

## ABSTRACT

BLINKA, KATE WHITLEY. Resin Flow in Clonal Loblolly Pine. (Under the direction of Fred P. Hain.)

Loblolly pines, *Pinus taeda* L., are a vital part of the timber industry throughout the southern United States and are susceptible to damage and mortality as a result of attack by southern pine beetles, *Dendroctonus frontalis* Zimmerman. The primary defense of loblolly pines against bark beetles is constitutive resin, which provides a physical barrier to beetle colonization. A study has been conducted to determine resin production heritability in clonal loblolly pines. Samples were taken in the spring and late summer of 2005 and 2006 in Milton, FL and Walterboro, SC. Each site included nine complete random blocks containing improved and unimproved checks and 115 and 149 sampled clones, respectively. Two resin samples were collected at breast height from each tree after resin was allowed to flow for 22 +/- 2 hours. Samples were then dried until a subset reached constant mass and sample mass data were used to determine total resin flow repeatability. Total resin flow was found to be significantly higher in one family than the improved check. Resin production by clonal loblolly pines was found to have moderately high repeatability and low positive correlation with tree volume. Results indicate that clone selection that includes resin production as well as desirable growth characteristics can result in loblolly pines that display decreased susceptibility to the southern pine beetle.

Resin Flow in Clonal Loblolly Pine

by  
Kate Whitley Blinka

A thesis submitted to the Graduate Faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the Degree of  
Master of Science

Entomology

Raleigh, North Carolina

2007

APPROVED BY:

---

Dr. John Monahan

---

Dr. Barry Goldfarb

---

Dr. Fred Hain  
Chair of Advisory Committee

## **Dedication**

I would like to dedicate this thesis to my husband, Eric Blinka, my parents, Jerry and Cindy Whitley, my brothers Zack and Ben, and my Grandma, Mary Parks. With out their love, faith, and support this thesis would not have been possible. This work is also dedicated to Arabella Michelle who gave me the extra push to finish.

## **Biography**

Megan Kate Whitley Blinka was born to Jerry and Cindy Whitley in Greensboro, NC. She graduated from Southeast Guilford High School in May of 2000. In August of 2000 Kate enrolled at the University of North Carolina at Chapel Hill, pursuing a degree in biology. Kate graduated from UNC-Chapel Hill in May of 2004 with a BS in biology and a minor in chemistry. Kate then enrolled at North Carolina State University, in August of 2004; to pursue a master's in entomology.

## Acknowledgements

I would like to acknowledge the members of my committee, Dr. Fred Hain, Dr. Barry Goldfarb, and Dr. John Monahan, for help with research, analysis, and the writing of this thesis.

I would like to express my appreciation to Dr. Fikret Isik for assisting with data analysis.

A special thanks to Anthony LeBude, David Brown, Donnie Flemming, and Phillip Dougherty who aided me in understanding the design of the plantations.

I would also like to thank all of those who assisted me with data collection: John Strider, Navdip Kaur, Manpreet Sandhu, Zack Whitley, Eric Blinka, David Titus, Britt Hart, Kenneth Dobyms, Pedro Alvarez, Robert J

etton, Adrian Duehl, Brian Strom, and the additional help from the USFS.

In addition I would like to gratefully acknowledge those who helped me secure or allowed me to use their driers for my resin samples: Alexander Krings, Jennifer Bennett, Sharon Freeman, and Dr. Joe Burns. Also Dr. Joel Kingsolver, Greg Ragland, and Matthew Smith for allowing me to utilize their laboratory at UNC-Chapel Hill.

Finally, I would like to acknowledge my family and friends who have given me unending support that made everything possible.

## Table of Contents

List of Figures .....	vi
List of Tables .....	vii
Literature Review.....	1
Introduction.....	2
Southern Pine Beetle Life History .....	2
Southern Pine Beetle Control.....	5
Host Resistance.....	7
Current Direction of Loblolly Pine Production .....	12
Chapter 2.....	15
Introduction.....	16
Materials and Methods.....	18
Experimental Procedure.....	18
Analytical Procedure.....	21
Results and Discussion .....	24
Works Cited .....	38
Appendices.....	43
Appendix I .....	44
Appendix II.....	46

## List of Figures

<b>Figure I.</b> Sampling of clonal loblolly resin, Milton, FL .....	20
<b>Figure II.</b> Differences in family LSMean resin mass for Florida .....	31
<b>Figure III.</b> Differences in family LSMean resin mass for South Carolina .....	31
<b>Figure IV.</b> LSMean resin mass for top ten resin producing clones for Florida .....	32
<b>Figure V.</b> LSMean resin mass for top ten resin producing clones for South Carolina .....	33
<b>Figure VI.</b> LSMean resin mass (g) by family by clone .....	34
<b>Figure VII.</b> Resin samples from control tree .....	45
<b>Figure VIII.</b> Resin samples from test tree .....	45

## List of Tables

<b>Table I.</b> Significance of fixed effects for model used in SC Proc Mixed analysis .....	30
<b>Table II.</b> Significance of fixed effects for model used in FL Proc Mixed analysis .....	30
<b>Table III.</b> Mean resin mass (g) for each site by season and year.....	30
<b>Table IV.</b> Data for top ten resin producing clones in Florida .....	32
<b>Table V.</b> Data for top fifteen resin producing clones in South Carolina.....	33
<b>Table VI.</b> Site Statistics.....	37
<b>Table VII.</b> Genetic correlations of resin mass (g) to growth traits, included the correlation and its associated standard error .....	37
<b>Table VIII.</b> Rank, LSMeans, and associated standard errors of all South Carolina clones and checks.....	46
<b>Table IX.</b> Rank, LSMeans, and associated standard errors of all Florida clones and checks .....	48



## **Literature Review**

## **Introduction**

Throughout the southern United States, forestry has been an important part of the economy (Wear and Greis, 2002). Due to their rapid growth, loblolly pines (*Pinus taeda*) are often the trees of choice in plantations ranging from Virginia to Texas (McKeand et al. 2003). Loblolly pine is a native species susceptible to southern pine beetle, *Dendroctonus frontalis* Zimmerman, especially when the trees are under stress. With an increasing demand for pulp and timber products, trees are being planted in large single species plantations to increase the economic feasibility of timber production. Ordinarily southern pine beetles are a pest in trees that have been weakened by lightning strikes, or a severe stress such as crown loss in high wind, but at high population densities, southern pine beetles can overcome the defenses of healthy mature trees. Southern pine beetle populations are known to cycle from low endemic levels to catastrophic epidemics, which can be devastating to the timber industry (Payne 1981). Losses from a single season may exceed \$350 million (Wullschlegel 2004) making it necessary to find ways of protecting the timber industry from this native pest.

## **Southern Pine Beetle Life History**

The southern pine beetle is in the family *Curculionidae*, subfamily Scolytinae, bark beetles. Adult beetles are 2-4mm in length and dark red to brown. When females mature and emerge from a host they will begin to search for a suitable host for gallery construction and egg laying. The preferred time of flight and host location is from 0900 to 2000 h. (Bunt 1980). When populations are at an endemic level southern pine beetles rely heavily on lightning struck and other highly stressed trees to maintain their population. Several factors

may aid females looking for a suitable host. Ozone, fermenting phloem, chemicals produced by microorganisms in the tree, oleoresin release, and quantitative changes in the monoterpenes from stressed trees may attract female beetles (Nebeker et. al 1993).

Once a female locates a suitable host, she releases the aggregation pheromone frontalin and begins excavation of an egg gallery. Resin is released from the tree, and the beetle works her way through the resin to begin gallery construction. Within the resin, a monoterpene,  $\alpha$ -pinene, reacts synergistically with frontalin, increasing the attraction of male and female southern pine beetles (Payne 1981).

Beetles that land, after the attempt at colonization has begun, explore the tree bole and any pitch tubes created by boring activity of other beetles (Bunt et al 1980). Females select locations to begin boring, while males look for females. Initially, males are attracted at a ratio of three to one, over females. However, once males have landed and found a mate, they release verbenone, which at low concentrations decreases the attraction of males to the tree (Renwick and Vité 1970, Renwick 1969). Once verbenone reaches higher concentrations it will also repel females, resulting in a 1 to 1 ratio of males to females. (Renwick and Vité 1970, Payne et al 1978) This mechanism allows beetles to avoid over crowding in individual hosts. Other beetles in the area are typically attracted to trees located in close proximity to the successfully attacked tree. (Renwick 1969)

The initially high male to female ratio may be necessary because of the methods used by males to locate females. Males search in one of two ways, either walking randomly until encountering female excavation sites or by following the sex-pheromone, trans-verbenol, released by females to attract a mate. Once a male locates a female's tunnel he chirps and works his way into the tunnel. If the tree is still producing resin the male attempts to swim

through the resin to reach the female. (Payne 1981) Males have been known to aid the female in resin removal when the female is in the process of boring (Bunt et al 1980). As the attack continues and more entry sites are created, the constitutive resin will decrease to a level at which the beetles will survive, overcoming “pitch-out”, and successfully invading the tree (Payne 1981).

The monogamous male and female beetle work cooperatively to create winding “s” shaped galleries in, with single egg niches created along the way. If females mate with a second male, that male will not aid in gallery construction (Payne 1981). In addition to creating galleries, beetles inoculate trees with fungi found in their mycangium, aiding larval development.

Southern pine beetle eggs hatch in three to eleven days if temperatures stay between 15° and 30° C. If temperatures drop below 10°C egg hatch may take up to 34 days. Larvae go through four instars, each lasting seven to thirteen days. First instar larvae are 2mm long, yellow-to-white, sub cylindrical, legless, with 10 abdominal segments, and well-developed mandibles. Late fourth instar, red-headed, larvae are 5-7 mm long. Larvae feed on cambial tissue and symbiotic fungi throughout development (Payne 1981).

*Ceratocystiopsis ranaculosus* and *Entomocorticium sp.* are symbiotic fungi associated with southern pine beetles. These two species grow around galleries, providing nutrients for developing larvae. Gallery lengths of larvae that develop in the presence of mutualistic fungi are nearly half the length of galleries developed in the absence of the fungi (Berisford 1981 USDA).

*Ophiostoma minus*, bluestain fungus, is also commonly associated with southern pine beetle. This fungus is not carried within the mycangium, but is found on the exoskeleton of

adult beetles. Bluestain fungus is highly competitive and may prevent successful growth of symbiotic fungi. In laboratory studies bluestain fungus out-competed both mutualistic fungi (Klepzig 1996). Beetle success in trees with bluestain fungus was lower when compared to success of beetles developing outside of the presence of bluestain (Reeve et al 1998, Hofstetter et al 2005).

Pupae are yellowish-white, 3-4mm long, and have visible wing pads and legs folded beneath the body, leaving the abdomen exposed. The pupal stage lasts 5-17 days, preceding the nupial stage, a period prior to sclerotization. After sclerotization, adults emerge and search for a suitable host (Payne 1981).

### **Southern Pine Beetle Control**

Southern pine beetles have been documented as a pest since the late 1700s. Even before the time of large pine plantations this insect has experienced epidemic outbreaks (Price and Doggett 1982). Southern pine beetle populations naturally fluctuate from very low, barely sustained levels, to outbreak levels that can strip the life from thousands of acres of pine stands. There has not been a reasonable control for the pest other than to enhance tree quality within its range (Metcalf et al 1962). The probability of a southern pine beetle attack to a loblolly pine under normal circumstances is  $4.59 \times 10^{-4}$ , but once the beetle is established within the stand, tree risk increases to  $1.81 \times 10^{-2}$  (Zhang and Zeide 1999). The increased risk to trees surrounding an infestation is even greater.

Overall, the best method for preventing southern pine beetle outbreaks is to practice good silviculture (Belanger 1981). Thinning trees to a maximum of 100 square feet of basal area per acre has been shown to increase tree vigor to the point that they are better able to defend against southern pine beetles (Brown et al 1987). Trees are known to be more

susceptible to southern pine beetle infestations when under severe stress and resin production is insufficient. Increasing tree vigor increases resistance, however, caution should be taken to avoid resource allocation to growth over resin production, as discussed under “host resistance”.

A complimentary control measure is cutting infested trees. Cut trees may be left, salvaged, burned, or sprayed with insecticides (Metcalf et al 1962). When “cut and leave” is used, broods are exposed to sunlight and subsequent temperature increases, leading to mortality (Metcalf et al 1962 and Billings 1981). Those beetles that do emerge cannot orient to a pheromone source emanating from a standing tree and will therefore disperse, reducing their likelihood of survival. Salvaged trees must be used before the brood emerges and spreads to additional hosts. Burning trees effectively kills brood, however, as with all cut methods, a buffer must be included to ensure no infested trees are left standing. In addition to insecticide use with cut trees, insecticides may be used on standing trees (Metcalf et al 1962), however this is typically discouraged due to ecological sensitivity and the lack of economic practicality (Billings 1981).

Within plantations, intensive management improves tree health and does not increase the risk of southern pine beetle infestation. However, during an epidemic healthy trees may be overcome. Therefore, in addition to sound silvicultural practices, it is important to select trees, if possible, that are genetically resistant to southern pine beetle (Belanger 1981).

Enhanced growth characteristics such as height, bole diameter, straightness, and resistance to fusiform rust are under genetic control and can be modified by selective breeding (Isik et al. 2005). Therefore, breeding for increased resin flow, as a form of southern pine beetle resistance, also seems reasonable. However, since the majority of tree

genetic improvement programs focus on production instead of pest resistance, there has been concern that fast growing trees may not produce large amounts of resin due to a trade-off in resources. In many systems it is assumed that resources must be allocated to growth and defense differentially. However, fast growing trees are often better at resource acquisition in general and are high resin producers (Roberds et. al. 2003) making them suitable for plantations and increasing resistance to southern pine beetles.

### **Host Resistance**

Pines, like all plants, have a variety of defense mechanisms. While there is no known combination of defenses that grants tree species immunity against southern pine beetles, some species and individuals may have lower degrees of susceptibility, or higher degrees of resistance (Hodges 1977). Defense mechanisms are not specific to individual pests, but are general responses mobilized if physical damage is experienced. Within *Pinus* there is a range of resistance to southern pine beetles, loblolly pines being one of the most susceptible species. However, because of good form and fast growth, loblolly pine is the preferred plantation species throughout the southeast (McKeand 2003).

*Pinus* species rely heavily on resin as a defensive mechanism. While the first defense encountered by southern pine beetles is the bark of the tree, it provides only minimal protection against infestation. Resin production is critical in preventing southern pine beetle colonization, but is a physiologically expensive process for the tree. Therefore, trees have two defense types, constitutive and inducible (Herms and Mattson 1992, Berryman 1972). In pines, the first defensive response is constitutive resin, which is always present in the tree and is released when wounding severs resin ducts. Following constitutive resin release, trees exhibit an induced response. This induced response may be a hypersensitive response to

fungi introduced to the tree. Cells surrounding the site of infection will begin to break down and produce toxic compounds that retard fungal growth. In addition to chemical release, the necrotic region formed around the wound creates a lesion, preventing the spread of fungal infection (Berryman 1972). In addition to the hypersensitive response, resin production from epithelial cells occurs in response to wounding and may serve to reinforce or replace constitutive resin, increasing tree resistance if subsequent southern pine beetle attacks occur (Ruel 1998).

According to the carbon allocation theory, defenses that utilize resin require carbon allocation from primary growth to secondary metabolism, which produces resin. Therefore, there is a trade off between growth and resin production at any given time (Herms and Mattson 1992). When all elements necessary for growth are available, primary metabolism will be the dominant use of carbon. However, when there is a limiting factor to growth, such as drought, secondary metabolism increases, producing more constitutive resin (Lombardero et al 2000). Mild to moderate droughts increase constitutive resin production because fewer resources are utilized for growth (Lombardero et al 2000, Lorio et al 1995, Lorio and Sommers (1986), Warren et al 1999, Bishop 1933).

Like water, fertilization impacts carbon allocation and resin production. Fertilization can reduce resin production by as much as 25-49%, due to an influx of nutrients leading to carbon allocation to growth over resin production (Warren et al 1999). In addition, rapid growth does not necessarily include development of vertical resin ducts, which are typically developed in late-wood. The lack of vertical resin ducts impedes resin mobilization and increases the risk of southern pine beetle colonization (Lorio et al 1995, Warren et al 1999). Results from Klepzig et al (2005) contradict the impacts of fertilization previously described.



However, Klepzig acknowledges that difference in tree age and the inoculation of study trees with blue stain fungus may have had an impact on resin production and carbon allocation. Furthermore, Klepzig's analysis of fertilization examined the impact of fertilizer applied for Warren's research in 1997-1998, and affects may not have been apparent in 2001. Therefore, reports of fertilization increasing or having no impact on resin production may not be accurate and require further examination to determine validity.

While there is a within tree trade off between primary and secondary metabolism, the tradeoff does not necessarily occur at a genetic level. Roberds et al (2003) found that trees with high growth rates are often those that also produce the highest levels of resin. Overall, these trees are better at resource acquisition and allocation, regardless of the environmental conditions.

When trees are attacked, there is an initial drop in resin flow due to a depletion of constitutive resin. Within two days after wounding, resin flow will return to the initial rate and is doubled within three days (Ruel et. al 1998). This response of inducible resin is not usually sufficient to allow recovery from a southern pine beetle attack. However, if the initial attack was unsuccessful it may help trees resist a subsequent attack.

The defensive properties of resin are two fold. The primary defense is found within the physical properties of the resin itself. In addition to its physical characteristics, resin contains volatile chemical compounds that may be toxic to southern pine beetles.

Physical properties of resin include crystallization rate, viscosity, duration of flow after wounding, and total resin flow. Overall, loblolly pines have been found to display a combination of these properties that leave them more susceptible to southern pine beetle

attacks. When loblolly pines were compared to other pine species it was found that loblolly pines were consistently less resistant than slash and longleaf pines (Hodges 1977).

Crystallization rate describes how rapidly resin crystallizes and hardens. Resin that has begun to crystallize is more difficult for beetles to move through (Hodges 1979). Crystallization rate of loblolly pine resin was found to be as fast as five to ten minutes, while it exceeded forty-eight hours in slash pine (Hodges et al 1977). Slash pine is considered more resistant, however the mechanism for resistance may differ from that used by loblolly pines. Loblolly pine rapid crystallization may be necessary due to a lower viscosity and total flow, as described in following sections. Slow crystallization of slash resin may increase the effectiveness of high viscosity and total resin flow.

Viscosity is known to be higher in slash and longleaf than in loblolly. It is believe that higher viscosity makes resin removal from galleries more difficult for southern pine beetles, decreasing fecundity and survival, due to the increased work required for successful gallery construction (Hodges 1977).

The rate and duration of flow are also important factors in bark beetle resistance and in 50% of loblolly pines there was no resin flow after 24 hours, while 100% of slash and 95% of longleaf pines still had resin flow after a full day. Rate of flow was also fastest in longleaf pines (Hodges 1977). A higher rate and longer duration of resin flow makes colonization more difficult. Longleaf pines did not succumb to southern pine beetle attack until beetle density reached 200-300 attacks per square meter (Hodges 1979). The average loblolly pine was successfully overcome at rates of only 100 attacks per square meter. This supports the suggestion that a high rate and duration of resin flow have a negative impact on southern pine beetle success.

Finally, Hodges (1977) found total resin flow significantly higher in longleaf pines than loblolly pines. The total flow is very important in southern pine beetle resistance. Progeny of loblolly pines that escaped infestation had 1.65 times the total average resin flow of typical loblolly pines (Strom 2002). Resin flow has a genetic (Nebeker et al 1992, Roberds et al 2003), and an environmental component (Matson et al 1987, Brown 1987). Vertical resin duct density increases in trees after thinning as well as trees under a water deficit, and results in increased total resin flow (Blanch et al 1992).

In addition to the physical properties of resin, there are also chemical properties that may increase the tree's attractiveness to southern pine beetles, or may act as a repellent or toxin. The two components that make up resin are monoterpenes and resin acids, or rosins.

Monoterpenes are highly volatile compounds that can have a variety of effects on southern pine beetles; the ratio of these compounds is hereditary (Squillace et. al 1980, Rockwood 1973). All monoterpenes are toxic to southern pine beetles to some extent, the generally accepted ranking of toxicity is as follows: limonene, myrcene,  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -phellandrene, and camphene (Cook and Hain 1985).

The monoterpene,  $\alpha$ -pinene, is known to react synergistically with the southern pine beetle aggregation pheromone frontalin (Renwick and Vité 1970). While  $\alpha$ -pinene is a toxin to southern pine beetles, its potential negative impacts are over shadowed by benefits to southern pine beetle aggregation. Thinning might reduce  $\alpha$ -pinene concentrations (Matson 1987), in addition to increasing overall resin flow, which suggests that thinning may increase resistance in multiple ways.

While the remaining monoterpenes do not benefit the southern pine beetle, they may not be strongly correlated with resistance, since loblolly pines have higher concentrations of

limonene, the most toxic monoterpene, than longleaf pines, which are known to be less susceptible (Hodges 1979). Myrcene concentration is higher in loblolly pines that have escaped southern pine beetle attack, than those that have succumbed (Cook and Hain 1985, and Gollob 1980). In addition, resistant trees had no apparent limonene present (Gollob 1980). Thus, simple monoterpene toxicity and concentration are not considered to have a strong relationship to loblolly pine resistance.

Resin acid, rosin, is the other chemical component of loblolly resin. Little research has been conducted regarding the repellent properties of rosin. However, early work with 4-allylanisole suggests that it can act as a repellent to male and female southern pine beetles. However, repellent qualities of 4-allylanisole are only apparent in laboratory tests and additional work is needed to determine if there is any basis for this chemical as a deterrent in the field. The retardation of the growth of symbiotic fungi is a more promising impact of four-allylanisole that has been observed, however more research is needed (Hayes et al. 1994). In addition to its chemical properties, resin acid left behind after monoterpene volatilization creates the characteristic pitch tubes visible on trees attacked by the southern pine beetle (Phillips and Croteau 1999). It is likely that the amount of resin acid contributes the majority of mass to total resin flow.

Overall, it is believed that the most important component of host resistance to southern pine beetle is the amount, duration, and rate of resin flow. The physical “pitch-out” of the pioneering beetles is the best line of defense for potential host trees.

### **Current Direction of Loblolly Pine Production**

When considering the susceptibility of loblolly pines to southern pine beetles, it is necessary to understand the importance of loblolly pines to the timber industry.

Approximately 1.2 billion loblolly pine seedlings are planted each year (McKeand 2003); by comparison only 150 million of the more resistant slash pines are planted. Eighty-four percent of all seedlings in the south are loblolly pine, and this percentage is not expected to decrease. With ever growing demands on the timber industry it will be necessary to increase productivity of plantations without increasing land use. It is believed that intensive management of loblolly pines will allow the timber industry to meet demands, without increasing land holdings (Yin and Sedjo 2001), by decreasing rotations from the current rate of 18-25 years to 12-15 years (Borders and Bailey 2001). Clones and improved tree stock may also increase productivity (Bishir and Roberds 1999), potentially by as much as 59% while also reducing rotation time (McKeand 2003).

When considering benefits provided by intensive management and improved tree stock it is clear that financial benefits will follow. There has been some concern expressed that clones will decrease genetic variability and increase plantation risk to infestation by various pests. While the risk of using only one clone may be offset by financial gains (McKeand 2003), it is also necessary to examine potential hazards. One concern is that a decrease in genetic variability will result in pests overcoming host resistance; however, natural populations of loblolly pines will provide reservoirs for pests (Bridgwater et al 2005) that will allow pests to avoid extreme evolutionary pressure and prevent development of widespread resistance to host defenses.

Considering the positive implications for productivity as a result of using clones in loblolly pine plantations, more research should be conducted to determine if it is possible to breed trees for southern pine beetle resistance. While plantations do provide optimal growing conditions and minimal competition due to tree spacing and thinning, plantations not confer

immunity to the southern pine beetle. The positive relationship between resin flow and growth rate (Roberds et al 2003) suggest that economically viable trees may also exhibit bark beetle resistance. As of 2003, there were no operational clonal plantations; however it is likely that industry will move in that direction in the near future (McKeand et al. 2003). One study of clonal loblolly pine growth characteristics found that within family clonal means of growth characteristics have moderately high repeatability and that desirable traits have a positive genetic correlation (Isik et al. 2005). Since resin production has a positive correlation to profitable growth traits in loblolly pine families (Roberds et al. 2003), it is important to know whether a similar relationship exists in loblolly pine clones within families. It is imperative that as the timber industry develops clones and improved families, resistance to pests is considered in addition to productivity and growth.

## **Chapter 2**

## Introduction

Throughout the southern United States the timber industry has grown rapidly over the past several decades, with more than 1.2 billion loblolly pine seedlings planted annually. As advances have been made in the field of genetics, loblolly pines have been bred and cloned to increase yields and resistance to pathogens such as fusiform rust. It is believed that improved families have the capacity to increase production 10-30%, while clones selected for superior growth characteristics will be able to increase production by as much as 50% (McKeand 2003). In addition to an increase in productivity, it is believed that clones will decrease rotation time, increasing the profitability of loblolly pines (Bishir and Roberds 1999).

The southern pine beetle, *Dendroctonus frontalis* (Zimmerman), is a serious pest affecting loblolly pines throughout the southern United States. Eighty-four percent of all seedlings in the south are loblolly, favored over more resistant species, such as slash, at a ratio exceeding seven to one (McKeand 2003). Losses in a single season from southern pine beetle damage to loblolly pines may exceed \$350 million (Wullschleger 2004) and suppression programs are considered economically justified (Clarke and Billings 2003). In addition to southern pine beetle suppression, increasing host defense can decrease economic loss.

Loblolly pines are considered to be susceptible to *D. frontalis*, but some individuals display high resin flow, an important characteristic in southern pine beetle resistance (Lorio and Hodges 1977, Hodges 1977, Hodges et al 1979). Resin flow is the first defense encountered by southern pine beetles when tree colonization begins. As beetles bore into the bole, resin is exuded from wounds providing a physical barrier to infestation. The presence of resin increases the energy expenditure for colonizing southern pine beetles, making



colonization of trees with high levels of resin flow difficult (Lorio and Hodges 1977). Longleaf pines, a more resistant species with higher total resin flow, succumb to southern pine beetle attack after beetle density reaches 200-300 attacks per square meter, as opposed to 100 attacks per square meter for loblolly pine colonization (Hodges 1979). Within loblolly pines, higher total resin flow has been found to correlate with resistance to southern pine beetle attacks (Lorio and Hodges 1977, Hodges et al 1979) and progeny of loblolly pines that have escaped infestation had 1.65 times the total average resin flow of typical loblolly pines (Strom 2002).

Resin flow is influenced by a genetic component (Nebeker et al 1992, Strom 2002, Roberds et al 2003), as well as an environmental component (Matson et al 1987, Brown 1987). Families with elevated levels of resin production are often faster growing (Roberds et al. 2003). Roberds examined resin flow data as well as growth characteristics of juvenile loblolly pines and suggested that resin production and growth rate may be pleiotropic, thus, selecting for families with rapid growth will typically result in families that also display resistance to southern pine beetle. This suggests that it is possible to have economically viable loblolly pines that are less susceptible to southern pine beetle. However, Roberds does caution that resin production should be included in selection parameters for plantation stock to ensure that high levels of resistance are expressed (Roberds et al 2003).

With the increasing possibility of using clones in plantations, resin heritability research has become even more important. While, as of 2003, there were no substantial commercial clonal plantations, it is believed that clonal plantations will become more common in the future (McKeand 2003). Therefore, it is important that clones selected for good growth characteristics also be examined for their resistance to pests and pathogens.

Loblolly pine clones demonstrate within-family repeatability of clonal means for characteristics associated with good growth (Isik et al 2005). As previously stated, resin flow is dependent upon genetics as well as environmental affects and breeding for good growth characters will typically also result in trees with high resin production. However, not all trees with acceptable growth characters are also high resin producers (Roberds et al 2003), so care must be taken to ensure that the most resistant clones are deployed.

While work has been conducted to determine that resin production is heritable in loblolly pine families, this research has not been extended to examining clones within families. In the work presented here, resin production in loblolly pine clones has been examined from field tests plots containing numerous clones from the same family. Resin samples were analyzed for resin flow between and within clones. The objective of this study was to determine the heritability of resin production and its genetic correlation to growth characteristics in loblolly pine clones.

### **Materials and Methods**

**Experimental Procedure:** Resin samples were collected from two clonal plantations originally established by the North Carolina State University Tree Improvement Cooperative. Full-sib families used to establish these sites were unrelated and chosen on the basis of good growth characteristics. Seeds were planted in 1996 and 15 stem cuttings were taken from each seedling in February 1998. Cuttings were rooted and a total of 450 clones, those with at least nine surviving ramets, were used to establish the field tests. The 282 clones from families F, H, K, and I were planted near Walterboro, South Carolina in November of 1998. The remaining 168 clones, from families A, C, D, and E, were planted near Milton, Florida in December of 1998. In addition to clones from these families, both sites contain check trees,

an improved check composed of a single family that has been planted widely in the southeast and an unimproved check of trees from multiple families, which has previously been used to quantify genetic improvement of selected stock. Both sites included nine complete randomized blocks with a single ramet of each clone present as well as individuals from the improved and unimproved checks (Isik et al 2005).

Clones for studying resin production were selected based on tree mortality and fusiform rust data from 2004 (Isik et al. 2005). At least five living ramets, free of fusiform rust, were required for the clone to be included in the study. Suitable clones were selected and each tree was given an individual identification number in the field. Trees that were found to be unhealthy or damaged in the field were removed from the study.

In Florida, 894 trees were selected for sampling. A total of 121 clones were sampled, each from one of four families A, C, D, or E. Samples were also taken from the improved and unimproved checks, 40 and 34 samples respectively. Sampling was conducted in the spring of 2005, as well as the spring and fall of 2006. A fall of 2005 data set was not collected due to hurricane activity during the late summer.

A total of 1076 trees were selected for the South Carolina site. A total of 151 clones were sampled, each from one of the four families F, H, K, or I. In addition to samples from the selected clones, 28 improved checks were sampled and 12 unimproved checks. Sampling was conducted in the spring and fall of 2005 and the spring and fall of 2006. The fall 2005 sample set was not complete and only consisted of five blocks due to adverse weather conditions.

Samples were collected using the same technique at both locations. Two samples were taken at or just below breast height on opposite sides of the tree. The tree was prepared

by shaving the bark with a paint scraper to create a flattened surface. Once all selected trees were scraped a size 10 leather punch and rubber mallet were used to create a small round hole through the bark of the tree to the cambium. Care was taken not to damage the cambium, and the hole was rejected and redone if damage did occur. A custom-made plastic sampler (Karsky 2004) was then screwed into place over the hole. The sampler was held in place by two screws and allowed for a 15mL centrifuge vial to be screwed into place to collect resin (Figure I). The vials were left in place for 22 (+/- 2) hours, and then removed and immediately capped.



**Figure I.** Sampling of clonal loblolly resin, Milton, FL.

Except for the fall 2006 samples, the resin samples were dried at approximately 42°C until a constant mass of a sub-sample was achieved. Sub-sample size was approximately 10% of samples in the drier at that time. Drying was necessary due to rain events during collection that contaminated samples. Samples that were full of water were rejected and all remaining samples were dried. The dry weight of each sample was measured to the thousandths of a gram and recorded.

For samples collected in the fall of 2006 no rain contamination occurred. These samples were not dried, but a wet weight measurement was taken for each.

**Analytical Procedures:** Data were analyzed to examine resin production for family and individual clones across all years and seasons for each site.

Analysis of family resin production included a transformation of data from the fall of 2006 for both sites. Data were transformed from obtained wet weights to estimated dry weights according to subset drying results. Samples, free of apparent water contamination, were weighed before and after drying. One sample was not used in  $R^2$  estimation, as it appeared to be an extreme outlier. According to SAS Proc GLM,  $R^2 = 0.99$ , indicating a strong relationship between wet and dry resin mass. The relationship was linear, following:

$$M_D = \beta_0 + \beta_1 M_w$$

$M_D$  = estimated dry mass (g)  
 $\beta_0 = 0.511$   
 $\beta_1 = 0.908$   
 $M_w$  = initial wet mass (g)

Using transformed data for fall 2006 and dry mass measurements for all other samplings, SAS Proc Mixed analysis was used to determine significant difference in mean family resin mass across all years and seasons for each site. Alpha values were transformed by the Bonferroni pair-wise comparison method, resulting in an experimental alpha-value of 0.05 and a pair-wise comparison value of 0.0033.

Progeny of loblolly pines that escaped southern pine beetle attack produced 65% more resin, by mass, than progeny of trees of control loblolly pines (Strom et al. 2002). Therefore, a threshold was established, based on an increase of 1.65 over mean site resin production. Exceeding this threshold may indicate an increased level of resistance to the southern pine beetle. However, exceeding this threshold is not considered a true indicator of resistance and is primarily used to determine which clones are superior resin producers at each site for future research. Clonal resin mass was examined using SAS Proc GLM, using

transformed dry mass for fall 2006 data and untransformed data for all other seasons and years. Clones were ranked according to LS Mean resin production and compared to the site mean and threshold.

The following linear mixed model was fitted to resin weight to partition phenotypic variance into causal components.

$$[1] R_{ijklmn} = u + Y_i + S(Y)_{j(i)} + B_k + BY_{ik} + BS(Y)_{kj(i)} + F_l + FY_{li} + FB(YS)_{lk(ij)} + FS(Y)_{lj(i)} + C_{m(l)} + CS(FY)_{m(li)} + E_{ijklmn}$$

Where,

$R_{ijklmn}$  = is the n-th

$u$  = overall mean

$Y_i$  is the fixed i-th year effect, (i=2),

$S(Y)_{j(i)}$  is the fixed j-th season effected nested within year i, (j=2),

$B_k$  is the fixed k-th block effect (k=9),

$BY_{ik}$  is the fixed k-th block by i-th year interaction,

$BS(Y)_{kj(i)}$  is the fixed k-th block by j-th season interaction nested within the i-th year,

$F_l$  is the fixed l-th family effect (l=4),

$FY_{li}$  is the fixed l-th family by i-th year interaction,

$FB(YS)_{lk(ij)}$  is the fixed l-th family by block effect nested within year and season

$FS(Y)_{lj(i)}$  is the fixed l-th family by j-th season effect nested within year

$C_{m(l)}$  is the random m-th clone effect nested within l-th family with expected zero mean and variance (0,  $\sigma_c^2$ ),

$CS(Y)_{m(li)}$  is the random m-th clone by j-th season effect nested within i-th year, with expected zero mean and variance  $(0, \sigma^2_{cs})$ ,

$E_{ijklmn}$  is the random error normally and independently distributed with expected mean zero and variance  $\sim NID(0, \sigma^2_e)$ .

Using the variance components from above model, repeatability of clone means for resin mass production was calculated.

$$[2] \quad H^2_c = \frac{\sigma_c^2}{\sigma_c^2 + \frac{\sigma_{cs}^2}{s} + \frac{\sigma_e^2}{sb}}$$

Where,

$H^2_c$  is the repeatability of clone means for resin mass,

$\sigma_c^2$  is the variance component explained by the clone differences,

$\sigma_{cs}^2$  is the clone by season interaction variance component,

$\sigma_e^2$  is the error variance,

s is the number of seasons (s=2),

b is the number of trees per clone (b=9).

The model was run with SAS Proc Mixed procedure (SAS Institute 1996). The variance component explained by the clones is genetic; clone by season interaction and the error variances describe environmental effects. Standard error of the repeatability was estimated using the Delta method and a SAS /IML code (Pers. comm. Dr. Fikret Isik, North Carolina State University, Raleigh, USA). Heritability could not be calculated in this study due to the inability of the experiment to break down genetic variation into additive and non-additive components.

In order to examine the relationship of resin mass to height, volume, branch angle, forking and ramicorn branching, product-moment correlations and genetic correlations were estimated. Phenotypic correlations were calculated using the CORR procedure of SAS (SAS Institute 1996). Genetic correlations of resin mass and continuous variables were estimated fitting a bivariate mixed model and using a SAS Proc Mixed procedure code (Pers. comm. Dr. Fikret Isik, North Carolina State University, Raleigh, USA), where genetic correlations of resin mass with binary variables were calculated using ASReml software (Gilmour et al. 2002). Standard errors of genetic correlations were estimated using the Delta method (Isik et al. 2005).

### **Results and Discussion**

Multiple factors had a significant effect on resin production (SAS Proc Mixed, alpha level 0.05). The greatest effect was due to seasons within a year followed by yearly effects. The impact of these factors was expected due to seasonal variation in resource allocation between primary and secondary metabolism as well as variation across years in resource availability. Family was also found to have a large effect on mean resin production, indicating a genetic component (Table I and Table II).

Mean resin mass demonstrated a consistent pattern of fluctuation across year and season, regardless of location. Resin mass was higher in the fall than the spring and in 2006 than 2005 (Table III). As previously suggested, meteorological data for sites could provide an explanation for observed fluctuations.

Significant differences existed between families in both South Carolina and Florida (SAS Proc Mixed). At both sites, mean resin production across families was different.



Florida families E and D, with mean resin masses  $8.26 \text{ g} \pm 0.02$  and  $8.18 \text{ g} \pm 0.03$  respectively, displayed superior mean resin production to families A, C, and the unimproved check. However, mean resin production of families E and D was not significantly greater than mean resin production by the improved check in Florida,  $8.12 \text{ g} \pm 0.10134$  (Figure II).

Within the four families in South Carolina, family F was found to have greater mean resin mass with an LSMeans of  $8.61 \text{ g} \pm 0.02$ . Resin production in family F was significantly different from all other families. The improved check had mean resin mass  $8.02 \text{ g} \pm 0.058$  and, with the exception of family F, was significantly higher than all other families in South Carolina including the unimproved check. All other families were different from one another; however, the unimproved check had a large standard error and did not differ significantly from families I, H, or K (Figure III).

In both Florida and South Carolina the improved check demonstrated not only high resin production, but also the largest trunk volume of the families used in the study. High resin mass families, D, E, and F were found to have larger mean trunk volumes than all other families, excluding the improved check (Isik et al 2005). The superiority of the improved check and families D, E, and F in both resin production and trunk volume, an indicator for rapid growth, suggests these two characteristics are compatible. These findings support Roberds et al. (2003) in suggesting that selecting trees for good growth characteristics may not require a decrease in resin production.

Clonal mean resin production was compared to the threshold determined by a 65% increase over site mean, excluding checks. In Florida LSMeans resin mass was  $7.96 \text{ g}$  with a standard deviation of  $0.48$ , which yielded a threshold value of  $8.70 \text{ g}$ . The LSMeans resin production, including vial weight for South Carolina was  $7.90 \text{ g}$  with a standard deviation of

0.66, thus the threshold value was 8.65 g. In Florida clones that exceeded the threshold were from the two superior families, D and E (Figure IV, Table IV). Resin mass rank by clone revealed thirteen clones from South Carolina that exceeded the threshold; at alpha level 0.05 (Figure V). All clones exceeding the threshold were from family F (Table V).

The improved check did not exceed the threshold at either location. This suggests that while overall family means for families D and E did not differ from the mean resin production of the improved check, individual clones of both families exceeded resin production by the improved check at a significant level.

Comparison of all clones to the threshold value demonstrates the impact of family on mean resin production by individual clones (Figure VI). While threshold value was determined based upon mean resin production for each site, it offers an indication of clone resin production that is clearly superior to the mean and illustrates family trends in resin production.

Repeatability, the proportion of phenotypic variance that may be attributed to genetics, was used to help determine the effectiveness of selection for increased resin production. Repeatability ranges from 0 to 1, with a high level of repeatability suggesting a high success rate of attempted selection. The overall repeatability of clone mean in South Carolina was  $0.866 \pm 0.018$  and in Florida  $0.815 \pm 0.029$ , indicating that additional ramets of high resin producing clones from this study can reasonably be expected to display high levels of resin production. Repeatability was broken down further to year and year-by-season results. Repeatability measurements ranged from  $0.629 \pm 0.053$  in the spring of 2005 at Florida to  $0.848 \pm 0.024$  in the fall of 2005 in South Carolina. While a range of repeatability was found, overall repeatability measurements were moderate to high,

suggesting that it is reasonable to expect desirable results from breeding programs for increased resin production. While no direct parallel may be drawn to narrow and broad sense heritabilities found by Roberds et al (2003), repeatability estimates do support that resin production is under moderate-to-strong genetic control (Table VI).

In addition to implications of genetic control, genetic correlations of resin production to other growth characteristics were also examined (Table VII). Genetic correlations for height across Florida and South Carolina were found to differ significantly,  $0.375 \pm 0.101$  and  $0.107 \pm 0.109$  respectively. Florida data suggests that there is a positive correlation between resin production and tree height, however no correlation was found for South Carolina. Reasons for this discrepancy are not known, however the Florida site was exposed to multiple hurricanes during the course of this study.

Genetic correlations of trunk volume and resin production were found to be significant at both sites and correlations were not different. A positive correlation was expected due to findings of Roberds et al. (2003), however values found here were lower than those found by Roberds et al. Volume may have a positive correlation due to a larger number of resin ducts found in trees with an increased dbh. The positive correlation of resin production and dbh (Roberds et al. 2003) supports the expectation that volume may have a positive correlation to resin production, even if a correlation is not found to tree height. It is expected that trees which exhibit high resin production and large trunk volume are superior at resource acquisition. Resources will preferentially be expended in growth, or primary metabolism, until nitrogen levels are insufficient for photosynthesis and photorespiration to be efficient. High levels of primary metabolism allow trees to out-compete others in their vicinity, increasing their total leaf area and root coverage, allowing them to secure additional

resources. When primary metabolism is limited by nitrogen availability secondary metabolism will increase, as it requires lower levels of nitrogen. Therefore, more resources are available for secondary metabolism and a decrease in nitrogen availability will increase resin production further, when energy cannot be used for growth. Hence, it is reasonable to expect trees that exhibit rapid growth to also have the capacity to produce large amounts of resin.

The genetic correlations between resin production and forking and ramicorn branches was not found to be significant, however, during tree selection, trees were eliminated if forking or a large ramicorn branch were located at or below dbh.

The heritability of resin production and consistent performance in relative total resin flow within a clone suggests that it is possible to develop clonal plantations of loblolly pine that are less susceptible to southern pine beetle attacks. While under outbreak conditions it would be possible for any host's defenses to be overcome by the southern pine beetle, it is expected that trees with high levels of total resin flow would be more capable of repelling attack than individuals with lower constitutive resin flow.

According to the results found in this study, resin production is expected to be repeatable for clonal progeny. However, before clonal production is taken to the plantation level it would be advantageous to conduct additional research in the area of clonal resin production. Desirable clones, such as those found in families F, D, and E should each be tested under a variety of environmental conditions and in different regions throughout the south to ensure that resin production will remain high. Clones should be selected based not only on growth or resin production, but also on the combination of these traits. Through careful selection and parent to progeny comparisons, clones may be developed with the best

combination of economic appeal for growth rate and a decreased susceptibility to the southern pine beetle when selection accounts for resin production.

**Table I.** Significance of fixed effects for model used in SC Proc Mixed analysis.

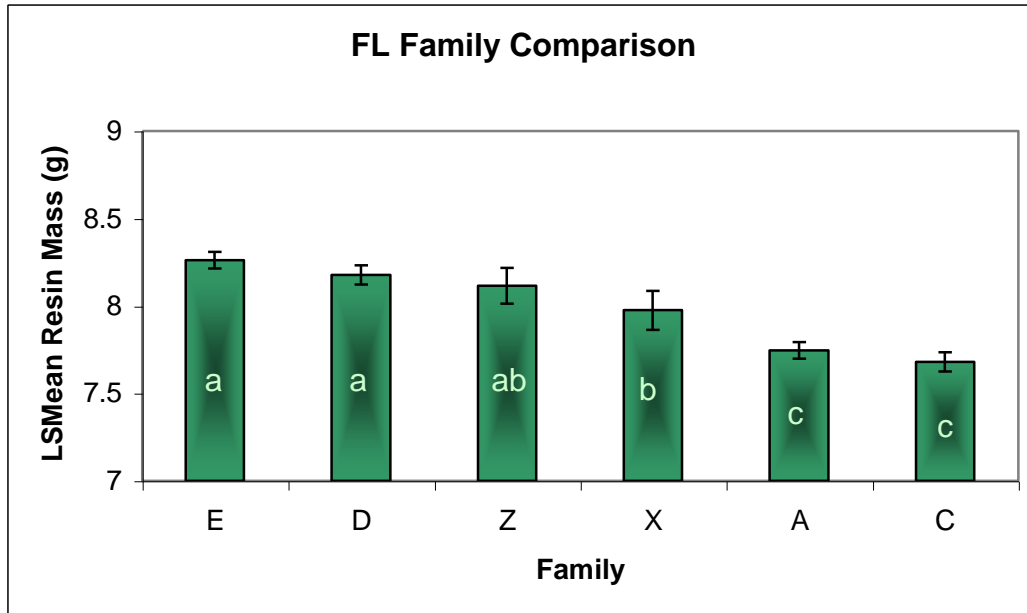
<b>South Carolina Fixed Effects</b>		
<b>Effect</b>	<b>F Value</b>	<b>Pr &gt; F</b>
year	106.03	<0.0001
season (year)	296.9	<0.0001
block	4.2	<0.0001
year*block	3.75	0.0002
season*block (year)	9.5	<0.0001
family	52.63	<0.0001
block*family (year*season)	1.49	0.0031
year*family	8.44	<0.0001
season*family (year)	53.37	<0.0001

**Table II.** Significance of fixed effects for model used in FL Proc Mixed analysis.

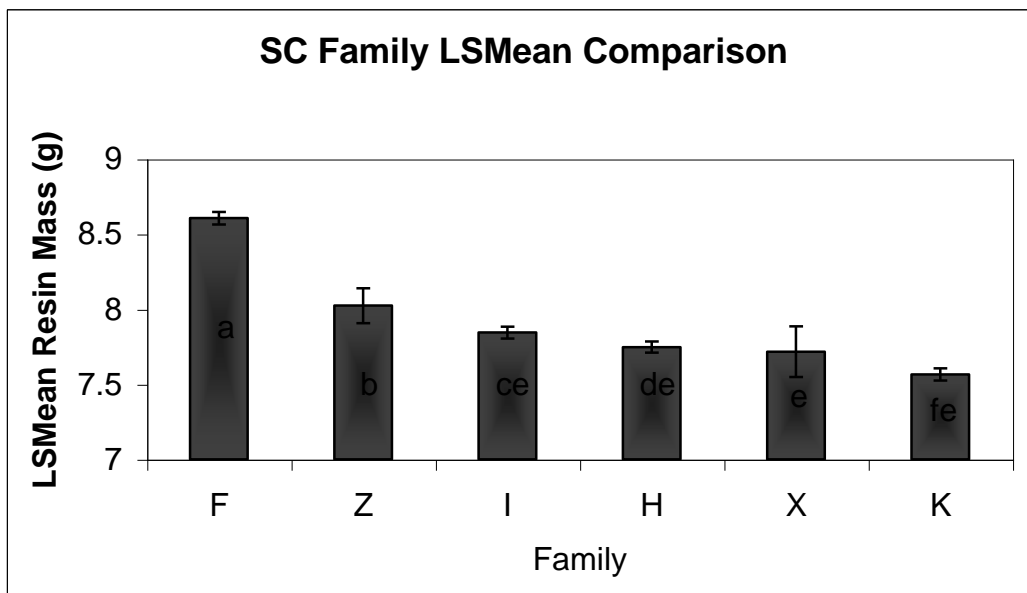
<b>Florida Fixed Effects</b>		
<b>Effect</b>	<b>F Value</b>	<b>Pr &gt; F</b>
year	133.34	<0.0001
season (year)	258.1	<0.0001
block	16.91	<0.0001
year*block	1.46	.1761
season*block (year)	1.61	.1182
family	20.16	<0.0001
block*family (year*season)	1.01	0.4541
season*family (year)	7.13	<0.0001

**Table III.** Mean resin mass (g) for each site by season and year. FL/SC combined indicates the overall mean resin mass for all years, seasons, and sites. Mean values do not included the improved or unimproved checks.

<b>Site</b>	<b>Season/Year</b>	<b>Mean Resin Mass (g) ± Stderr</b>
<b>Florida</b>	2005	7.710 ± 0.022
	2006	8.161 ± 0.020
	Spring	7.763 ± 0.014
	Fall	8.521 ± 0.032
<b>South Carolina</b>	2005	7.807 ± 0.022
	2006	8.160 ± 0.028
	Spring	7.689 ± 0.015
	Fall	8.388 ± 0.034
<b>FL/SC combined</b>	Overall	8.008 ± 0.012



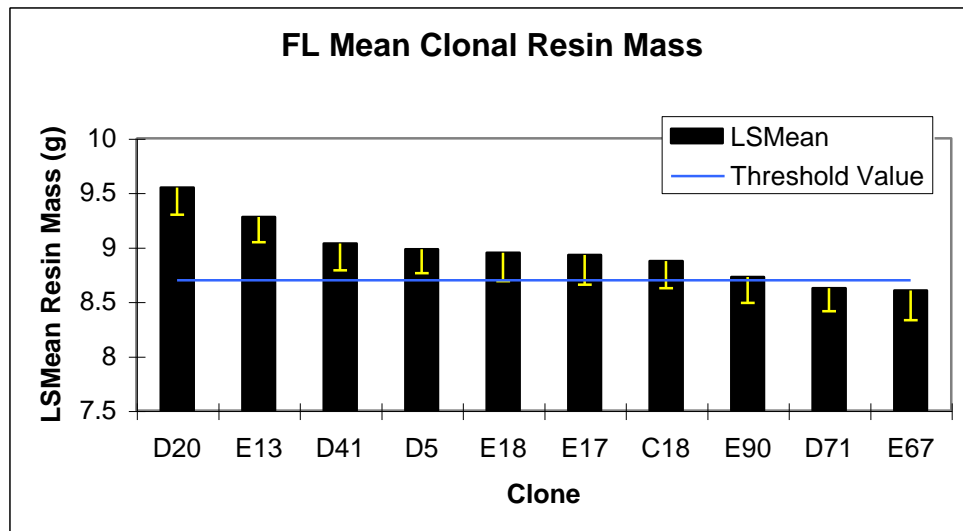
**Figure II.** Differences in family LSMean resin mass for Florida. Family Z represents the improved check and family X represents the unimproved check. Error bars are based on two standard errors from the mean. Significant differences between family LSMeans are indicated by letters on the bars.



**Figure III.** Differences in family LSMean resin mass for South Carolina. Family Z represents the improved check and family X represents the unimproved check. Error bars are based on two standard errors from the mean. Significant differences between family LSMeans are indicated by letters on the bars.

**Table IV.** Data for top ten resin producing clones in Florida. Family refers to family, of which clone is a member. Clone indicates the individual clone id. Rank is the rank of the clone's LSMeans resin mass in relation to all other clones from Florida. LSMeans refers to the LSMeans resin mass (g) and stderr is the associate standard error.

Rank Data Milton, FL				
Family	Clone	Rank	LSMean	Stderr
D	20	1	9.55	0.13
E	13	2	9.28	0.12
D	41	3	9.04	0.12
D	5	4	8.98	0.11
E	18	5	8.95	0.13
E	17	6	8.93	0.14
C	18	7	8.87	0.13
E	90	8	8.73	0.12
D	71	9	8.63	0.11
E	67	10	8.60	0.14

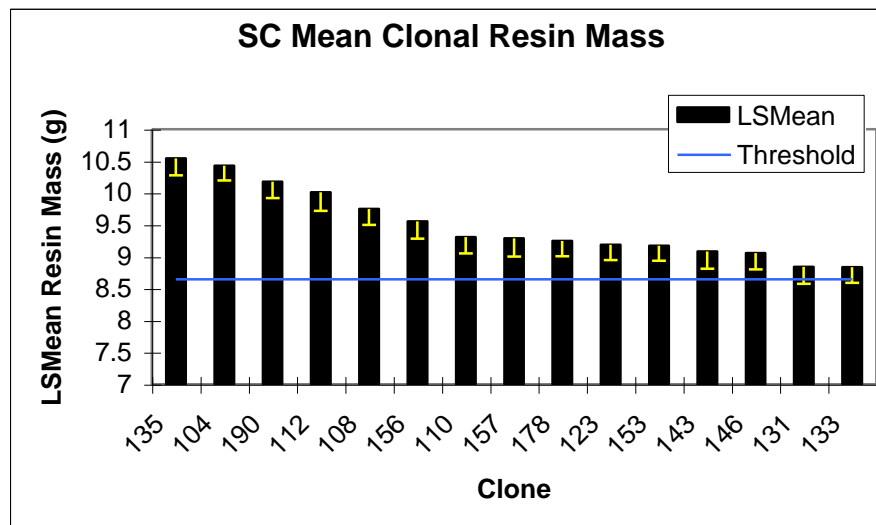


**Figure IV.** LSMeans resin mass for top ten resin producing clones for Florida. Threshold value (8.70 g) based on 65% increase over the LSMeans resin production for all Florida clones at alpha level 0.05.



**Table V.** Data for top fifteen resin producing clones in South Carolina. Family refers to family, of which clone is a member. Clone indicates the individual clone id. Rank is the rank of the clone's LSMean resin mass in relation to all other clones from South Carolina. LSMean refers to the LSMean resin mass (g) and stderr is the associate standard error.

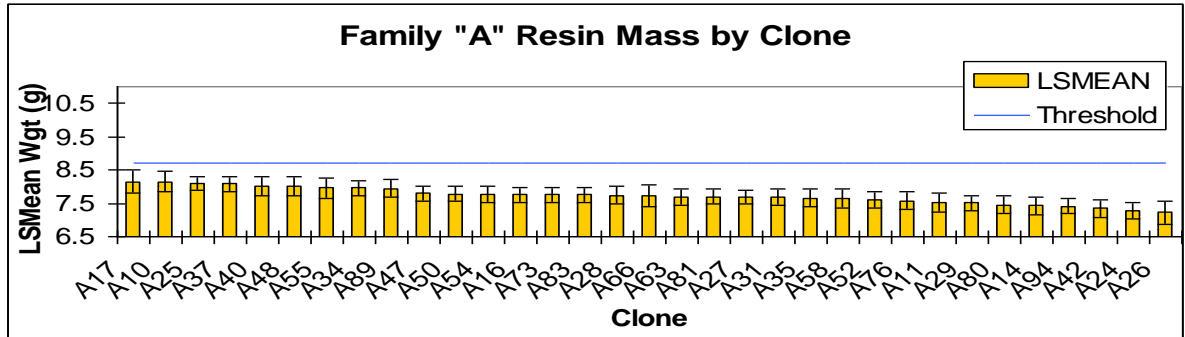
Rank Data Walterboro, SC				
Family	Clone	Rank	LSMean	Stderr
F	135	1	10.54	0.13
F	104	2	10.43	0.11
F	190	3	10.18	0.13
F	112	4	10.01	0.14
F	108	5	9.76	0.13
F	156	6	9.56	0.14
F	110	7	9.31	0.13
F	157	8	9.29	0.14
F	178	9	9.25	0.12
F	123	10	9.19	0.12
F	153	11	9.17	0.12
F	143	12	9.08	0.14
F	146	13	9.06	0.13
F	131	14	8.84	0.13
F	133	15	8.84	0.12



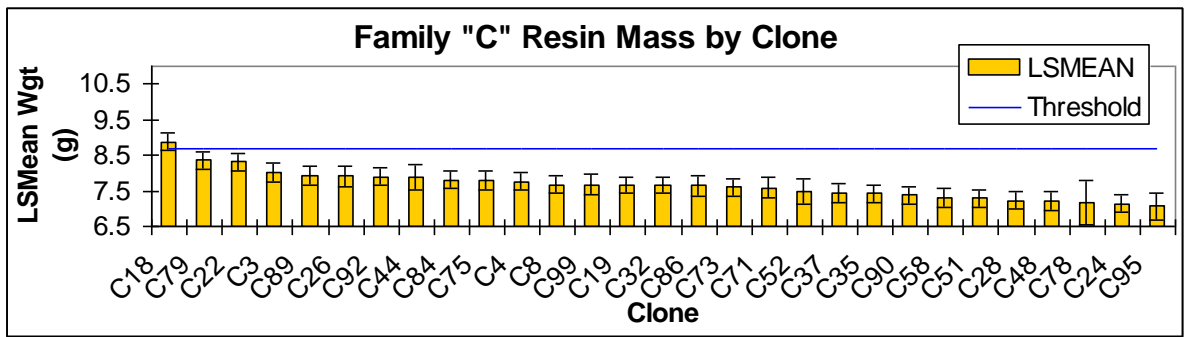
**Figure V.** LS Mean resin mass for top ten resin producing clones for South Carolina. Threshold value (8.65 g) based on 65% increase over the LS Mean resin production for all South Carolina clones at alpha level 0.05.

**Figure VI.** LS Mean resin mass (g) by family by clone. Threshold indicated is 8.70 g for FL families (a-d). Threshold indicated is 8.65 g for SC families (e-h).

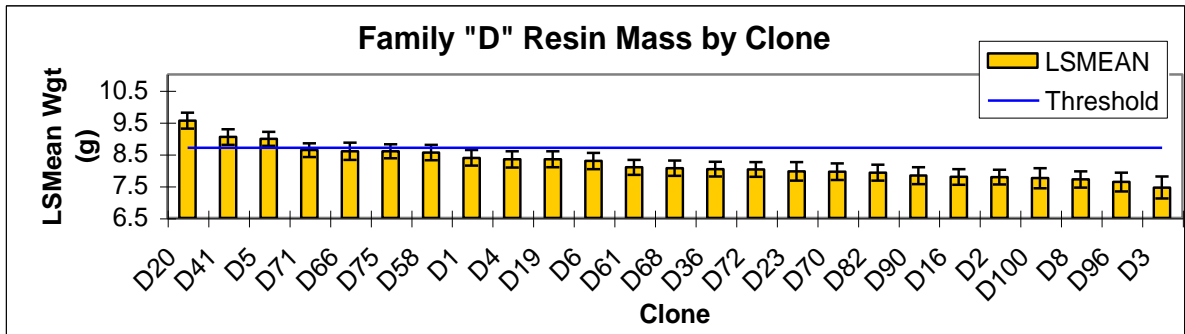
a.



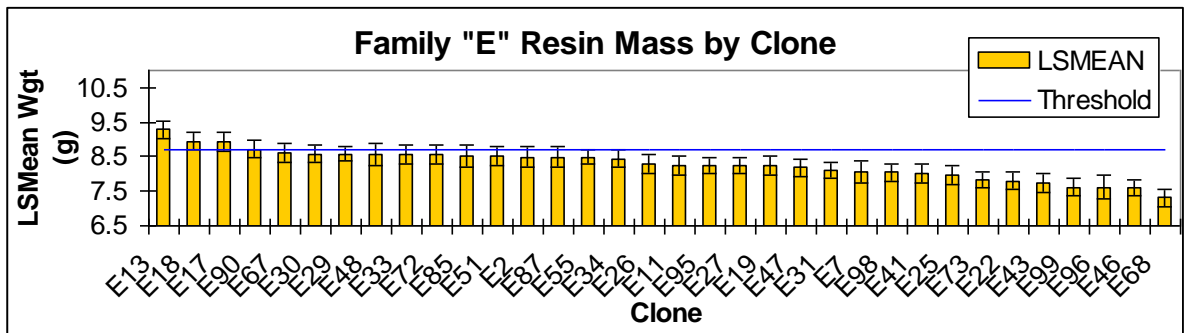
b.



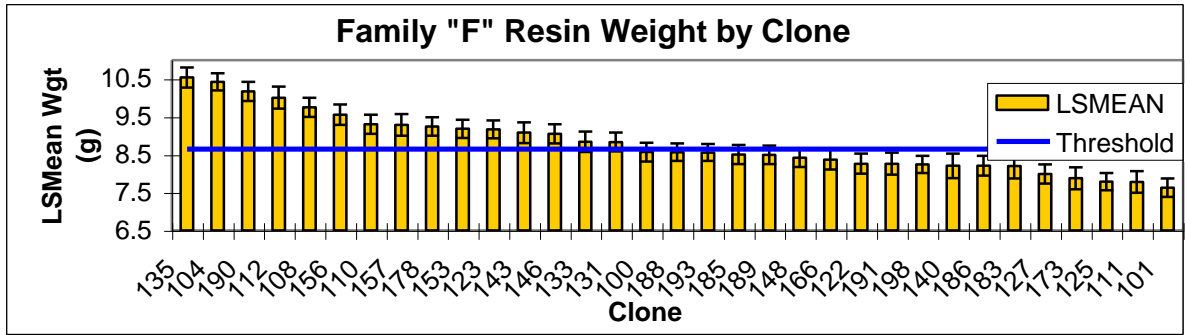
c.



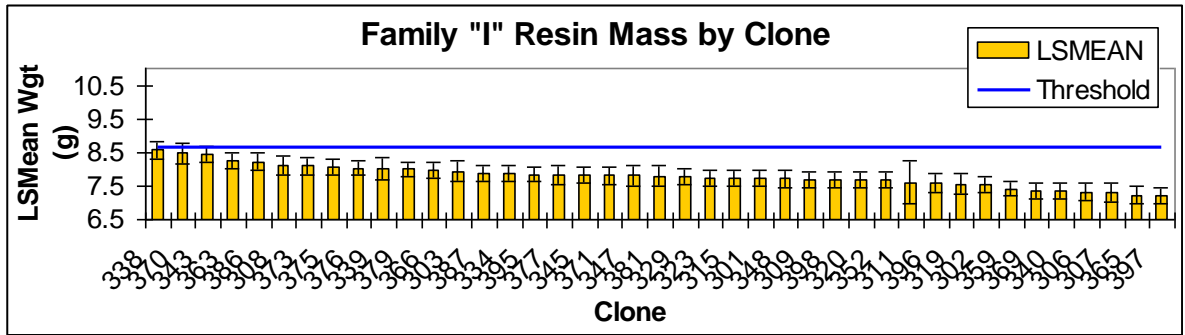
d.



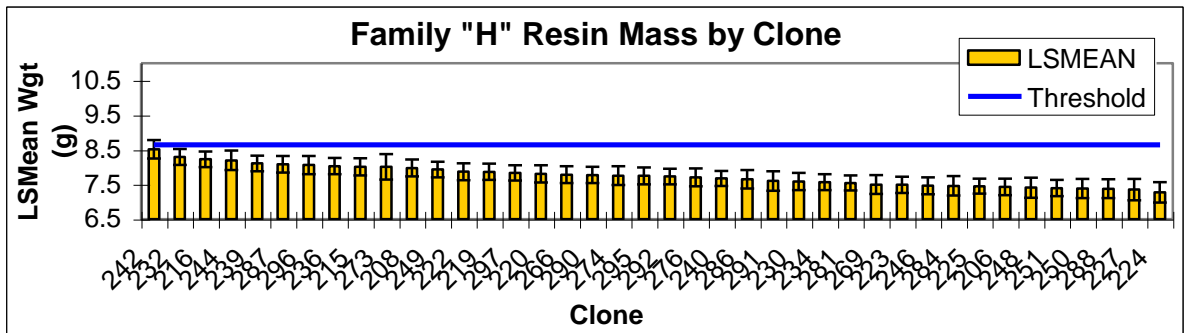
e.



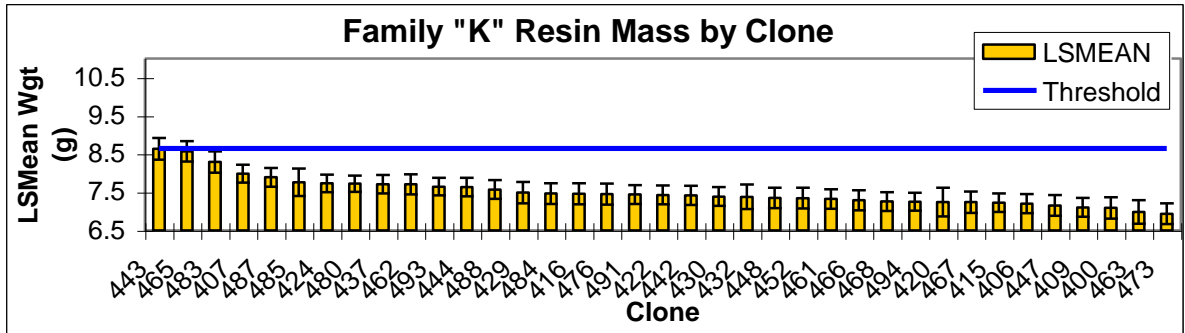
f.



g.



h.



**Table VI.** Site Statistics. LS Mean resin mass refers to the LS mean resin mass, excluding checks, for each site, and its associated standard deviation. Phenotypic variance is the combined influence of genotype and environment. Repeatability refers to the repeatability of mean clonal resin mass.

Site	Season/Year	Phenotypic Variance	Repeatability
<b>FL</b>	Overall	0.167	0.815 ± 0.029
	2005	0.135	0.625 ± 0.053
	2006	0.239	0.784 ± 0.030
	Spring 2005	0.135	0.629 ± 0.053
	Spring 2006	0.107	0.638 ± 0.052
	Fall 2006	0.520	0.810 ± 0.028
	<b>SC</b>	Overall	0.231
2005		0.200	0.794 ± 0.026
2006		0.292	0.748 ± 0.031
Spring 2005		0.161	0.676 ± 0.042
Fall 2005		0.341	0.848 ± 0.024
Spring 2006		0.120	0.692 ± 0.039
Fall 2006		0.661	0.814 ± 0.024

**Table VII.** Genetic correlations of resin mass (g) to growth traits, correlation and its associated standard error. Height5 refers to the height of the tree. Volume5 is trunk volume, taking into account height and dbh. Branch5 refers to the branch angle from the trunk of the tree. Forks5 and Ramicorn5 are binary data. Forks5 refers to the presence or absence of a forked trunk and Ramicorn5 refers to the presence or absence of a large branch that could be considered a small fork. All growth traits are based on data gathered at stand age five.

<b>Genetic Correlation between Mean Resin Mass and Defined Trait</b>					
Site	Height 5	Volume 5	Branch 5	Forks 5	Ramicorn 5
<b>SC</b>	0.107 ± 0.109	0.359 ± 0.101	-0.153 ± 0.208	0.035 ± 0.165	0.031 ± 0.130
<b>FL</b>	0.375 ± 0.101	0.384 ± 0.098	-	0.152 ± 0.153	-0.100 ± 0.177

## Works Cited

- Belanger, R. P. (1981). Silvicultural guidelines for reducing losses to the southern pine beetle. The Southern Pine Beetle. R. C. Thatcher, Searcy, Coster, and Hertel, Technical Bulletin 1631: 165-178.
- Berisford, C. W. (1981). Natural enemies and associated organisms. The Southern Pine Beetle. R. C. Thatcher, Searcy, Coster, and Hertel: 31-52.
- Berryman, A. A. (1972). "Resistance of conifers to invasion by bark beetle-fungus associations." BioScience **22**(10): 598-602.
- Billings, R. F. (1981). Direct control. The Southern Pine Beetle. R. C. Thatcher, Searcy, Coster, and Hertel, Technical Bulletin 1631: 179-194.
- Bishir, J., and J.H. Roberds (1999). "On numbers of clones needed for managing risks in clonal forestry." Forest Genetics **6**(3): 149-155.
- Bishop, G. N. a. G. D. M. (1933). "Some factors influencing resin concentration in loblolly and slash pines." Journal of Forestry **31**(8): 951-960.
- Blanche, C. A., P.L. Lorio Jr., R.A. Sommers, J.D. Hodges, and T.E. Nebeker (1992). "Seasonal cambial growth and development of loblolly pine: xylem formation, inner bark chemistry, resin ducts, and resin flow." Forest Ecology and Management **49**: 151-165.
- Borders, B. E., and R.L. Bailey (2001). "Loblolly pine-pushig the limits of growth." Southern Journal of American Forestry **25**(2): 69-74.
- Bridgwater, F., T. Kubisiak, T. Byram, S. McKeand (2005). "Risk assessment with current deployment strategie for fuisform rust-resistant loblolly and slash pines." Southern Journal of American Forestry **29**(2): 80-87.
- Brown, M. W., T.E. Nebeker, and C.R. Honea (1987). "Thinning increases loblolly pine vigor and resistance to bark beetles." Southern Journal of American Forestry **11**: 28-31.
- Bunt, W. D., J.E. Coster, and P.C. Johnson (1980). "Behavior of the southern pine beetle on the bark of host trees during mass attack." Annals of the Entomological Society of America **73**: 647-652.
- Cook, S. P. and F. P. Hain. (1985). "Qualitative examination of the hypersensitive response of loblolly pine, *Pinuse taeda* L., inoculated with two fungal associates of the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae)." Environmental Entomology **14**: 386-400.

- Clarke, S. R., and R.F. Billings (2003). "Analysis of the southern pine beetle suppression program on the national forest in Texas in the 1990s." Southern Journal of Applied Forestry **27**(2): 122-129.
- Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J., and Thomson, R. 2002. ASREML user guide, release 1.0. VSN International Ltd, Hemel Hempstead, UK.
- Gollob, L. (1980). "Monoterpene composition in bark beetle-resistant loblolly pine." Naturwissenschaften **67**(7): 409-410.
- Hayes, J. L., B.L. Strom, L.M. Roton, and L.L. Ingram Jr. (1994). "Repellent properties of the host compound 4-allylanisole to the southern pine beetle." Journal of Chemical Ecology **20**(7): 1595-1613.
- Herms, D. A., and W.J. Matson (1992). "The dilemma of plants: to grow or defend." The Quarterly Review of Biology **67**(3): 283-335.
- Hodges, J. D., W.W. Elam, and W.F. Watson (1977). "Physical properties of the oleoresin system of the four major southern pines." Canadian Journal of Forest Research **7**: 520-525.
- Hodges, J. D., W. W. Elam, W. F. Watson, and T. E. Nebeker (1979). "Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks." Canadian Entomology **111**: 889-896.
- Hofstetter, R. W., J.T. Cronin, K.D. Klepzig, J.C. Moser, M.P. Ayers (2006). "Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle." Oecologia **147**: 679-691.
- Isik, F., B. Goldfarb, A. LeBude, B. Li, and S. McKeand (2005). "Predicted genetic gains and testing efficiency from two loblolly pine clonal trials." Canadian Journal of Forest Research **35**: 1754-1766.
- Karsky, D., B. Strom, and H. Thistle. (2004) "An improved method for collecting and monitoring pine oleoresin." Technology and Development Program. USDA 0434-2306-MTDC.
- Klepzig, K. D., and R.T. Wilkens (1996). "Competitive interactions among symbiotic fungi of the southern pine beetle." Applied and Environmental microbiology **63**(2): 621-627.
- Klepzig, K. D., D.J. Robinson, G. Fowler, P.R. Minchin, F.P. Hain, and H.L. Allen (2005). "Effects of mass inoculation on induced oleoresin response in intensively managed loblolly pine." Tree Physiology **25**: 681-688.

- Lombardero, M. J., M.P. Ayres, P.L. Lorio, Jr., and J.J. Ruel (2000). "Environmental effects on constitutive and inducible resin defences in *Pinus taeda*." Ecology Letters **3**: 329-339.
- Lorio, P. L. J. and J. D. Hodges. (1977). "Tree water status affects induced southern pine beetle attack and brood production." USDA For. Serv. Res. Pap. **SO-135**: 7.
- Lorio, P. L. J., and R.A. Sommers (1986). "Evidence of competition for photosynthates between growth processes and oleoresin synthesis in *Pinus taeda* L." Tree Physiology **2**: 301-306.
- Lorio, P. L. J., F.M. Stephen, and T.D. Paine (1995). "Environment and ontogeny modify loblolly pine response to induced acute water deficits and bark beetle attack." Forest Ecology and Management **73**: 97-110.
- Matson, P. A., F.P. Hain, and W. Mawby (1987). "Indices of tree susceptibility to bark beetle vary with silvicultural treatment in a loblolly pine plantation." Forest Ecology and Management **22**: 107-118.
- McKeand, S., E.J. Jokela, D.A. Huber, T.D. Byram, H.L. Allen, B. Lee, T.J. Mullin (2006). "Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs." Forest Ecology and Management **227**: 178-184.
- McKeand, S., T. Mullin T. Byram and T. White (2003). "Deployment of genetically improved loblolly and slash pines in the south." Journal of Forestry **101**(3): 32-37.
- Metcalf, C. L., W.P. Flint, and R.L.Metcalf (1962). Insects Attacking Shade Trees and Shrubs: Bark Beetles and Ambrosia Beetles. Destructive and Useful Insects: Their Habits and Control: 846-848.
- Nebeker, T. E., J.D. Hodges, C.A. Blanche, C.R. Honea, and R.A. Tisdale (1992). "Variation in the constitutive defensive system of loblolly pine in relation to bark beetle attack." Forest Science **38**(2): 257-266.
- Nebeker, T. E. J. D. H., and C.A. Blanche (1993). Host response to bark beetle and pathogen colonization. Beetle-Pathogen Interactions in Conifer Forests. T. D. S. a. G. M. Filip, Academic Press Inc: 157-169.
- Payne, T. L. (1981). Life history and habits. The Southern Pine Beetle. R. C. Thatcher, Searcy, Coster, and Hertel: 7-30.
- Payne, T. L., J.E. Coster, J.V. Richerson, L.J. Edson, and E.R. Hart (1978). "Field response of the southern pine beetle to behavioral chemicals." Environmental Entomology **7**: 578-582.



- Phillips, M. A., and R.B. Croteau (1999). "Resin-based defenses in conifers." Trends in Plant Science **4**(5): 184-190.
- Price, T. S., and C. Doggett (1982). A history of southern pine beetle outbreaks in the southern United States. Macon, GA, The Georgia Forestry Commission: 1-35.
- Reeve, J. D., D.J. Rhodes, and P. Turchin (1998). "Scramble competition in the southern pine beetle, *Dendroctonus frontalis*." Ecological Entomology **23**: 433-443.
- Renwick, J. A. A. (1969). "Bark beetle attractants: mechanism of colonization by *Dendroctonus frontalis*." Nature **224**(5225): 1222-1223.
- Renwick, J. A. A., and J.P. Vite (1970). "Systems of chemical communications in dendroctonus." Contributions of the Boyce Thompson Institute **24**(13): 283-292.
- Roberds, J. H., B.L. Strom, F.P. Hain, D.P. Gwaze, S.E. McKeand, and L.H. Lott (2003). "Estimates of genetic parameters for oleoresin and growth traits in juvenile loblolly pine." Canadian Journal of Forest Research **33**: 2469-2476.
- Roberds, J. H. and B. L. Strom. (2006). "Repeatability estimates for oleoresin yield measurements in three species of the southern pines." Forest Ecology and Management **228**: 215-224.
- Rockwood, D. L. (1873). "Variation in the monoterpene composition of two oleoresin systems of loblolly pine." Forest Science **19**(2): 147-153.
- Ruel, J. J., M.P. Ayres, and P.L. Lorio, Jr. (1998). "Loblolly pine responds to mechanical wounding with increased resin flow." Canadian Journal of Forest Research **28**: 596-602.
- SAS Institute Inc. (1996) SAS/STAT Software: changes and enhancements (through release 6.11). SAS Institute Inc., Cary, N.C.
- Squillace, A. E., O.O. Wells, and D.L. Rockwood (1980). "Inheritance of monoterpene composition in cortical oleoresin of loblolly pine." Silvae Genetica **29**(3-4): 141-151.
- Strom, B. L., R.A. Goyer, L.L. Ingram Jr., G.D.L. Boyd, and L.H. Lott (2002). "Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle." Forest Ecology and Management **158**: 169-178.
- Warren, J. M., H.L. Allen, and F.L. Booker (1999). "Mineral Nutrition, resin flow, and phloem phytochemistry in loblolly pine." Tree Physiology **19**: 655-663.
- Wear, D. N. a. J. G. G. (2002). "Southern forest resource assessment: summary of findings." Journal of Forestry **100**(7): 6-14.

- Wullschleger, S. D., S.B. McLaughlin, and M.P. Ayres (2004). "High-resolution analysis of stem increment and sap flow for loblolly pine trees attacked by southern pine beetle." Canadian Journal of Forest Research **34**: 2387-2393.
- Yin, R., and R.A. Sedjo (2001). "Is this the age of intensive management?" Journal of Forestry **99**(2): 10-17.
- Zhang, Y. a. B. Z. (1999). "Which trees and stands are attracted by the southern pine beetle?" Southern Journal of Applied Forestry **23**(4): 217-223.

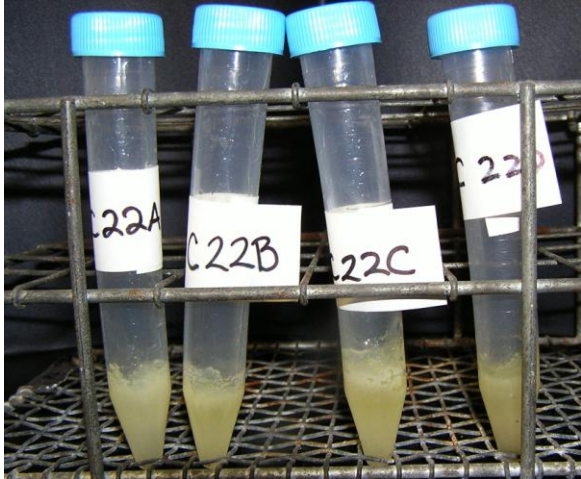
## **Appendices**

## Appendix I

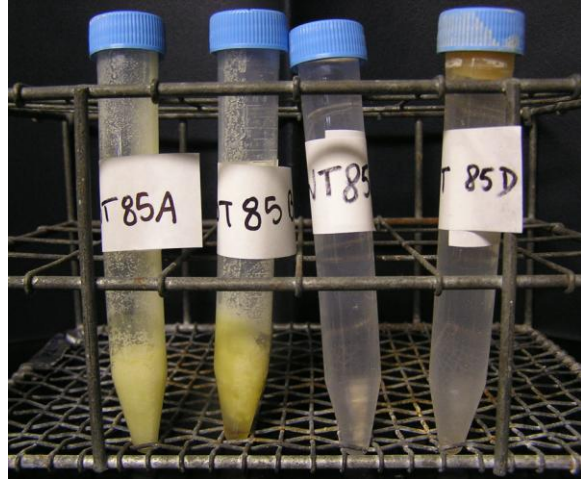
A preliminary study was conducted in July 2006 to examine the impact of rainwater on resin collection. This study was prompted by concerns regarding samples filled with water, due to rain, over the 24-hour collection period in South Carolina.

Loblolly pines located in Schenck forest of Raleigh, NC were used in this study. Four samples were taken from 91 trees. Twenty-three trees were full control trees, with no water in any vials used. For the remaining 59 trees, two control samples were empty vials and two samples were vials filled with water. Vials were placed on trees according to random design, determined prior to entering the field. Trees were prepared by scraping the bark to create a flat surface. Holes were then punched in the bark, to the cambium, using a size 10 leather punch. Custom samplers were then loosely screwed into place and vials attached. If vials contained water, samplers were filled using a water bottle, then tightened into place. If control vials were attached, samplers were tightened without adding water. Samples were collected 24-hours after attachment.

According to observations of samples, vials filled with water collected significantly less resin than controls (Figure VIII). Variation of resin collected from single trees, as seen in control trees, cannot account for the difference in resin collected (Figure VII). All resin collected in water filled vials was located at the top of vials. While the presence of resin may have been due to insufficient water in samplers, it suggests that resin is less dense than water, and in situations where water is present in vials; resin would be unable to displace water.



**Figure VII.** Resin samples from control tree, (A-D) no water included in any samples.



**Figure VIII.** Resin samples from test tree, samples on left (A and B) control samples, samples on right (C and D) vials filled with water before samples collected.

Overall, this study aided in deciding to eliminate samples full of water from the primary study. Water contamination was a problem on multiple sampling dates, leading to the drying of all samples except for those collected in the fall of 2006. While vials filled with water were completely eliminated from the study, it was considered necessary to dry all vials to prevent inaccurate mass measurements due to low-level water contamination.

## Appendix II

**Table VIII.** Rank, LSMeans, and associated standard errors of all South Carolina clones and checks. Family Z is the improved check and family X is the unimproved check.

Rank	clone	family	LSMEAN	STDERR	Rank	clone	family	LSMEAN	STDERR
1	135	F	10.5431	0.13353	44	287	H	8.0833	0.1194
2	104	F	10.4271	0.1148	45	375	I	8.07433	0.11701
3	190	F	10.1766	0.12723	46	296	H	8.0613	0.13033
4	112	F	10.009	0.14492	47	376	I	8.03653	0.11377
5	108	F	9.7517	0.12582	48	339	I	8.03286	0.16893
6	156	F	9.5557	0.13523	49	236	H	8.03134	0.11589
7	110	F	9.3082	0.12728	50	215	H	8.00814	0.12448
8	157	F	9.2892	0.14285	51	273	H	8.00698	0.18444
9	178	F	9.2477	0.12062	52	379	I	8.00328	0.11172
10	153	F	9.1864	0.12059	53	756	Z	7.99051	0.06334
11	123	F	9.1724	0.11701	54	127	F	7.98926	0.12735
12	143	F	9.084	0.137	55	407	K	7.98764	0.11589
13	146	F	9.0559	0.12724	56	208	H	7.9769	0.12176
14	133	F	8.8441	0.13353	57	366	I	7.96049	0.11812
15	131	F	8.8379	0.12312	58	303	I	7.93233	0.14936
16	443	K	8.6388	0.14073	59	249	H	7.93163	0.11271
17	338	I	8.5765	0.12882	60	487	K	7.89266	0.12312
18	465	K	8.5703	0.13522	61	173	F	7.87896	0.14492
19	100	F	8.5695	0.12312	62	387	I	7.87211	0.12737
20	188	F	8.5682	0.117	63	334	I	7.86852	0.12058
21	193	F	8.5603	0.11169	64	222	H	7.86714	0.12057
22	242	H	8.5109	0.13344	65	219	H	7.8629	0.11699
23	185	F	8.5059	0.12446	66	297	H	7.83732	0.1098
24	189	F	8.4978	0.12052	67	395	I	7.83579	0.11074
25	370	I	8.4682	0.15424	68	377	I	7.82624	0.137
26	343	I	8.4406	0.12716	69	345	I	7.8235	0.1218
27	148	F	8.4209	0.12052	70	371	I	7.80815	0.12584
28	166	F	8.3711	0.12871	71	347	I	7.80546	0.14923
29	232	H	8.2985	0.11483	72	220	H	7.80341	0.12445
30	483	K	8.2928	0.13883	73	381	I	7.7929	0.14936
31	122	F	8.2648	0.13353	74	125	F	7.78715	0.11276
32	191	F	8.264	0.14491	75	266	H	7.7823	0.12314
33	198	F	8.2449	0.11475	76	111	F	7.77962	0.14284
34	363	I	8.2343	0.11701	77	290	H	7.77185	0.11704
35	216	H	8.2262	0.11277	78	329	I	7.76918	0.12186
36	386	I	8.2228	0.12178	79	485	K	7.76333	0.18016
37	140	F	8.2088	0.16268	80	274	H	7.75341	0.13523
38	186	F	8.2077	0.13023	81	295	H	7.74898	0.12314
39	183	F	8.1994	0.16268	82	323	I	7.73223	0.11275
40	244	H	8.197	0.1388	83	424	K	7.73191	0.11377
41	239	H	8.1068	0.11378	84	292	H	7.73068	0.1127
42	308	I	8.10536	0.13355	85	315	I	7.7267	0.12309
43	373	I	8.08732	0.13522	86	301	I	7.72662	0.12184

Rank	clone	family	LSMEAN	STDERR
87	480	K	7.7248	0.10711
88	348	I	7.72271	0.13344
89	437	K	7.71115	0.12177
90	462	K	7.7072	0.13181
91	276	H	7.70457	0.12874
92	309	I	7.68208	0.12186
93	398	I	7.68188	0.11378
94	320	I	7.67897	0.12313
95	240	H	7.67501	0.10889
96	352	I	7.6719	0.11931
97	286	H	7.64988	0.13183
98	493	K	7.64766	0.11484
99	444	K	7.63733	0.12187
100	101	F	7.629	0.12315
101	664	X	7.61457	0.09511
102	311	I	7.60236	0.31989
103	291	H	7.60125	0.13882
104	396	I	7.59769	0.13883
105	230	H	7.58788	0.12445
106	488	K	7.57222	0.12312
107	234	H	7.5681	0.11588
108	319	I	7.54985	0.15423
109	281	H	7.54355	0.10798
110	302	I	7.53826	0.1117
111	269	H	7.49644	0.13699
112	223	H	7.49222	0.11811
113	429	K	7.49088	0.14073
114	484	K	7.46543	0.13523
115	246	H	7.46251	0.12183
116	416	K	7.46225	0.13698
117	284	H	7.45896	0.14074
118	476	K	7.45001	0.13703
119	225	H	7.44963	0.10799
120	491	K	7.44018	0.12441
121	422	K	7.43029	0.12185
122	206	H	7.42944	0.1193
123	442	K	7.41892	0.12595
124	359	I	7.41643	0.11073
125	248	H	7.40754	0.14483
126	251	H	7.39552	0.11699
127	430	K	7.38602	0.12306
128	250	H	7.38221	0.13698

Rank	clone	family	LSMEAN	STDERR
129	432	K	7.38035	0.15978
130	288	H	7.37731	0.13698
131	227	H	7.35535	0.15426
132	448	K	7.35157	0.13191
133	452	K	7.34734	0.13515
134	369	I	7.34292	0.12314
135	340	I	7.33944	0.11701
136	306	I	7.32475	0.12726
137	461	K	7.32321	0.12583
138	307	I	7.30971	0.14708
139	466	K	7.28908	0.13023
140	224	H	7.27219	0.14697
141	468	K	7.25589	0.12449
142	494	K	7.25492	0.11704
143	420	K	7.24389	0.18914
144	467	K	7.23915	0.14085
145	415	K	7.22656	0.12176
146	365	I	7.22523	0.12725
147	397	I	7.22418	0.1148
148	406	K	7.20333	0.12446
149	447	K	7.15513	0.13701
150	409	K	7.10389	0.12186
151	400	K	7.08885	0.14085
152	463	K	6.98159	0.15426
153	473	K	6.93538	0.13521

**Table IX.** Rank, LSMeans, and associated standard errors of all Florida clones and checks. Clone Z is the improved check and clone X is the unimproved check.

Rank	clone	family	LSMEAN	STDERR	Rank	clone	family	LSMEAN	STDERR
1	20	D	9.5492	0.12507	44	7	E	8.06648	0.15752
2	13	E	9.27847	0.11615	45	68	D	8.05985	0.12189
3	41	D	9.0356	0.12339	46	98	E	8.04913	0.12344
4	5	D	8.98277	0.11009	47	36	D	8.03218	0.11364
5	18	E	8.94885	0.13009	48	72	D	8.02217	0.11364
6	17	E	8.92785	0.1363	49	40	A	8.01681	0.13628
7	18	C	8.87393	0.12505	50	48	A	8.00908	0.14574
8	90	E	8.72629	0.11889	51	3	C	8.00169	0.13228
9	71	D	8.62508	0.10684	52	41	E	7.99629	0.1363
10	67	E	8.60427	0.13631	53	25	E	7.96989	0.14078
11	66	D	8.59028	0.13625	54	55	A	7.96796	0.15111
12	75	D	8.5902	0.10789	55	X	X	7.96453	0.05737
13	30	E	8.5852	0.12502	56	23	D	7.96056	0.14309
14	29	E	8.57979	0.10901	57	34	A	7.95314	0.11888
15	48	E	8.5641	0.15118	58	70	D	7.95015	0.12843
h16	33	E	8.55661	0.1431	59	89	A	7.94079	0.1322
17	58	D	8.55331	0.12033	60	82	D	7.91519	0.12505
18	72	E	8.54621	0.13844	61	89	C	7.90908	0.13223
19	85	E	8.53137	0.16452	62	26	C	7.9053	0.13619
20	51	E	8.51884	0.13421	63	92	C	7.89631	0.12188
21	2	E	8.49715	0.14574	64	44	C	7.88701	0.17237
22	87	E	8.49633	0.15409	65	73	E	7.82592	0.12193
23	55	E	8.49197	0.10897	66	90	D	7.82533	0.13018
24	34	E	8.44326	0.12187	67	47	A	7.80613	0.11363
25	1	D	8.38305	0.12189	68	22	E	7.80449	0.1189
26	79	C	8.35711	0.12841	69	84	C	7.80401	0.12498
27	4	D	8.33711	0.12843	70	16	D	7.78523	0.12194
28	19	D	8.33592	0.12505	71	2	D	7.78057	0.11363
29	22	C	8.31021	0.12188	72	75	C	7.77888	0.13631
30	6	D	8.28584	0.12855	73	50	A	7.77137	0.11363
31	26	E	8.28436	0.14574	74	54	A	7.76582	0.12021
32	11	E	8.26168	0.1363	75	16	A	7.76154	0.11362
33	95	E	8.24818	0.11898	76	73	A	7.75736	0.11365
34	27	E	8.24603	0.12189	77	83	A	7.75508	0.10684
35	19	E	8.23123	0.1363	78	4	C	7.75127	0.12184
36	47	E	8.17901	0.12497	79	100	D	7.74597	0.15751
37	17	A	8.15541	0.16442	80	28	A	7.74482	0.12855
38	10	A	8.14486	0.15111	81	43	E	7.73702	0.13631
39	31	E	8.10938	0.12349	82	66	A	7.72239	0.16436
40	25	A	8.09533	0.10897	83	8	D	7.70779	0.12844
41	Z	Z	8.09035	0.05202	84	63	A	7.69719	0.12188
42	61	D	8.08607	0.11754	85	81	A	7.69428	0.11619
43	37	A	8.07908	0.11888	86	27	A	7.6895	0.10897



<b>Rank</b>	<b>clone</b>	<b>family</b>	<b>LSMEAN</b>	<b>STDERR</b>
87	A31	A	7.68234	0.12667
88	C8	C	7.66999	0.11889
89	C99	C	7.66836	0.14085
90	C19	C	7.66432	0.11899
91	A35	A	7.66235	0.12674
92	C32	C	7.65994	0.10684
93	C86	C	7.64629	0.14085
94	A58	A	7.63788	0.14558
95	D96	D	7.62521	0.14574
96	E99	E	7.62384	0.12183
97	E96	E	7.62006	0.1683
98	C73	C	7.60527	0.12505
99	E46	E	7.60194	0.11888
100	A52	A	7.59006	0.12187
101	C71	C	7.57844	0.14086
102	A76	A	7.57637	0.13619
103	A11	A	7.51174	0.14563
104	A29	A	7.51045	0.10789
105	C52	C	7.47664	0.17254
106	A80	A	7.46078	0.13422
107	D3	D	7.45049	0.17232
108	C37	C	7.4388	0.13223
109	A14	A	7.43844	0.13207
110	C35	C	7.42705	0.12188
111	A94	A	7.41697	0.10786
112	C90	C	7.3804	0.12506
113	A42	A	7.34939	0.13223
114	E68	E	7.30778	0.12498
115	C58	C	7.2997	0.12855
116	C51	C	7.28042	0.13229
117	A24	A	7.27923	0.12671
118	C28	C	7.23387	0.12189
119	A26	A	7.22448	0.17237
120	C48	C	7.20135	0.13223
121	C78	C	7.15941	0.31524
122	C24	C	7.14112	0.12511
123	C95	C	7.07111	0.18717