floor as it covers the capture portion. The seal used during rearing and experimentation was achieved using Velcro® and suffered the same problem described for the bolt containment portion. Velcro® seems to create a fantastic bond that completely prevents SPB escape, as evinced by the dozen or so SPB found dead within the seal on the capture portion. The problem arose in the attachment and subsequent detachment of the Velcro® to the organza material. After the Velcro® seal failed, duct tape was used and found to be satisfactory for a time. The problem arose when daily monitoring of the capture portion required constant removal and reattachment of the duct tape resulting in the persistent need for replacement. Any large-scale SPB trapping operation would require a more

flexible and long-term solution. As mentioned above hot glue or a zipper could have been utilized as an attaching agent, or perhaps another method should be employed altogether.

While initial observations on the new-style traps coupled with much higher numbers of useable SPB seemed to indicate a marked improvement in trapping technology, testing was needed to confirm. The trapping experiment consisted of four total traps, two old-style and two new style. The reason that only two traps of each type were tested is that only two of the new-style traps were produced during the 2009 research season. The new design was simply an extension of the hypothesis that spatial separation, increased surface area, and multiple refugia would result in less conspecific mutilation and cannibalism. Since the hypothesis had yet to be tested, the choice to only spend time constructing two traps was made in an effort to preserve time spent on a project that did not concern SPB development or biology directly. The trapping experiment showed that the new-style traps did capture significantly more SPB than the old-style traps and helped confirm the hypothesis.

An interesting and unexpected result of this experiment came in the form of more total SPB captured alive at the 24 hr. rate than the 12 hr. rate. This result is counterintuitive in that it does not make sense that more consistent monitoring would not help capture and preserve more live SPB. The only way to confirm this result is through further testing. Future research on this new-style SPB trap should include at least 5 traps of each type and could also include an 8 hr. and 36 hr. monitoring time. This would help

to understand the point at which one has waited too long to remove the SPB from the trap. Considering the fact that the new-style traps added at least two new types of refugia (the verti-cel® and the organza material), additional testing of the usage of these structures compared to the shredded paper and the bottom of the container (this sentence needs work). An attempt to record this kind of data was made during the trap experiment but inconsistencies rendered the data unusable. With a few improvements to the design of the new-style trap and a more thorough experimental design, the efficacy of the trap could be better tested and understood. Regardless, the new-style traps netted enough live and usable SPB that some successful SPB development experiments could be accomplished.

SPB Biology and Developmental Experiment

The goal of the SPB development experiment was to compare several parameters of SPB attack, oviposition, and offspring success between loblolly pine and white pine. Additionally temperature was included as a factor with bolts held at 25°C or 30°C. The initial range of temperatures for this experiment also included testing at 10°C, 15°C, and 20°C. The reason for this is that it represents the entire developmental temperature range for SPB. Due to low numbers of SPB overall only the two highest temperatures were chosen so as to maximize the chance of SPB development and survival. The prime developmental temperature for SPB lies between 25° and 30°C according to Wagner (1984), the author whose methods are the basis for this experiment. Future research of this type should include the full range of temperature so that a more complete picture of

SPB development can be attained. Additionally some bolts should be tested at 27.5°C and 31.5°C because the latter represents the point at which SPB development suffers due to heat, and the former would help clarify the prime developmental temperature for SPB.

Another factor incorporated into these experiments was the presence or absence of surface-sterilization on SPB individuals used in the infestation of the experimental units (pine bolts). A solution of 4:1:95, ethanol to bleach to water, was utilized as the surface sterilizing agent. The method of sterilization was to place the SPB in Pasteur pipettes and pass the solution over the SPB 10 times. This method was developed by Dr. Yasmin Cardoza and chosen based on testing showing that it did not remove the mycangial fungi from SPB (Cardoza, personal communication). A shortcoming of this experiment is that none of the fungi found inside the bolts was isolated or identified. Future research on the effect of surface sterilization must include a thorough examination of all fungi and bacteria present on the SPB, in the host pre-infestation, and in main galleries, larval galleries, and egg niches. Testing of eggs and larvae for fungi could also provide useful information. Obtaining this information would have greatly benefitted the interpretation of our results.

Despite surface-sterilization, all bolts were found to contain Blue-stain fungi. Since the bolts were not tested for fungal contamination prior to infestation there is no way to know whether the Blue-stain came from the SPB or somewhere else. Future research in this area should include testing of uninfested material for fungi before it

enters the lab, after entering the lab, and post-infestation. Part of the problem with the surface-sterilization treatments may lie in the solution itself. The ethanol-bleach-water combination was tested on SPB and was not found to cause mortality out to seven days post sterilization. This testing should have included daily plating of SPB on fungal media to determine the longevity of the sterilization treatment as well. Other sterilization options such as White's solution could have been tested. Future research on SPB surface sterilization should include several different options tested over time to determine the most effective method. Any of these hypothetical future experiments could also include a treatment wherein the mycangia are excised from the SPB. This would serve as a negative control and could provide a good baseline for SPB development in the presence or absence of fungal symbionts.

Earlier in this section mention was made of the putative weakness and small size of SPB. Testing this as part of this experiment could have provided additional insight into the health of the SPB colony. SPB were inspected for any signs of mutilation prior to use in the experiment and this would have provided a perfect opportunity to garner some basic information on the size and weight of the individual SPB.

Beyond methodological issues there are other aspects of this experiment that merit further discussion. The results showed that SPB produce significantly shorter egg galleries in white pine compared to loblolly pine. It was also found that they lay significantly more eggs per millimeter of main gallery in white pine than in loblolly pine.

This indicates a higher degree of crowding in the galleries of white pine. These results seem to indicate that SPB are behaving differently inside white pine in terms of gallery construction and oviposition behavior. One would expect these differences to be borne out in the proportional hatch rates and adult emergence rates between the two species. The proportional hatch rate in loblolly pine was over 70% and almost twice that of SPB in white pine. This may indicate that the micro climate of an egg niche in loblolly pine is more conducive to egg development and even better at preventing desiccation. To use a metaphor it appears that SPB are better out of the starting gate in their traditional host, loblolly pine, than in white pine. The difference between species disappears, however, when adult emergence rates are considered. Neither host species had an adult emergence rate above 5%, meaning that the vast majority of SPB offspring died before completing development. This makes the obvious differences in main gallery length, oviposition density, and proportional hatch rates difficult to reconcile with the concept of host suitability. These results seem to indicate that our experimental design was not good in fostering full SPB development.

The reasons for why most of the SPB failed to develop are most likely plentiful. It is possible that the presence of Blue-stain fungi in all treatments created an inhospitable environment for SPB development. Another potential explanation may lie in unforeseen consequences arising from the genetics of the population from which we selected. The groups of SPB used in each experimental unit were unsexed as per Wagner's (1984) methods. Typically the sex ration during SPB emergence is 50:50 but it is possible that a

preponderance of males were selected inadvertently. The bolts were checked daily and the number of SPB on the outside was constantly changing. This points to the potential of reemergence of some SPB. Since the attacks of the SPB were not videoed or constantly observed, it is impossible to determine how many, if any, attacks arose due to reemergence. Additional research of this type should include some form of analysis of reemergence behavior in order to quantify its prevalence. Reemergence rates should also be compared between host type, across temperatures, and sterilization treatments. Due to restrictions on the number of available SPB only two treatments of this experiment were replicated. This makes it more difficult to notice differences between host, temperature treatment, and sterilization treatment. Each of the treatment combinations should have been replicated at least twice, and would have given enough useable SPB.

On the whole this project represents an attempt to do the best with what was provided. The goal of improving our SPB capture rate was realized through a new trap design which, in turn, allowed some form of developmental experiments to be performed. There were certainly many areas for improvement in the methods and techniques employed, but the whole experience has served as an example of just how difficult and intriguing it can be to work with living creatures.

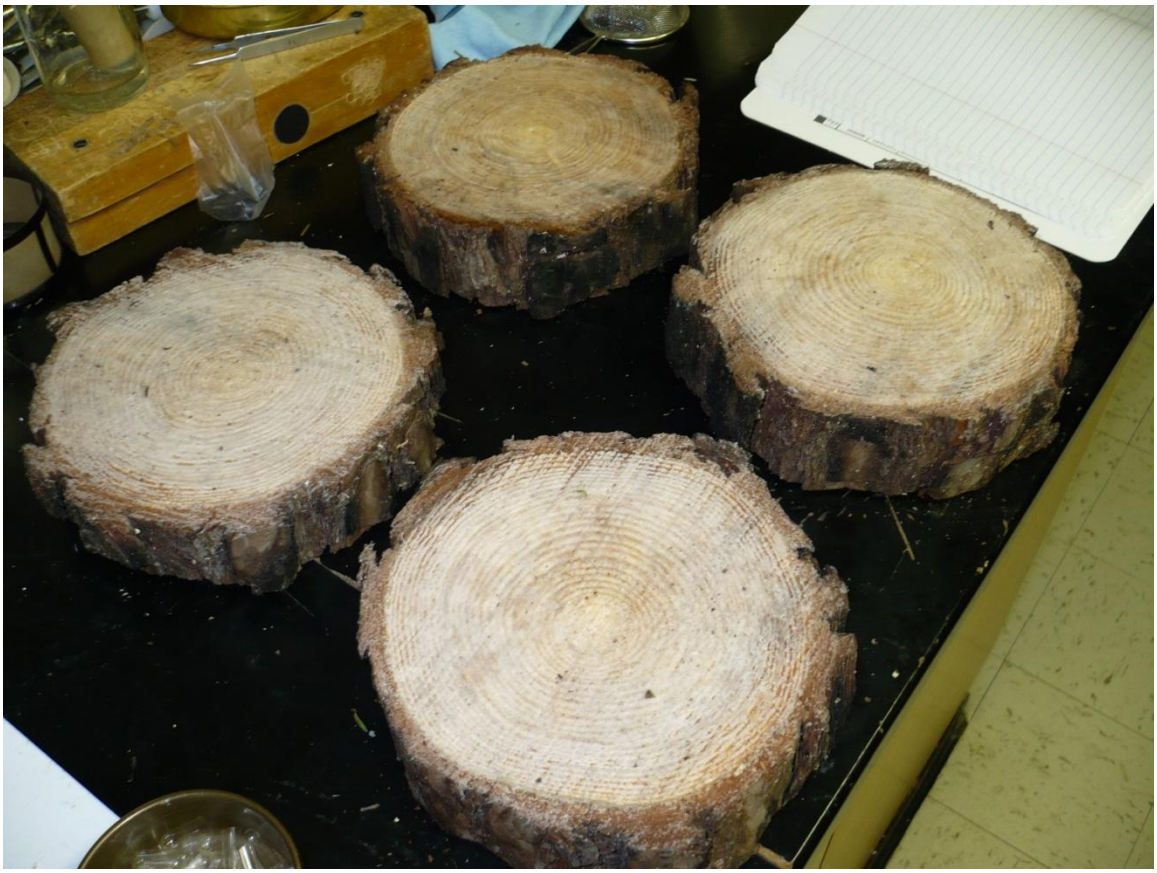

Figure 3.1 Loblolly pine wafers

Figure 3.2 White pine wafers

Figure 3.3 White pine wafer with bark islands removed

Figure 3.4 Loblolly pine wafers with bark islands removed

Figure 3.5 Profile view of wafers and bark islands

Figure 3.6 Loblolly and white pine wafers with gel caps inserted

Figure 3.7 Profile view of gel caps inserted into wafers with SPB inside

Figure 3.8 The upper portion of the SPB development cage based on Wagner's lard can design

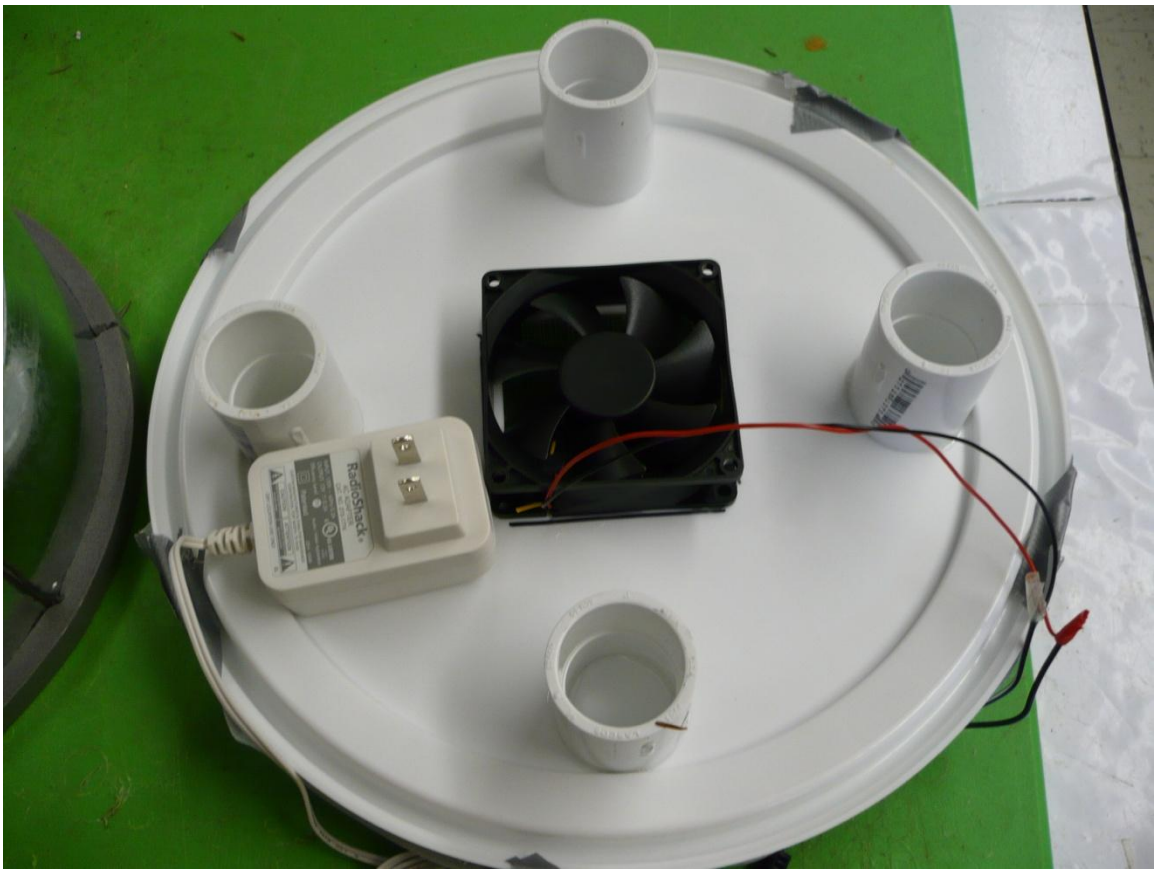

Figure 3.9 The bottom portion of the SPB development cage complete with PVC stands, CPU fan, and variable amperage adapter

Figure 3.10 The complete SPB development cage

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