

## ABSTRACT

DENHAM, SANDER OLIVIA. Quantifying Site Treatment Differences of Loblolly Pine Tree Resin Flow Defense and Bark Beetle Attack Density During an Induced *Ips* Beetle Infestation. (Under the direction of Dr. Bronson Bullock, Dr. John King, Dr. Fred Hain, and Dr. Kari Heliövaara).

Loblolly pine (*Pinus taeda*) is an important timber species both economically and ecologically. In past years there have been severe economic losses, as well as ecological disruption, due to epidemic outbreaks of the southern pine beetle (*Dendroctonus frontalis*). Resin flow is the first line of defense within conifer species acting as both a physical and chemical barrier to invading pests. We demonstrated the effectiveness of utilizing aggregation pheromones to attract *Ips* spp. bark beetles to loblolly pine plantation stands in order to study the resin flow defense mechanism. Individual trees were selected to be baited with aggregation pheromones. Trees in close proximity to the baited tree were labeled as monitor trees, and a control was established. Results of a general linear model for the aggregation pheromones attracting *Ips* beetles indicate that there was a significant difference ( $p < 0.0001$ ) between the baited and control trees. Using a repeated measures ANOVA, we considered differences of resin flow exudation in *Pinus taeda* among varying stand conditions (fertilizer, fire, and control plots) during the induced *Ips* bark beetle attack. This study illustrates that different stand conditions elicited more or less of a response of *Ips* to the baited trees; however, site treatment did not significantly affect resin flow. We conclude that utilizing pheromones to attract *Ips* spp. bark beetles is an effective technique for studying the resin flow defense in conifers. From a management perspective, it is concerning to see differences in bark beetle activity among different stand conditions while simultaneously seeing no difference in resin flow defense, making this an important aspect of an integrated pest management study, and an area in need of further research.

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Quantifying Site Treatment Differences of Loblolly Pine Tree Resin Flow Defense and Bark  
Beetle Attack Density During an Induced *Ips* Beetle Infestation

by  
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## **DEDICATION**

I would like to dedicate this to my brother, Jeremy Joseph Maristany, who passed away before he got the chance to read and approve my work. And to my beloved blue heeler, Booger T. Washington. His kindness and patience, not to mention loyalty, could not go unnoticed. He kept me company while doing field work whether it was through brutal heat, or torrential down pours.

## **BIOGRAPHY**

Sander O. Denham was born and raised in Western North Carolina. She received her Bachelors of Science in Geography & Planning from Appalachian State University in 2008. Before beginning her graduate career at North Carolina State University, she held positions as a forestry technician as well as a research technician with the United States Forest Service in Idaho, Alaska, Montana, and North Carolina. As part of her Master's degree, she participated in Trans-Atlantic Dual Master's degree program in collaboration with the University of Helsinki in Finland and with Sveriges Lantbruksuniversitet in Sweden. Currently she is working with the Timber Stand Improvement staff on the Kootenai National Forest in Northwest Montana. She is also working to develop a non-profit organization in Las Delicias, Costa Rica specializing in sustainable forest management, land conservation, research, and education with the hope of improving the lives of those living in poverty of this rural community.

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## I. INTRODUCTION

Loblolly pine (*Pinus taeda*) is an important tree species, both ecologically and economically. It is the most extensively planted commercial timber species in the southeastern United States and the most widely planted conifer in the world (Whetton et al., 2001). Roughly 19 million hectares (47 million acres) are planted in the United States alone (Shultz, 1997) and the area of pine plantations is forecasted to have a 67 percent increase to approximately 22 million hectares (54 million acres) by 2040 (Wear and Greis, 2002). It is the most important forest species in the southern United States, dominant on close to 11.7 million hectares (29 million acres) making up over one half of all standing pine volume (Burns and Baker, 1983).

Loblolly pine is considered a secondary successional species throughout the southeastern United States (Spring et al., 1974) and can reach reproductive age at seven years (Harlow and Harrar, 1968). It is a shade intolerant species (Spring et al., 1974) but tolerates variable soil and moisture conditions (McQuilken, 1940). When natural regeneration occurs, colonization is gradual which produces a continuous age class distribution (McQuilken, 1940).

With this species being managed using intensive silviculture, loblolly pine is an ideal candidate to study in order to enhance and improve management. It has the ability to regenerate quickly and easily, as well as having a fast growth rate. These factors contribute to making it the favored species throughout the southeastern United States, where it grows naturally in fifteen southern and Mid-Atlantic States (Shultz, 1999). The adaptability of the

species renders it capable of being planted along the periphery of its natural geographical range and it has been introduced into other continents with varying levels of success (Burns, 1983). It has become one of the favored conifers in parts of Asia, Africa and South America (Shultz, 1999). Its wide range of successful habitats demonstrates the ability for adaptation and economic value in many different environments (Shultz, 1999).

Monetary benefits to society from past regeneration investments have been (Shultz 1999) substantial. While it is difficult to predict future economic trends and timber values, the present annual value of loblolly pine to the southern US economy is about \$30 billion, this value includes direct and indirect employment. However, pest and disease have the potential to negatively impact this value. These disturbances are unpredictable and have the potential to cause massive losses in revenue as well as ecosystem services. Management practices could effectively curb or reduce these losses if there is adequate understanding of the mechanisms that contribute to the defense.

The southern pine beetle (*Dendroctonus frontalis*, SPB) is considered one of the most destructive pests in loblolly pine forests (Elmaghraby, 1990). From the 1970s to 1990s, SPBs destroyed timber valued at over US\$1.5 billion (Price et al., 1998). SPB accounts for roughly 80 percent of total economic losses caused by insects (Schultz, 1999). The number of outbreaks has declined in recent years (Friedenberg et al., 2008) for unknown reasons, although the effect of management practices and the cyclical nature of SPB population growth (which has a period of 6-9 years) have been suggested as causes for the decline. News reports in the popular press suggest outbreaks are increasing in places like New Jersey and North Carolina, and given the expected increases in brood development in warmer

climates (Gan, 2004), regional-scale outbreaks of SPB have potential to severely impact southern pine ecosystems in the future.

Raffa et al. (2008) describe bark beetles as “eruptive species”, and stress the importance of studying these types of systems due to the economic and environmental consequences of the damage caused by anthropogenic activities. Insects are now affecting species of trees that were previously unaffected and expanding into habitats where previously it was uncommon to be affected by these outbreaks (Raffa, 2008). Considering the ability for loblolly pine species to expand into new territories, this increase in epidemic populations of bark beetles may have serious implications on the future of these forest resources. Currently, the absence of large-scale SPB outbreaks, and that less than one percent of bark beetle species undergo broad-scale outbreaks, complicates efforts to better understand southern pine forest- bark beetle dynamics. The tree killing genera of bark beetles are mostly within *Dendroctonus*, *Ips*, and *Scolytus* (Raffa, 2008). In order for a forest stand to breach thresholds and experience eruptive populations, a combination of host availability and suitability, beetle population density, weather, and escape from natural enemies are required for the ability of beetle populations to reach this state (Raffa, 2008).

Bark beetle threats are complex and contribute to economic loss, ecosystem function, challenges to natural resource policy, and environmental threats arising from anthropogenic change. This can occur because large areas of tree mortality can lead to change in forest structure, composition, and function (Raffa, 2008). The threat of large scale outbreaks is increasing and repercussions of these outbreaks are massive both economically and ecologically. Loblolly pine should be considered to be a high priority for understanding the

threats to the species and mechanisms for defense to aid in the prevention of environmental and economic losses.

*Ips* species are one of the bark beetles of concern in pine forests in the southern US (Drooz, 1985; Riley and Goyer, 1988). *Ips* beetles are functionally very similar to SPB, but with population dynamics that prevent large-scale outbreaks. However, they are still among the genera of bark beetles that cause tree mortality. *Ips* infestations create more localized pockets of mortality. In general, bark beetles locate their host trees and aggregate in large numbers by utilizing pheromonal communication, where the initial landing beetle communicates to other beetles where the host tree is located.

Pheromones assist in stimulating colonization, making other beetles aware of the presence of suitable host material to colonize (Birch, 1978). In the current study, a pheromone is defined as a mixture of insect-produced chemicals that evokes a natural response within a species. Advantages of locating, attacking, and colonizing host trees quickly is that dead timber desiccates rapidly or is then used by other organisms, making it unsuitable for beetle breeding (Birch, 1978).

Bark beetles normally breed in the phloem tissue or bark and then deposit their eggs in galleries that are excavated in the phloem, cambium, and outer sapwood (Birch, 1978). Successful brood production is dependent upon the death of these tissues. Most species of bark beetle are only able to successfully colonize trees that are damaged or already dead; however, some are able to attack and kill living, healthy trees (Birch, 1978; Christiansen, 1987). The infestation of healthy trees can be attributed to large populations of beetles in need of more breeding habitat. If the populations of beetles are large enough then the insects

will move on to attack other trees. This is one way in which infestations spread. In the southeastern United States, as in other areas, these attacks can result in severe timber loss (Thatcher, 1960). There is an optimal density of beetle activity above which developing larvae will interfere with one another's growth and food supply. With an interruption in food supply, beetle progeny size is lessened so the attack is shifted to adjacent trees (Birch, 1978).

Much like the production of pheromones to attract others, beetles also produce pheromones that indicate that a host is fully colonized to deter further infestation. This effectively diverts the incoming beetles to adjacent hosts in the vicinity of already colonized hosts, contributing to the spread of infestations. Bark beetles use a technique called 'mass attack' referring to a concerted rapid attack by large numbers of beetles in order to kill a tree swiftly. It is during this 'mass attack' that pheromonal activity commences. The initial beetle selects suitable breeding habitat and generates an attractant pheromone which both sexes respond to. Aggregation pheromones are thought to be enhanced by insect stridulation, the rubbing together of their wings creating a sound, as well as the addition of host tree resin.

In combination with pheromones released by the beetles, the tree itself also releases chemicals indicating that it is stressed which also act as an attractant for beetles. It is unclear what the underlying chemical message is that the beetles are responding to. It would require separating each individual volatile compound of the pheromones in order to understand what message is sent by which pheromone, which is difficult to do with the high variability in natural environments (Birch, 1978). Seasonality is a major factor to production of pheromones as well as the insect response to pheromones. There is ambiguity in the literature about how a bark beetle locates and attacks the initial host tree. It is also unclear as

to whether mortality of the host is due to the girdling of the tree as the phloem is excavated around the stem, or if death is caused by the associated fungus that arrives along with the beetles or some combination of factors. Studies have been conducted where mechanical wounding is utilized to simulate the attack of hosts as well as the utilization of fungal inoculation to simulate the associated fungus. Due to the lack of aggression among the *Ips* species, small pockets of mortality are more likely than large outbreaks. None of the species in this genus are as aggressive as the SPB in terms of mortality of healthy trees and as a result, there is less published literature available for review (Frolze et al., 1984). Using baits to attract these bark beetles could be used as a tool for studying interactions between bark beetles and their hosts (Coyle, pers comm.) without risking the potential for the large-scale mortality that is generally associated with SPB outbreaks. *Ips* primarily attack stressed trees, specifically those on unmanaged lands, which can be beneficial in terms of naturally thinning a pine forest, leaving more vigorous trees and potentially decreases the risk of subsequent SPB infestations (Thatcher, 1960; Paine et al., 1981; Clarke et al., 2000; Ward and Mistretta, 2002).

Miller et al.(2005) studied the attraction of southern pine engravers and associated bark beetles to Ipsenol, Ipsdienol, and Lanierone in the Southeastern US. Miller et al. (2005) concluded that in North Carolina, there is a significant interaction of Ipsenol and Ipsdienol for attracting *Ips avulsus*. Ipsenol, Ipsdienol, or a combination of the two, caught more *Ips grandicollis* than the control traps. *Ips calligraphus* that were captured by using Ipsdienol was significantly greater than zero (Miller et al., 2005). Previous work conducted by Allison et al. (2012) has concluded that Ipsdienol was effective in capturing *Ips calligraphus*, and

that a combination of Ipsenol and Ipsdienol was effective in capturing *Ips avulsus* and *Ips grandicollis*. This would suggest that researchers would need to utilize a combination of different pheromone lures to effectively attract different species of pine engravers.

The damages from pests and other environmental stress have become an increasing problem in southern pine forests over the past 50 years (Schultz, 1999). This can be attributed to the human change of ecosystems from mixed species to fast growing even-aged plantations of a single species, which is common practice for loblolly pine. This change has altered natural balances that have previously kept pathogenic organisms moderated (Schultz, 1999). While these changes to the natural balance can be deemed necessary in order to optimize forest productivity and economic returns of forest resources, it is important to recognize the necessity of responsible management practices to protect stands from some of the preventable devastation that is accelerated by these systems.

An effective way of producing fast growing, quality timber is by managing for pine plantations. This allows for a managed environment that can optimize growth by managing for shorter rotation periods as well as producing high quality and high value timber. In terms of the increasing amount of forested landscapes globally, plantations are highly important in providing the ability to produce more timber on a smaller amount of land (Stephens and Wagner, 2007). With the reduction of nutrients by many individuals up-taking from the soil, the stand could become stressed and thus less resistant to unexpected disturbances. Fertilization can be used in order to replenish nutrients in the soil. Previous research on the effects that fertilizers have on resin flow has conflicting results.

Pest and disease disturbances are common occurrences in forested ecosystems and can have extensive detrimental impacts both ecologically and economically. These disturbances are expected to increase as temperatures fluctuate and storm events become more frequent and severe due to the effects of climate change. Considering the impact that climate change has on our environment, it is important to carefully study and understand the mechanisms to which our forests can be protected in terms of tree physiology and the management techniques that can be implemented in order to sustain our forests. Tree physiology is a complex field of study, particularly in terms of defensive strategies against invading bark beetles. These defense mechanisms and how they are employed have been increasingly studied over the past 20-30 years, mainly in Europe and North America. However, there are still large gaps in the research that are necessary to explore in order to comprehensively understand how individual environmental factors affect the host tree's response. It is important to understand how these environmental factors interact with one another and affect the corresponding tree response. This paper aims to address conifer resin flow as a role in tree defense and how this relates to bark beetle attacks.

Of the four major southern pine species (loblolly, slash, shortleaf, and longleaf pine), loblolly and shortleaf pines are much more susceptible to attack by the SPB and it is suspected that differences are related to differences in their oleoresin systems (Hodges, 1977). Resin flow is a primary defense against insect infestation (Hodges et al., 1977; Ruel et al., 1998). Oleoresin may have evolved as a defense against herbivores. This resin is primarily synthesized by epithelial cells that form membrane walls of resin ducts in the xylem and is stored within these same resin ducts at positive pressure (Barbosa and Wagner,

1989). Some conifers rely on an induced response in the phloem tissue once the constitutive resources (resin reserves) have been depleted. The induced, secondary, resin contains toxic levels of monoterpenes in addition to acting as a physical barrier. Both types of resin (constitutive and induced) require a lot of energy so typically are only produced to full capacity by trees that are vigorous and have substantial reserves of energy (Franceschi et al., 2005). On the contrary, stressed or weakened trees typically reserve this energy for vital functions as opposed to defense purposes (Franceschi et al., 2005). It has been suggested that southern pine species do not depend on the induced response due to their well-developed resin ducts in the xylem (Lewinsohn, 1991; Nebeker, 1993). For loblolly pine, about 90% of resin flow occurs within 24 hours of wounding (Hodges, 1977).

Lower constitutive resin flow has been connected with lower resistance to bark beetle attack. Knebel (2008) demonstrated that constitutive resin flow increases with fertilization and may suggest that fertilization will decrease loblolly pine susceptibility to attack. This is contradictory to findings by Warren et al. (1999) that suggest that fertilizer decreases constitutive resin flow. One possible explanation of the decrease in constitutive resin flow with the addition of fertilizer is the growth-differentiation balance model, which states that when nutrients are available that these resources are allocated towards growth rather than secondary metabolism (Warren et al., 1999). However, it has also suggested that *Pinus* species generally have higher constitutive levels of monoterpene synthesis do not respond to wounding with increased synthesis, but rather depend on translocation of preformed resin (Lewinsohn, 1991). The resin flow response, while becoming better understood in terms of

defense against bark beetles, has not been greatly studied in how different factors (such as fertilizer and fire) affect the response.

Physical ejection or entombment of beetles by constitutive resin flow, and the containment of beetle infestation of fungal infection, or both, through induced resin defenses, allow trees the ability to withstand a low density beetle attack (Knebel, 2008). Considering the hypothesis that lower constitutive resin reserves are connected to a lower resistance to bark beetle attacks, it is reasonable to hypothesize that constitutive resin flow depletion and newly synthesized resin is correlated with beetle attack densities. Additionally, if lower resin flow increases a tree's susceptibility to beetle attack (Snyder, 1992), it would be expected that there would be greater mortality in trees that produce less resin.

Fire has also been associated with an increase in resin flow (Knebel, 2008; Lombardero, 2000). Fire damage is associated with increased stress in tree species. The presence of massive wildfires has been increasing, especially in western states such as Colorado, over the past few decades. Westerling et al. (2006) compared a land use versus climate hypothesis as competing explanations for increased fire severity. Though the two may not be independent, the climatic explanation poses that increasing variability in moisture conditions described by wet and dry oscillations that promote biomass growth and then burning, a trend of increasing drought frequency, and warming temperatures, have led to increase wildfire activity (Westerling et al., 2006). Historic wildfires observations exhibit an abrupt transition in the mid-1980s from a trend of infrequent, large, short duration fires to one with more frequent and longer burning fires which is marked by a shift towards warmer springs and longer summers (Westerling et al., 2006). It is expected that this trend will

continue and that these fires will consume much of the land in the West. It is important to understand the physiological implications of the effect that these fires will have on conifers both in terms of resin flow as well as potential bark beetle infestations. Many studies have investigated the implications of bark beetle infested stands on fuels and fire behavior, but none have addressed the implications that fire scorched land has on bark beetle activity within those stands (i.e. post fire).

If pine trees produce oleoresin as a defense mechanism, under a bark beetle attack scenario, I hypothesize that sampled pine trees will undergo an induced resin flow response. Furthermore, if stressed trees are more susceptible to bark beetle attack, then trees that have already undergone a stressor (fire) will attract more beetles and produce higher amounts of resin until resources are depleted.

The objectives of this study were 1) to demonstrate there is an induced resin flow defense response in loblolly pine during an *Ips* bark beetle attack; 2) to compare differences in resin flow of loblolly pine under different stand treatments and conditions (fertilizer, no fertilizer at the Savannah River Site; and fire/ no fertilizer, fire/ fertilizer, no fire/ fertilizer, control (no fire/no fertilization) at the Southeastern Tree Research and Education Site); and 3) to compare differences of beetle attack on these same stand conditions.

## II. STUDY SITES

### *Savannah River Site*

The study took place at the U.S. Department of Energy's Savannah River Site (SRS), a National Environmental Research Park, near Aiken, South Carolina, in the Carolina sand-hills physiographic region. The climate is humid continental with warm summers, mild winters and an average annual temperature of 17.9 degrees Celsius (Coleman et al., 2004). The main soil type is of the Blanton soil series and previous vegetation on the study site was pine plantations with an upland *Quercus* spp. understory. Sampled loblolly pine were 12 years old.

### *Southeastern Tree Research and Education Site*

In November- December of 1993, The North Carolina State University Cooperative Tree Improvement Program established a research study with planted Atlantic Coastal Plain loblolly pine families and drought-hardy Texas loblolly pine families at the Southeastern Tree Research and Education Site (SETRES II) in Scotland County, North Carolina (McKeand, 2012, pers. comm.). Loblolly pine at 19 years of age in both fertilized and non-fertilized populations, as well as fire exposure and no fire exposure, were sampled simultaneously for comparison.

### III. METHODS

#### *Experimental Design*

At the SRS research site plots were established within a 12 year old loblolly pine plantation. Plots were 0.22 hectares (0.54 acres) in size with a tree spacing of 2.5m X 3 m for a density of 1,333 trees/ha (540 trees/acre). Fertilized plots received 120 kg N ha<sup>-1</sup> yr<sup>-1</sup> concentrated liquid fertilizer (7-7-7 nitrogen-phosphorus-potassium (N-P-K) plus 0.22 percent boron (B), 0.01 percent copper (Cu), 0.05 percent manganese (Mn), 0.001 percent molybdenum (Mo), and 0.03 percent zinc (Zn)). Between April and September of each year, the total applied fertilizer was divided into 26 equal doses that were applied weekly (Coleman et al., 2004). Three study plots were located within each treatment plot and were used to monitor resin flow and onset *Ips* activity. Each of these plots consisted of 5 trees from which resin flow samples were collected. One tree was baited with *Ips* aggregation pheromones (ipsenol, ipsdienol, alpha-pinene, and ethanol), 3 trees located close to the baited tree were termed monitor trees, and one tree selected at a distance between 10 and 15 m from the baited tree was identified for use as the control tree. Ninety trees in total were sampled. Of these, 18 were baited with pheromones, 54 were located close to a baited tree (termed as monitor trees), and 18 were control trees (Kelsey et al. 2014). Monitor trees were designated to capture beetle wounds and resin flow differences once the baited tree had reached capacity of colonization and beetles were forced to attempt to colonize adjacent trees.

The experimental design differed minimally at the SETRES II site with additional factors considered for analysis. A randomized split plot design consisting of 4 plots and 4 treatments (no fire/ fertilizer, fire/ no fertilizer, fire/ fertilizer, control) was used. Each plot

within the treatment block consisted of 10 X 10 trees, with 1.5m X 2.1m (5 X 7 foot) spacing for a density of 3,076 trees/hectare (1,245 trees/ acre). Within each plot, 5 trees were sampled. The 5 trees in each plot were randomly selected for a control tree, a bait tree, and 3 monitor trees surrounding the bait tree. These trees were of variable diameter classes. The site was planted in 1993 using different families of Atlantic coastal plain Loblolly pine.

### *Resin Flow Sampling*

**SRS:** Resin flow was measured by installing samplers with TD Falcon tubes over a  $24 \pm 1.5$  hour period for each sampling date. Two samples per tree were collected at approximately 180 degrees apart at an approximate height of 1.3 m (4.5 ft). A baseline resin flow measurement was collected prior to baiting. Four subsequent samples were collected throughout the summer from June-September of 2012. To install the samplers, a leather-hole punch was utilized to remove outer bark and puncture the phloem. Samplers were drilled in over the newly created circular hole. TD Falcon tubes were then screwed into the samplers and left for  $24 \pm 1.5$  hours. Once collected, tubes were labeled, sealed and taken to the lab to be weighed. Measurements were recorded to the nearest hundredth of a gram.

**SETRES II:** Sampling of oleoresin took place from April through August at SETRES II. Trees in each treatment (no fire/fertilizer, fire/no fertilizer, fire/fertilizer and control) were sub-divided using plots set up containing different families from the Atlantic Coastal Plain provenance of loblolly pine. Within each of these plots, one tree was selected for baiting using a combination of alpha-pinene, ethanol, ipsenol, and ipsdienol bubble packets (from Contech). As a general rule, each baited tree was at least 25 meters away from another baited tree (Coyle, pers comm.). Three monitor trees in proximity to the baited tree

were sampled and a control tree was located approximately 25 meters from the baited tree. In total, five trees in each plot were sampled. At each sampling occasion, two samples were taken from each tree located approximately 180 degrees apart around the bole at an approximate height of 1.3 m (4.5 ft). This method was utilized to help reduce the error in the variability of resin flow based on crown structure. The location of the sample was moved laterally around the stem of the tree for each different sampling event to avoid sampling at a prior location where radial ducts were previously severed. In order to obtain samples, the outer bark was scraped down to inner bark. A leather-hole punch was used to penetrate the phloem and specialized resin samplers were then installed over the hole. The samplers are internally threaded and 15 mL BD Falcon plastic tubes were then screwed into the resin samplers and left for a 24-hour period ( $\pm 1.5$  hours). After the 24 hour period, plastic tubes were labeled, sealed, collected and taken to the lab to obtain weight measurements to the nearest hundredth of a gram.

#### *Occurrence and Damage*

**SRS:** To determine the level of bark beetle damage, entry wounds were assessed visually based on the type of entry into bark on all trees where resin flow was collected. Secondary pests were also accounted for including *Dendroctonus terebrans* (black turpentine beetle) and *Monochamus titillator* (southern pine sawyer). Entry wounds were identified and amount of entries were recorded. To ensure thorough identification of insect arrival to trees, Gentry sticky traps (a basic glue based trap) were installed on all baited and control trees. This was also used to determine if insects were present whether or not they proceeded to attack the tree. These traps were installed approximately 2.4 to 3 meters (8-10 feet) from the

base of the tree and mounted directly on the bole. Sticky traps were collected at different time intervals throughout the duration of the study and a microscope was used to identify the different species of *Ips* beetles that were collected by the traps (*Ips avulsus*, *Ips calligraphus*, *Ips grandicollis*).

**SETRES II:** To determine the level of bark beetle damage, entry wounds were assessed visually based on the type of entry into bark on all trees where resin flow was collected. Secondary pests were also accounted for including *Dendroctonus terebrans* (black turpentine beetle) and *Monochamus titillator* (southern pine sawyer). Entry wounds were identified and amount of entries were recorded. To ensure thorough identification of insect arrival to trees, Scentry sticky beetle traps (a generic glue based trap) purchased from Gemplers, were mounted on baited and control trees in order to identify and quantify beetle type and amount that were landing on the selected trees. These traps were also used to detect SPB landing in addition to *Ips* species. These traps were installed approximately 2.4-3 meters (8-10 feet) from the base of the tree and mounted directly on the bole. The sticky traps installed on all baited trees and controls trees were collected over the course of the study. The first set of traps was hung on April 7 and collected on May 23 (46 nights). Traps were replaced on May 23 and collected on June 7 (46 nights). Beetle attack was assessed visually based on entry wounds detected. Secondary pests consisting of *Monochamus titillator* (black turpentine beetle) and *Dendroctonus terebrans* (pine sawyer) were also accounted for under the assumption that additional wounding from these pests would contribute to the depletion of constitutive resin flow and the synthesis or translocation of new resources (Coyle, pers comm).

## Statistical Analysis

For all analyses, an alpha-level of 0.05 was used to determine significance.

**SRS:** A repeated measures mixed model analysis of variance (ANOVA) is used (SAS 9.3, Cary, NC) to test for the effects of fertilizer treatment and baiting for *Ips* beetles on amount of resin flow and followed the statistical model:

$$Y_{ijkl} = \mu + R_i + T_j + RT_{ij} + B_k + \varepsilon_{ijkl} \quad \text{Where:}$$

$Y_{ijk}$  is the resin flow for the  $i^{\text{th}}$  replication effect and the  $j^{\text{th}}$  treatment effect of the  $k^{\text{th}}$  observation

$\mu$  is the grand mean over all resin flow measurements

$T_j$  is the  $j^{\text{th}}$  treatment effect ( $j=1, 2$ )

$R_i$  is the replication effect ( $i=1,2,3$ )

$RT_{ij}$  is the interaction of the  $j^{\text{th}}$  treatment effect and the  $i^{\text{th}}$  replication effect

$B_k$  is beetle effect at the  $k^{\text{th}}$  replication ( $k=1,2,3$ )

$\varepsilon_{ijkl}$  is the error associated with the model with expectations of  $NID \sim (0, \sigma^2_\varepsilon)$

Autoregressive and compound symmetry covariance structures were examined to determine the best fit model and based on AIC fit statistics, autoregressive covariance structure was utilized. Initial results indicated that there was no significant replication effect and was thus removed from the model. To analyze the success of aggregation pheromones in attracting *Ips* bark beetles, a general linear model approach was used to see if there were significant differences between baited trees and control trees in both treatments (fertilized, control).

**SETRES II:** Repeated measures mixed model analysis of variance (ANOVA) was used to determine effects of baiting, fertilizer, and historic fire treatments, as well as their interactions, on the resin flow of individual trees. A linear regression approach was used to

determine if there is any correlation between resin flow and beetle attack density. Baseline resin flow samples taken from day one sampling event were analyzed using a general linear model in order to detect if there were significant differences among the families within each treatment. A full model with all effects and their interactions was run through SAS 9.3:

$$y_{ijkl} = \mu + T_i + F_j + B_k + TF_{ij} + FB_{jk} + TB_{ik} + TFB_{ijk} + \varepsilon_{ijkl} \text{ where:}$$

$y_{ijkl}$  = each single observation

$\mu$  = the overall average of all observations

$T_i$  = the  $i^{\text{th}}$  site treatment (control, Fire/Fertilizer, No Fire/Fertilizer, Fire/No Fertilizer)

$F_j$  = the  $j^{\text{th}}$  family (A,B,C,D,E)

$B_k$  = the  $k^{\text{th}}$  beetle treatment (control, monitor, bait)

$TF_{ij}$  = the interaction between  $i^{\text{th}}$  site treatment and  $j^{\text{th}}$  family

$FB_{jk}$  = the interaction between  $j^{\text{th}}$  family and the  $k^{\text{th}}$  beetle treatment

$TB_{ik}$  = the interaction between the  $i^{\text{th}}$  site treatment and the  $k^{\text{th}}$  beetle treatment

$TFB_{ijk}$  = the interaction between the  $i^{\text{th}}$  site treatment,  $j^{\text{th}}$  family and the  $k^{\text{th}}$  beetle treatment

$\varepsilon_{ijkl}$  = the error term (assuming NID)

The family types of *Pinus taeda* were replaced with letters A-E in order to protect the genetic science produced by North Carolina State University.

## IV. RESULTS

**SRS:** DBH distribution with standard error bars within beetle treatments are shown in Figure 1 and DBH distribution with standard error bars within site treatment are shown in Figure 2. Fitting the main effects to the model indicates that there was a significant beetle treatment effect on weight of resin flow ( $F_{2, 440}=28.74$ ; p-value  $<0.0001$ ) from samples collected at the SRS site. The interaction between fertilizer and baiting for *Ips* beetles was not significant and was removed from the model. Beetle treatment remained a significant factor in explaining resin flow ( $F_{2,442} = 28.55$ ; p-value  $<0.0001$ ). Fertilizer did not have a significant effect on loblolly pine resin flow amounts in these plots (Table 1). Constitutive resin reserves were depleted and an induced response was achieved in the baited trees by sample day 86 as is noted by the increase in baited tree resin flow weight and standard error bars not intersecting with standard error bars of either control or monitor trees (Figure 3). Resin flow in the control trees and monitor trees did not appear to have spiked to a level that would indicate full depletion of resin reserves to initiate either new synthesis or translocation of resin. The model indicate significant effects for *Ips* beetles present on trees (Table 2; SRS,  $p<0.0001$ ).

Beetle treatment (baiting of trees) had a significant effect on number of *Ips* species ( $p < 0.0001$ ) observed on sticky traps; but neither fertilizer nor the interaction of baiting and fertilizer had any effect on attractiveness to pheromones (p-value 0.9647 and 0.9547 respectively; Table 3). The average and standard error of *Ips* beetles landing on sticky traps of baited and control trees as approximately 72 beetles landing on baited trees compared to an average of less than one on the control trees (Table 4).

**SETRES II:** On May 20, 2013 preliminary analysis was conducted for differences in resin flow (g) among 4 different families of loblolly pine under different site conditions (No Fire/Fertilizer, Fire/No Fertilizer, Fire/Fertilizer, control). The results indicate that there was no significant differences of initial resin flow (Table 5,  $p = 0.2007$ ). Beetle treatment and the associated interaction effects were removed from the model because it was not significant ( $p = 0.5652$ ). This was expected since the beetle treatment had just been installed therefore no time had passed for the treatment to have an effect. Type III sums of squares (Table 6) of this GLM with non-significant factors of beetle treatment and associated interactions removed show non-significance of family ( $p = 0.0662$ ), site treatment ( $p = 0.4083$ ) and their interaction ( $p = 0.4296$ ).

DBH distributions with standard error bars across site treatments are shown in **Figure 4** and

DBH distribution with standard error bars across beetle treatments are shown in **Figure 5**.

Fitting the main effects to the model using the repeated measure mixed model ANOVA

illustrate similar results as were reported from SRS with beetle treatment being a significant factor in explaining resin flow ( $F_{2, 493} = 6.04$ ;  $p = 0.0026$ ). Resin reserves were depleted and

an induced response achieved in the baited trees by sample day 117 as is noted by the spike in resin flow and standard error bars of baited tree not intersecting with standard error bars of control or monitor trees (**Figure 6** and **Figure 7**).

Results indicate significantly more bark beetles on baited trees (Table 7,  $p = 0.0005$ ) were consistent with results reported from SRS. Approximately one *Ips* landing on control trees and approximately 47 on baited trees suggests that attraction of beetles to the study site was due to the pheromone traps that were installed on the baited trees (**Table 8**).

A general linear model was used to determine if there were differences among site treatments and beetle treatments for total species of *Ips* entry wounds. All pairwise differences were considered with a Tukey-Kramer method for adjustment. Results of the GLM for aggregation pheromones attracting *Ips* beetles at SETRES II indicate that there was a significant difference ( $p < 0.0001$ ). *Ips* damage at SETRES II was explained by beetle treatment, silviculture treatment and their interaction (Table 9,  $p = 0.0002$ ,  $p = 0.0190$ , and  $p = 0.0245$  respectively). Pairwise differences using the Tukey-Kramer method of adjustment was used to explore significant differences within the interaction effect. Results indicate that baited trees *Ips* damage was significantly different than both control and monitor trees ( $p = 0.0016$  and  $p = 0.0003$ , respectively).

Due to low numbers of *Ips* wounds observed on individual trees, total *Ips* species were considered collectively in the analysis. Low counts of *Ips* from 3.04 meters (10 feet) and below could be explained by the different portions of the bole that are utilized by different species, some of which prefer the tops of the hosts, which were inaccessible for observation.

The higher numbers of secondary pests could be explained by the weakening of the tree due to *Ips* attacking the tops releasing stress chemicals, effectively attracting new pests. Results of the GLM for *Dendroctonus terebrens* damage indicate a significant beetle treatment effect ( $p < 0.0001$ ) as well as a significant interaction effect ( $p = 0.0006$ ). Results showed no significant silviculture treatment effect ( $p = 0.0663$ ). Using Tukey-Kramer comparisons for least squares, synonymous with results of *Ips*, damage illustrating a significant difference between baited and control trees ( $p < 0.0001$ ) and also significant differences between baited and monitor trees ( $p < 0.0001$ ). Results of the Tukey-Kramer

method for adjustment investigating pairwise differences of silviculture treatments indicated that the site treatment Fire/Fertilizer is significantly different than No Fire/Fertilizer ( $p = 0.0017$ ).

Arrival of *Ips* beetles and *Dendroctonus terebrens* were not observed until day 48 of the study (Figure 8 and Figure 9). This does not explain the increase of resin flow on day 15 but does support the subsequent depletion of resin at day 48.

A general linear model approach was taken to detect if there were significant differences of DBH between trees selected for each beetle treatment (bait, control, monitor) as well as differences between site treatments (fire/no fertilizer, fire/ fertilizer, no fire/ fertilizer, control) where site treatment and beetle treatment were used as main effects. The model showed that there was a significant difference present among both site treatments and beetle treatments ( $p < 0.0001$  and  $p = 0.0014$  respectively). To test to see if these differences in DBH would have affected the resin flow, a linear regression model with DBH being used as an explanatory variable for the prediction of resin flow was used to determine if DBH was a significant factor in explaining baseline flow of resin. The model was not significant ( $p = 0.8388$ ) therefore differences of DBH among site treatments was not considered to effect the overall results of differences in resin flow.

## V. DISCUSSION

The study performed at the two different sites during subsequent summer seasons demonstrated similar results. Given the similarities between site conditions, overall conclusions can be drawn from the sites.

Data analysis demonstrates that beetle treatment was consistently a significant effect in explaining increased resin flow sampled in loblolly pine trees. An explanation for this is that the use of aggregation pheromones is successful in attracting *Ips* beetles to these trees, as well as secondary pests (*Dendroctonus terebrens*, *Monochamus titillator*). The attacked trees then respond to beetle wounding by utilizing resin as a defense mechanism and increased resin flow over the study period. The results that fertilizer did not have a significant effect on loblolly pine resin flow is consistent with the work of Klepzig et al. (2005) which found that fertilizer did not significantly affect the constitutive resin yield of trees, even after more than three months of treatment. However, these results are contradictory to the findings of Knebel et al. (2008) which found that fertilized trees had an increase in constitutive resin flow. This increase was only sustained by the younger trees after the mechanical wounding and inoculation treatments (Knebel et al. 2008). For trees sampled at SRS, the age was the same as the older trees in Knebel et al.'s (2008) study, being 12 years old. These contradictory results could be explained by fertilizer no longer being applied at SETRES II and thus no longer having an effect on tree growth or vigor, or possibly that the older trees being 19 years of age, not producing the same fertilizer effect as younger trees.

The results of the resin flow analysis following pheromone use to attract bark beetles support previous studies of tree wounds and increased resin flow. Studies conducted by

Knebel et al. (2008) using mechanical wounding and fungal inoculation also resulted in increased resin flow exudation. The initial substantial increase in resin flow at sample day 15 (Figure 6, Figure 7) at the SETRES II site was unexpected and raises questions as to why. While no chemical analysis was conducted on resin samples, visual observation of samples suggest that resin flow is less viscous on sample day 15 than any other samples taken, including those of the previous study site, Savannah River. Field notes from the sample day indicate that a rain event occurred during the evening after plastic sample tubes were installed and when they were collected the following day. It is possible that there was some sample contamination during the rain event.

The suggestion of an induced resin flow response was achieved at different times at the two sites. The observed resin induction occurred on sampling day 86 at Savannah River Site and on sampling day 117 at SETRES II. The resin flow differences of baited trees compared to monitor and control trees on the final sample event was greater at Savannah River. This is much longer than the induction that was achieved by Knebel et al. (2008) which occurred at approximately 30 days of wounding. This could indicate the induced resin response is more due to the fungal inoculation as increased resin flow was no longer observed in the wound-only individuals of Knebel's study. One explanation for these differences is that wounding occurs at these sites whenever the beetles are attracted to their hosts, not at a specified time with a mechanical wounding tool. Based on sticky trap data and insect wound data at SETRES II, beetles were observed at sample day 48 (Figure 8, Figure 9). It would then require beetle excavation of larvae galleries after the arrival to the host before the physical wound would be present and begin to affect resin flow.

Other possible explanations of result differences between published literature and the current study could be the differences of ages of the sampled trees as well as their total heights and DBHs, any differences in water stress, or climatic variations. Tree physiology is affected by many environmental factors that need to be considered individually as well as how they interact with one another. Therefore to pinpoint the exact difference in results would be rather complex. Testing for effects of DBH on resin flow showed that while the DBH are significantly different when comparing trees within a site treatment as well as within a beetle treatment is not a significant factor in explaining resin flow. It should be considered however, that a larger DBH tree has more breeding material to excavate for beetles as well as more feeding material. The method of resin flow collection should also be considered as sample tubes are installed directly on the stem of the tree and moved laterally around the bole at each sampling event. Smaller diameter trees with less perimeter around the bole in which to sample may be stressed additionally by the percentage of resin ducts that had been severed.

Higher numbers of *Ips* beetles present on baited trees compared with those of control trees offers support that ipsenol and ipsdienol attract *Ips* beetles. Additionally, these higher numbers of *Ips* beetles contributed to the significant differences in resin flow among baited and control trees. This difference could be explained by the increase in beetle attack density. It should be noted that the monitor trees did not appear to experience an induced resin response and had lower levels of resin flow than that of control trees even while experiencing low occurrences of beetle damage. *Ips* species and *Dendroctonus terebrens* arrival did not

occur until day 48 (Figure 8 and Figure 9). This does not explain the increase of resin flow on day 15 but does support the subsequent depletion of resin at day 48.

Given the unexpectedly low overall count of *Ips* beetles observed and the high occurrence of *Dendroctonus terebrens* pitch tubes, it is speculated that the majority of *Ips* beetle activity was occurring higher up on the bole of the tree out of sight of the observer. It is generally accepted that *Dendroctonus terebrens* is primarily a secondary pest that arrives to individual hosts after initial attack.

Analysis of the attracted *Ips* species as well as *Dendroctonus terebrens* suggest that fertilizer treatments, both with fire and without fire, produce larger numbers of entry wounds. This could be explained by the fertilizer providing the necessary nutrients to produce healthier trees with larger breeding material areas that are sought out by pests in order to effectively reproduce (Boone et al., 2011). Arrival of the *Monochamus titillator* was minimal and showed no significance in the study between beetle treatments or site treatments.

Fertilizer not having a significant effect on the amount of *Ips* beetles at that SRS site was surprising since insects would likely gravitate towards hosts consisting of more breeding material for successful brood production as presented in the work of Boone et al. (2011) where it is demonstrated that as beetle populations increase, beetles tend to select trees with larger diameters to invade regardless of that host's better defenses, while low populations of beetles tend to exploit trees that are experiencing stress. The time since fertilizer application was not expected to be a factor in explaining this result; the effects of the fertilizer increasing the diameter growth would already have taken place, increasing the size of the bole of the fertilized trees.

## VI. CONCLUSION

The results of the increased beetle activity on trees that were baited with aggregation pheromones is encouraging for future studies involving bark beetle/ host tree interactions and the defense mechanisms involved. These results were expected and suggest that using aggregation pheromones to attract *Ips* beetles could be used as an alternative approach to investigating resin flow defense responses in pines. Previous studies have utilized other methods, such as mechanical wounding and fungal inoculation to better understand the physiological processes of pine trees once attacked by bark beetles. Attracting *Ips* instead of SPB may be a more promising and a more natural way to understand the interaction between bark beetles and conifers during a beetle outbreak. It is encouraging to note that we did not observe that the monitor trees located in close proximity to the baited trees experienced a depletion of resin reserves and an induction of resin flow, suggesting that these mass attacks of bark beetles are extremely localized; only those trees which had baits directly installed showed a depletion of resin reserves.

When utilizing pheromones for a controlled simulation of bark beetle attack, it should be considered that the behavioral nature of the beetles cannot be controlled whereas physically wounding and inoculating fungus into the tree, a specific amount of inoculation as well as a predetermined wound site can be controlled. This is one explanation as to why there are differences in this study when compared to what was observed in studies of mass inoculation and physical wounding (Klepzig et al., 2005; Knebel et al., 2008).

A general linear model approach was taken to detect if there were significant differences of DBH at SETRES II between trees selected for each beetle treatment (bait,

control, monitor) as well as differences between silviculture treatments (fire/no fertilizer, fire/ fertilizer, no fire/ fertilizer, control) where site treatment and beetle treatment were used as main effects. The model showed that there was a significant difference present among both silviculture treatments and beetle treatments ( $P < 0.0001$  and  $P = 0.0014$ , respectively). To test to see if these differences in DBH would have effect on the resin flow study, a linear regression model was used with DBH being used as an explanatory variable for the prediction of resin flow. The model was not significant ( $p = 0.8388$ ), therefore differences of DBH among site treatments was not considered to effect the overall results of differences in resin flow. When normalizing DBH data and using this as a covariate within the repeated measures ANOVA, DBH was not a ( $p = 0.0509$ ).

On day 117, there was a difference in resin flow between baited trees and both control and monitor trees, and also that there was a difference between monitor and control trees (Figure 6). One possible explanation for this difference is that the beetles successfully colonized the baited trees and were looking for new breeding grounds and moved to those trees that were in close proximity to the initial host tree. In the repeated measures ANOVA, silviculture treatment was not found to have a significant effect on differences in resin flow. The difference in resin flow between control trees and monitor trees in resin flow that is observed at the SETRES II study site was not observed at SRS.

Analysis of Variance determined that the beetle treatment of trees (baited with pheromones or not baited) was a significant indicator of resin flow. This would suggest that baiting of the trees was successful in attracting bark beetles and that aggregation of these bark beetles and the damage to the individual baited trees elicited the defense response of the

pine increasing the resin flow in order to protect against attack. This result is further supported by the general linear model for number of *Ips* damage recorded on individual trees and illustrated in Table 2. Sample day was also a significant factor ( $p < 0.0001$ ) in explaining resin flow, which could be explained by the increase in number of beetle attacks throughout the study.

Based on analyses conducted, it is not apparent that resin flow is significantly different on the four treated sites (Fire/Fertilizer, Fire/No Fertilizer, No Fire/Fertilizer, and Control) at SETRES II. This could be explained by the amount of time since the fire or fertilization treatment had taken place on these sites, the effects of each treatment could have already passed and the site returned to a more natural (no treatment) setting. However, the quality of breeding material of each individual may be decreased in the trees where fire had been present in the plot thus decreasing their appeal for beetle attack.

A general linear model approach was utilized to assess the effect of using the pheromone bubble traps to attract *Ips* beetles. When using the silviculture treatment, beetle treatment, and sample day as explanatory variables, the model illustrates that the results were significant in explaining number of entry wounds ( $p < 0.0001$ ). When comparing all pairwise differences, the results indicate that baiting of the trees was significant ( $p < 0.0001$ ) while not having a bait is non-significant, including the monitor trees that are in close proximity to baited individuals. The monitor trees showing an effect illustrates that the beetle attack was primarily concentrated to the baited trees.

Results of both the Savannah River Site in Aiken, SC and the Southeastern Tree Research and Education II Site in Scotland County, NC indicate that using pheromone traps

to attract *Ips* beetles is an effective way to study an induced resin flow defense response in loblolly pine. Based on the results of the individual trees baited with pheromones being a significant factor in explaining beetle damage, and neither the control or the monitor trees (those not baited) demonstrates that this is also a safe, low-risk means to study defense responses. The monitor trees, which were located no more than three trees away (or approximately six meters) from the baited trees, did not show significant results when analyzed for beetle damage. This is a promising result that suggests that it is a relatively inexpensive way to study defense mechanisms as close to naturally as possible without a concern of causing major insect outbreaks that could lead to larger scale mortality.

Two related but slightly opposing views on host selection by invading pests is that stressed or weakened trees are more vulnerable to attack due to decreased defenses such as resin production. However, stressed or weakened trees also provide less breeding material for the pests to feed and lay their brood. It has been demonstrated by Boone et al. (2011) that as the beetle populations increased, the beetles selected trees with larger diameters to invade regardless of the host's better defenses while low populations of beetles tend to exploit trees that are stressed (Boone et al., 2011). This theory is supported by this study with higher number of beetles being observed on the trees located in plots with fertilizer treatment whether fire was present or not at the SETRES II site. This is interesting to note when considering the application of fertilizer to plantations of pine. While there may be an increase in resin flow defenses, there is also a higher likelihood of attack due to the amount of breeding material available to invading pests making them more attractive for attack. Additional studies could investigate fertilizer application time and examine whether a

modified time schedule could be applied in order to consider flight patterns of insects or seasonal variations in outbreaks.

Understanding differences in resin flow defenses under different stand conditions could better equip land managers in implementing suitable management practices for both plantations and natural stands. For future studies involving fire disturbance as a factor, it would be beneficial to install pheromone baits to attract beetles directly following the fire. It is also important to note the optimal flight season of the beetles being attracted to the host trees and plan for installing pheromones immediately prior to this for the most effective colonization of trees. This would not be a possibility in terms of using this technique directly following a fire event. This could be taken into consideration when setting up study after a fire event and using the lag time between fire and beetle flight to select the trees and arrange the study plan.

These mechanisms of defense and how environmental factors interact and play a role in pest disturbances, as well as tree defenses, are critical when considering the future of forest ecology and species composition. Unlike trees, which are unable to adapt and evolve rapidly, bark beetles and other pests, make up a biotic agent that can actively adapt and evolve on a much shorter time scale (Dunn and Crutchfield, 2009). The implication of this ability of bark beetles to behave in such a way has the potential to contribute to rapid deforestation of forests. As environmental factors and their interactions change, so do the techniques of forest managers in order to maintain an ecological balance in these areas.

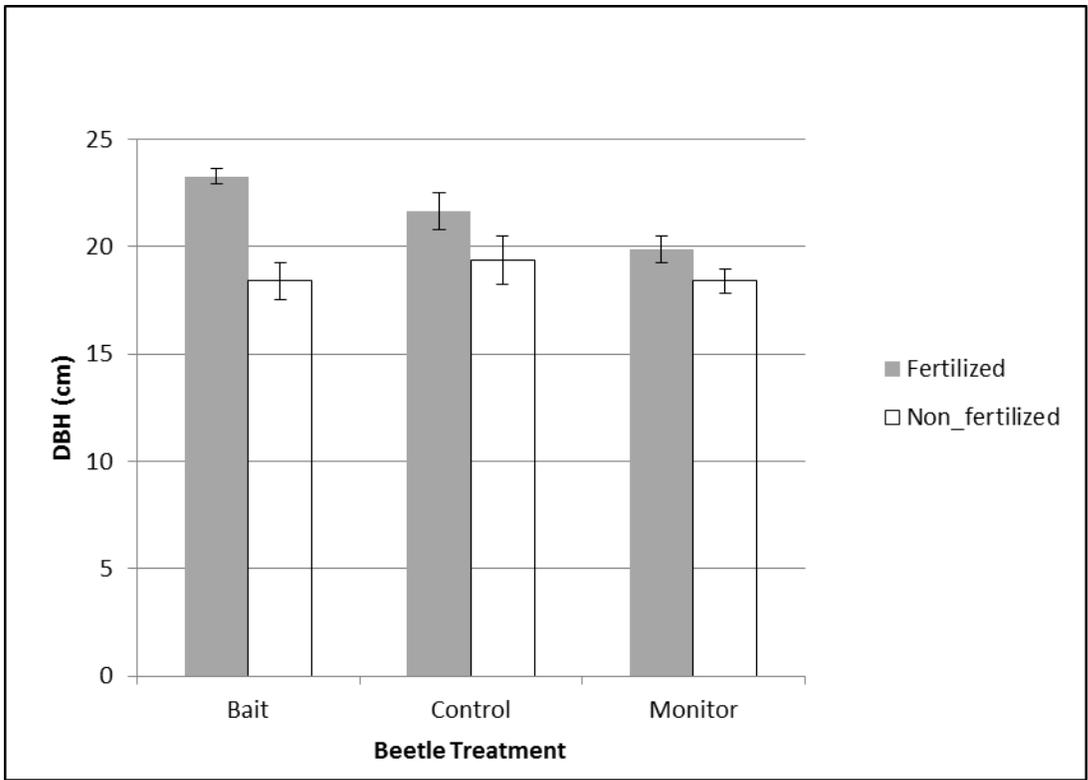


Figure 1: Average DBH of sampled trees at SRS across silviculture treatment with standard error bars.

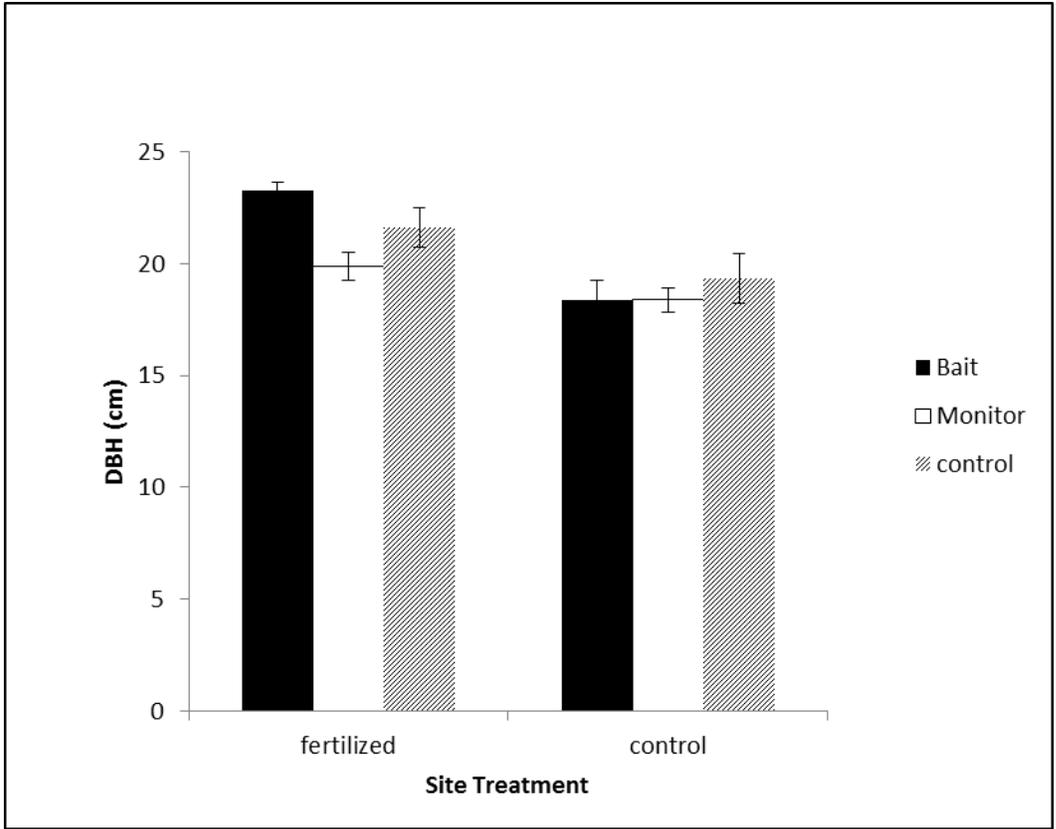


Figure 2: Average DBH of sampled trees at SRS across beetle treatment with standard error bars.

Table 1: Repeated Measures mixed model results for full model of resin flow response to baiting in fertilized and non-fertilized treatments at SRS.

<b>Type III Tests of Fixed Effects</b>				
Effect	Num DF	Den DF	F Value	Pr>F
Fertilizer	1	446	0.94	0.3334
Beetle	2	446	28.55	<0.0001
Treatment				

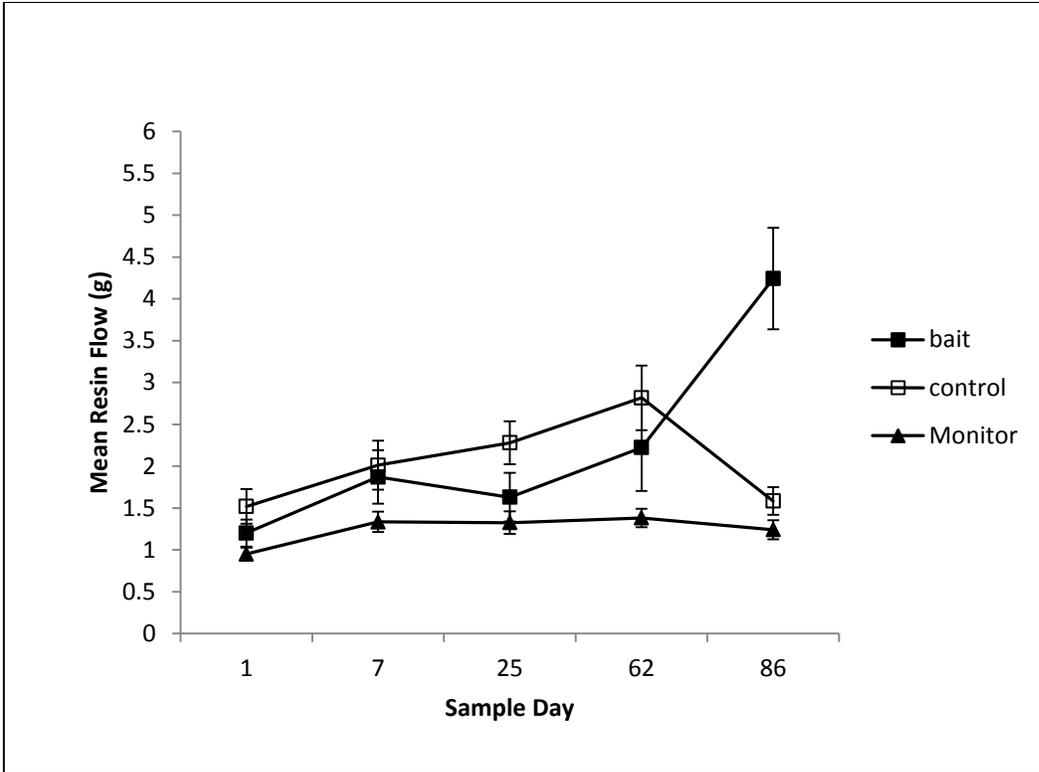


Figure 3: Average resin flow of sample trees at SRS with standard error bars.

Table 2: General linear model of effect of baiting on total number of *Ips* species for fertilized and control treatments at SRS.

Source	DF	Mean Square	F Value	P value
Model	3	15625	22.8	<0.0001
Error	32	685		
Total	35			

Table 3: General Linear model testing effect of baiting on total number of *Ips* species for beetle treatment, fertilizer treatment, and their interaction effect at SRS.

Source	DF	Mean Square	F Value	P-value
Beetle Treatment	1	46872	68.38	<0.0001
Fertilizer Treatment	1	1.36	0.00	0.9647
Beetle*Fertilizer	1	2.25	0.00	0.9547

Table 4: Average *Ips* species counts and standard error on baited and control trees for SRS site.

		<b>Average <i>Ips</i> counts</b>	
Beetle Treatment	n	Mean	Std. Error
Bait	18	72.22	8.46
Control	18	0.055	0.05

Table 5: General linear model for sample day 1 effects on resin flow with beetle treatment and associated interactions removed at SETRES II.

Source	DF	Mean Square	F Value	Pr>F
Model	16	0.2526	1.34	0.2007
Error	68	0.1888		
Total	84			

Table 6: Type III Sum of Squares of GLM testing for effects on resin flow with non-significant beetle treatment and associated interactions removed for day 1 resin samples, SETRES II.

Source	DF	Mean Square	F Value	Pr>F
Family	4	0.4368	2.31	0.0662
Site Treatment	3	0.1846	0.98	0.4083
Family*Treatment	9	0.1934	1.02	0.4296

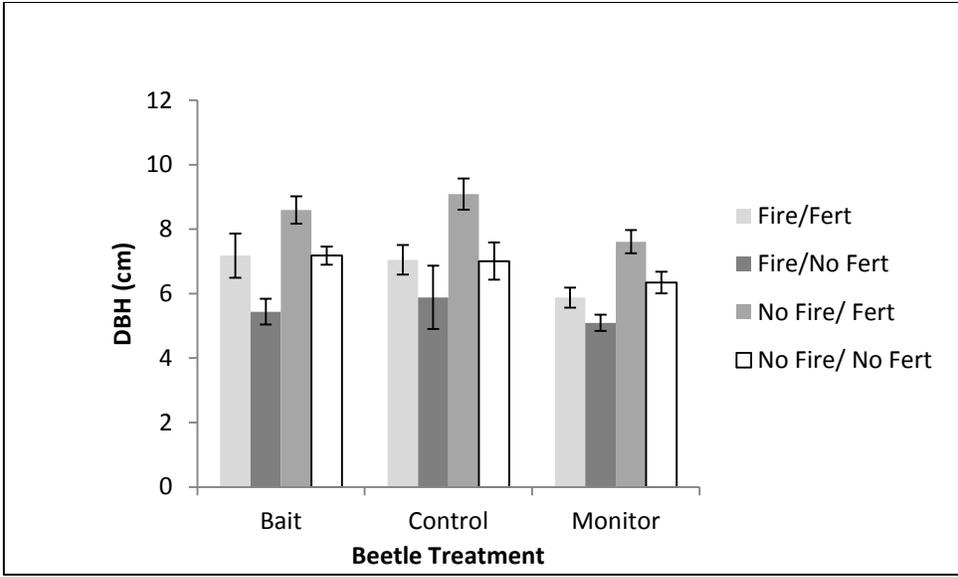


Figure 4: Average DBH of sampled trees at SETRES II with standard error bars across silviculture treatments.

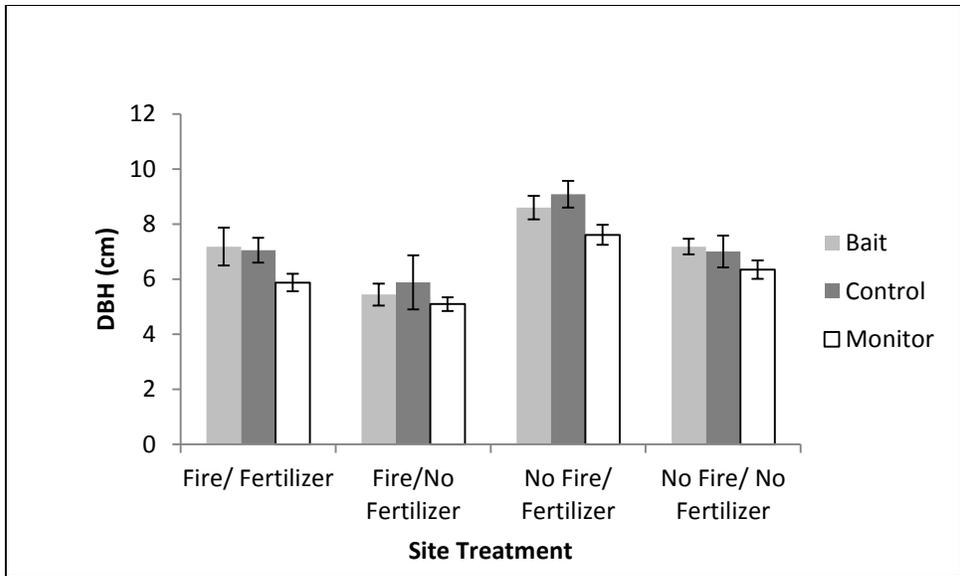


Figure 5: Average DBH of sampled trees at SETRES II with standard error bars across beetle treatments.

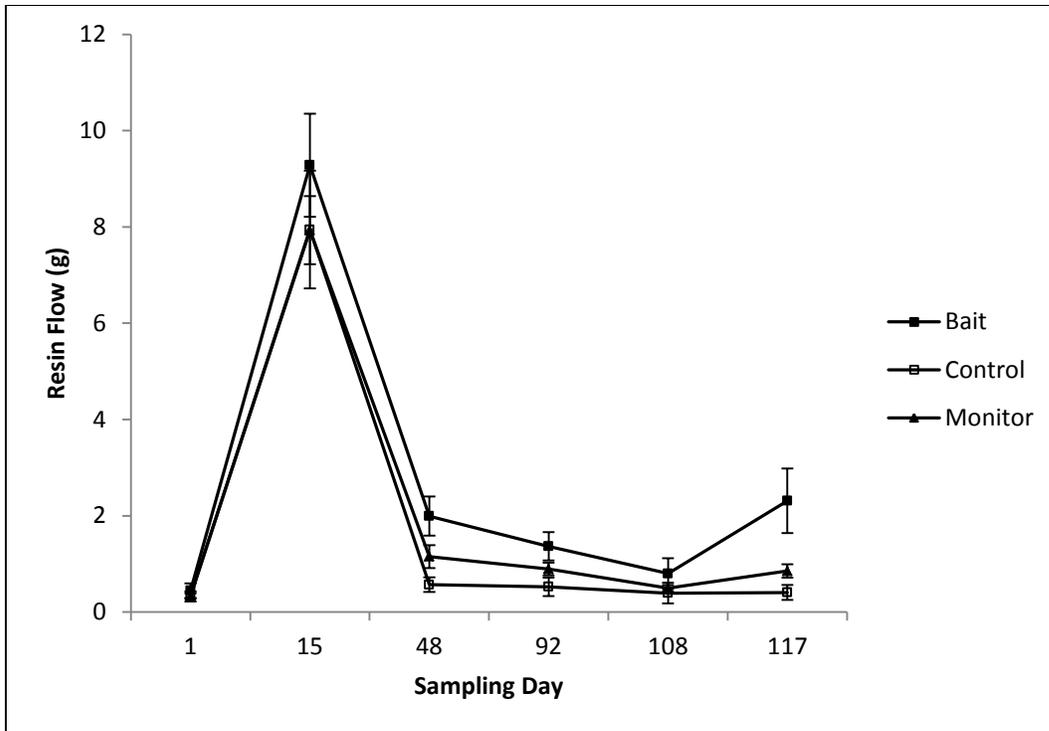


Figure 6: Average resin flow of sampled trees at SETRES II with standard error Graph indicates that an induced response was achieved in the baited trees between day 108 and 117.

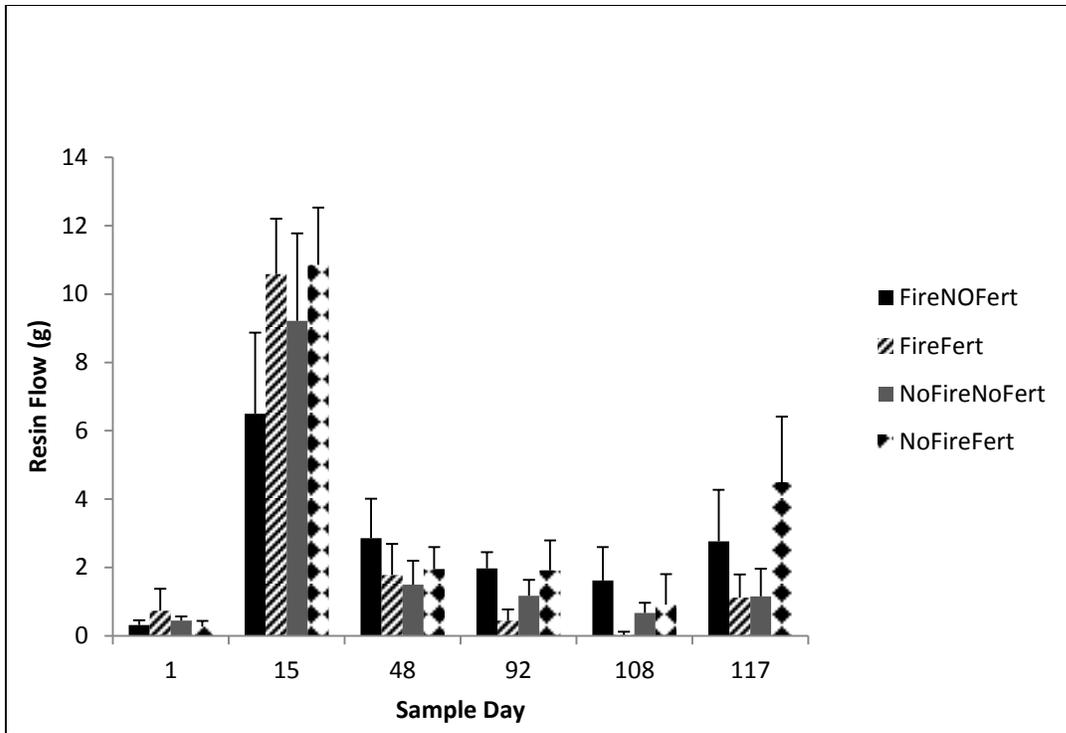


Figure 7: Resin Flow Bar graph with standard error from SETRES II

Table 7: GLM for *Ips* damage including all effects (site treatment, beetle treatment, and interaction) on day 117 at SETRES II.

Source	DF	Mean Square	F Value	Pr>F
Model	11	116.29	3.56	0.0005
Error	73	32.65		
Total	84			

Table 8: Average *Ips* collected on sticky traps for SETRES II.

Beetle Treatment	n	Mean	Standard Error
Bait	17	47.53	7.03
Control	17	1.12	0.26

Table 9: General Linear Model for *Ips* damage, SETRES II.

Source	DF	Mean Square	Pr>F
Silviculture	3	115.21	0.0190
Treatment			
Beetle Treatment	2	311.91	0.0002
Site*Beetle	6	84.83	0.0245

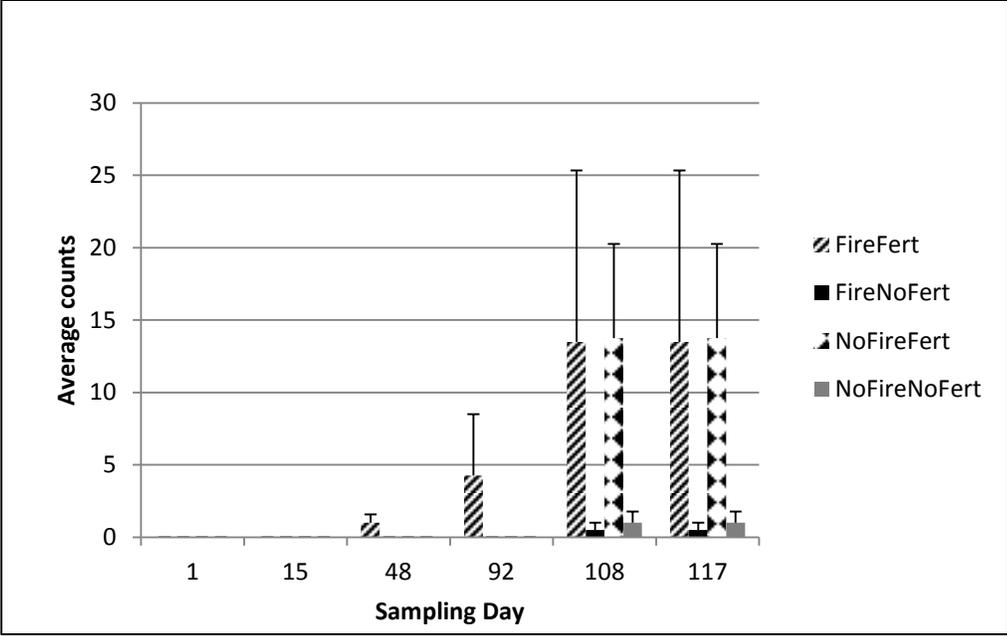


Figure 8: Averages of *Ips* beetles entry/ emergence holes (collective of *avulsus*, *grandicolis*, *calligraphus*) averages of baited trees for each treatment with standard error bars.

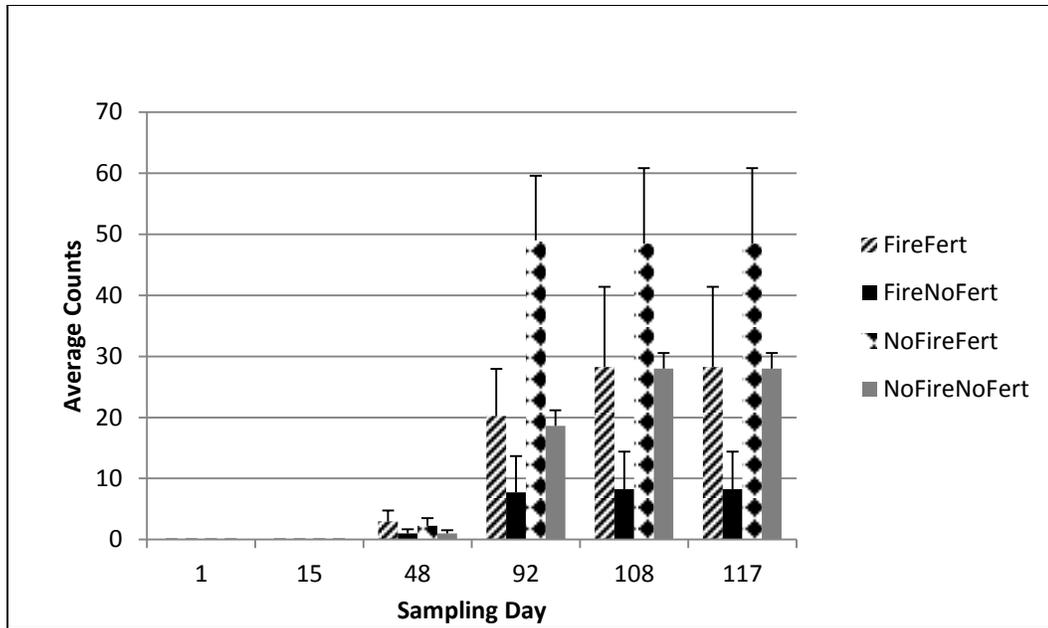


Figure 9: Averages of Black Turpentine Beetle (*Dendroctonus terebrens* -BTB) at SETRES II of baited trees in for each treatment with standard error bars.

## IX. REFERENCES

- Allison, J.D., J.L. McKenney D.R., Miller and M.L.Gimmel. "Role of Ipsdienol, Ipseol, and cis-Verbenol in Chemical Ecology of Ips avulsus, Ips calligraphus, and Ips grandicollis (Coleoptera: Curculionidae: Scolytinae)." Journal of Economic Entomology 105(3) (2012): 923-929.
- Barbosa P., and M.R. Wagner. Intoduction to forest and shade tree insects. San Diego: Academic Press pp. 150-167, 1989.
- Birch, M. C. "Chemical Communication in Pine Bark Beetles." American Scientist Volume 66 (1978): 409-419.
- Burns, R. M. "Sivliculture Systems for the Major Forest Types of the US." Baker, J.B. and W. E. Balmer. Loblolly Pine. Washington DC: United States Department of Agriculture, 1983 (revised). 148-152.
- Christiansen, E., R. H. Waring, and A. A. Berryman. "Resistance of conifers to bark beetle attack: Searching for general relationships." Forest Ecology and Management Volume 22, Issues1-2 (1987): 89-106.
- Clarke, S.R., R.F. Billings, and R.E. Evans. "Influence of pine bark beetles on the West Gulf Coastal Plain." Texas Journal of Science 52 (4 Supplement), (2000): 105-126.
- Coleman, M.D, D.R. Coyle, J. B, K. Britton, M.Buford, R.G. Campbell, J. Cox, B. Cregg, and D. Daniels,. Production of Short-Rotation Woody Crops Grown with a Range of Nutrient and Water Availability: Establishment Report and First Year Responses. Asheville, NC: USDA Forest Service Southern Research Station, 2004.
- Drooz, A. "Insects of Eastern Forests." 1985.
- Dunn, D. D. and J. P. Crutchfield. "Entomogenic Climate Change: Insect Bioacoustics and Future Forest Ecology." Leonardo June 2009: 239-244.
- Elmaghraby, S. E. "Optimal control of the southern pine beetle (SPB) infestation." Applied Mathematical Modelling Vol. 14, Issue 13 (1990): 153-164.
- Franceschi, V. R., P. Krokene, E. Christiansen, and T. Krekling. "Anatomical and Chemical defense of conifer bark against bark beetle and other pests." New Phytologist (2005): 167:353-376.

- Friedenberg, N. A., S.Sarkar, N. Kouchoukos, R. F. Billings, and M. P. Ayres. "Temperature Extremes, Density Dependence, and Southern Pine Beetle (Coleoptera: Curculionidae) Population Dynamics in East Texas." Environmental Entomology 37(3) (2008): 650-659.
- Froitz, J.L., E.P. Merkel, and R.C. Wilkinson. Annotated Bibliography of Dendroctonus terebrans (Olivier), Ips avulsus (Eichhoff), Ips grandicollis (Germar) in the Southeastern USA Monograph 12. Gainesville, FL: Agricultural Experiment Station, University of Florida, 47 pp, 194.
- Gan, J. "Risk and damage of southern pine beetle outbreaks under global climate change." Forest Ecology and Management 191 (2004): 61-71.
- Harlow, W. H. and Harrar, E.S. Textbook of Dendrology. New York: McGraw-Hill, 1968.
- Hodges, J. D., W. W. Elam, W. F. Watson, and T. E. Nebeker. "Physical properties of the oleoresin system of the four major southern pines." The Canadian Entomologist 111 (1977): 889-896.
- Kelsey, R. G., D. Gallego, F.J.Sanchez-Garcia, and J.A. Pajares. "Ethanol accumulation during severe drought may signal tree vulnerability to detection and attack by bark beetles." Canada Journal of Forest Research: 44 (2014): 554-561.
- Klepzig, K. D., D. J. Robison, G. Fowler, P. R. Minchin, F. P. Hain, and H. L. Allen. "Effects of mass inoculation on induced oleoresin response in intensively managed loblolly pine." Tree Physiology 25 (2005): 681-688.
- Knebel, L and T.R. Wentworth. "Influence of Fire and Southern Pine Beetle on Pine-Dominated Forests in the Linville Gorge Wilderness, North Carolina." Castanea 72(4) (2007): 214-225.
- Knebel, L., D. J. Robison, T. R. Wentworth, and K. D. Klepzig. "Resin flow responses to fertilization, wounding and fungal inoculation in loblolly pine (*Pinus taeda*) in North Carolina." Tree Physiology 28 (2008): 847-853.
- Lewisohn, E., M. Gijzen, and R. Croteau . "Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species." Plant Physiology 96 (1991): 44-49.

- Lombadero, Maria J., Matthew P. Ayres, Peter L. Lorio Jr. Jonathan J. Ruel. "Environmental effects on constitutive and inducible resin defenses of *Pinus taeda*." Ecology Letters (2000): 329-339.
- McQuilken, W.E. "The natural establishment of pine in abandoned fields in the Piedmont Plateau region." Ecology 21 (1940): 135-147.
- Miller, D.R, C. Asaro, and C.W. Berisford. "Attraction of Southern Pine Engravers and Associated Bark Beetles (Coleoptera: Scolytidae) to Ipsenol, Ipsdienol, and Lanierone in Southern United States." Journal of Economic Entomology 98 (6) (2005): 2058-2066.
- Nebeker, T.E., J.D.Hodges, and C.A. Blanche. "Host response to bark beetle and pathogen colonization." Filip, T.D. Schowalter and G.M. Beetle-pathogen interactions in conifer forests. London: Academic Press, 1993. 157-173.
- Paine, T.D., M.C. Birch, and P.Svihra. "Niche breadth and resource partitioning by four sympatric species of bark beetles (Coleoptera: Scolytidae)." Oecologia 48 (1981): 1-6.
- Price, T., C. Doggett, J. Pye, and B. Smith. "A history of southern pine beetle outbreaks in the southeastern United States." Georgia Forestry Commission (1998): 71 pp.
- Raffa, K. F., B. H. Aukema, B. J. Bentz, A. L. Carroll, J. A. Hicke, M. G. Turner, and W. H. Romme. "Cross-scale Drivers of Natural Disturbances Prone to Anthropogenic Amplification: The Dynamics of Bark Beetle Eruptions." BioScience 58(6) (2008): 501-517.
- Riley, M. A. and Goyer, R. A. "Seasonal abundance of beneficial insects and Ips spp. engraver beetles in felled loblolly and slash pines in Louisiana." Journal of Entomological Science: 23 (1988): 357-365.
- Ruel, J.J., M.P. Ayers, and P.L. Lorio. "Loblolly pine responds to mechanical wounding with increased resin flow." Canadian Journal of Forest Research 28 (1998): 596-602.
- Schultz, R. P. "Loblolly- the pine for the twenty-first century." New Forests (1999): 17: 71-88.
- Shultz, R. Loblolly Pine The Ecology and Culture (Pinus taeda L.). Agriculture Handbook 713. New Orleans, Louisiana: USDA Southern Forest Experiment Station, Dec. 1997.

- Snyder, M.A. "Selective herbivory by Abert's squirrel mediated by chemical variability in ponderosa pine." Ecology, 73: (1992): 1730-1741.
- Spring, P. E., M. L. Brewer, J. R. Brown, and M. E. Fanning. "Population Ecology of Loblolly Pine *Pinus taeda* in an Old Field Community." Oikos Vol. 25, Fasc. 1 (1974): 1-6.
- Stephens, S. S. and M. R. Wagner. "Forest Plantations and Biodiversity: A Fresh Perspective." Journal of Forestry (2007): 307-313.
- Thatcher, R. Bark beetles affecting southern pines: a review of current knowledge. Asheville, NC.: USDA Forest Service South Forest Experimental Station Paper 180, 1960.
- Ward, J. D. and Mistretta, P. A. "Impact of pests on forest health." Wear, D. N. and Greis, J. G. Southern Forest Resource Assessment GTR-SRS-53. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station, 2002. 635.
- Ward, J.D. and P.A. Mistretta. Impact of pests on forest health. In: Wear, D.N., J.G. Greis (Eds.). Asheville, NC: Southern Forest Resource Assessment. GTR-SRS-53. USDA Forest Service Southern Research Station, 2002.
- Warren, J. M., H. L. Allen, and F. L. Booker. "Mineral Nutrition, resin flow and phloem phytochemistry in loblolly pine." Tree Physiology 19(10) (1999): 655-663.
- Wear, D. N. and J. G. Greis. "Southern Forest Resource Assessment Summary of Findings." Journal of Forestry (2002): 6-14.
- Westerling, A.L., H. G. Hidalgo, D.R. Cayan, and T.W. Swetman. "Warming and Early Spring Release Increase Western U.S. Forest Wildfire Activity." Science (2006): 940-943.
- Whetton, R., Ying-Hsuan Sun, Yi Zhang and R. Sederoff. "Functional genomics and cell wall biosynthesis in loblolly pine." Plant Molecular Biology 47: (2001): 275–291.