

ABSTRACT

KNEBEL, LARISSA. Resin flow induction in southern pines: Implications for defense against southern pine beetle. (Under the direction of Dr. Thomas R. Wentworth.)

The interactions between conifers, bark beetles and their common fungal associates have been widely studied, in part due to the severe economic and natural losses that occur annually. Host resistance, involving constitutive and induced defensive measures, and the factors associated with pest success are now widely understood and some losses can be avoided with proper management. Recent research with Norway spruce (*Picea abies*) has provided additional insight into the chemical pathways involved in host resistance, and has determined that trees exposed to mechanical wounding or fungal inoculation show acquired resistance to future pathogen attacks. This aspect of defense in loblolly pine (*Pinus taeda*) has not been adequately investigated. However, our recent studies indicate that fungal inoculation results in elevated resin flow levels that last up to one year after treatment. Further research in southern Appalachian pines showed that elevated resin flow occurs in response to low intensity fire, and that this response is still present at 18 months after burning. These studies indicate that acquired resistance through induced resin flow in southern pines is a possibility. Further research with beetle or fungal challenges could determine new possibilities for management of both natural and planted stands in order to maximize host defenses against southern pine beetle.

RESIN FLOW INDUCTION IN SOUTHERN PINES:
IMPLICATIONS FOR DEFENSE AGAINST SOUTHERN PINE BEETLE

by
LARISSA KNEBEL

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

BOTANY

Raleigh

2005

APPROVED BY:

Chair of Advisory Committee

Personal Biography

Larissa Knebel was born and raised in Abingdon, VA. She graduated from Abingdon High School in 1992. She earned a B.S. in Environmental Studies with an emphasis in Biology and a minor in Spanish from Lenoir-Rhyne College in 1996. She married Jason Knebel two weeks after the graduation ceremony. They took a year off from school and spent part of that time teaching English as a Second Language at Universidad Peruana Los Andes in Huancayo, Peru. She returned to North Carolina and earned a M.S. in Biology from Western Carolina University under the direction of Dr. Dan Pittillo in 1999. She began her doctoral studies in Dr. Tom Wentworth's Plant Ecology lab that same year at North Carolina State University. Her first son, Sebastian, was born in December 2002 and she is currently awaiting the birth of her second son in July 2005.

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
RESIN FLOW RESPONSES TO FERTILIZATION, WOUNDING, AND FUNGAL INOCULATION IN LOBLOLLY PINE (<u>PINUS TAEDA</u>) IN NORTH CAROLINA.....	1
Abstract.....	1
Introduction.....	2
Methods.....	5
Results.....	8
Fertilizer effects on trees.....	8
Fertilizer effects on resin flow responses.....	9
Wounding/inoculation effects on resin flow responses.....	9
Effects of location of resin sampling in relation to wounded area....	10
Age and seasonal effects.....	11
Discussion.....	11
Fertilizer effects.....	11
Wounding/inoculation treatment effects.....	14
Time since wounding/inoculation treatment and seasonal effects	16
Literature cited.....	18
SOUTHERN PINE BEETLE AND FIRE IN LINVILLE GORGE WILDERNESS AREA.....	33
Abstract.....	33
Introduction.....	34
Study Site.....	36
Methods.....	36
Results.....	38
Plant composition.....	38
Resin flow.....	39
Discussion.....	39
Plant composition.....	40
Resin flow.....	42
Literature cited.....	46
THE POTENTIAL FOR ACQUIRED RESISTANCE TO <u>DENDROCTONUS FRONTALIS</u> IN SOUTHERN PINES.....	56
Abstract.....	56

Introduction.....	57
Bark beetle / conifer / fungus system.....	57
Recent research.....	58
Acquired resistance in loblolly pine.....	60
Recommendations and conclusions.....	62
Literature cited.....	64

LIST OF TABLES

	Page
RESIN FLOW RESPONSES TO FERTILIZATION, WOUNDING, AND FUNGAL INOCULATION IN LOBLOLLY PINE (<u>PINUS TAEDA</u>) IN NORTH CAROLINA	
1. Mean nitrogen (%) in foliage and phloem of fertilized and unfertilized loblolly pine (<i>Pinus taeda</i>).....	23
2. Effects of fertilization on the resin flow (g/day) of loblolly pines (<i>Pinus taeda</i>) in Hill Forest, North Carolina.....	24
3. Effects of artificial wounding and <i>Ophiostoma minus</i> inoculation treatment on the resin flow (g/day) of loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina.....	25
4. Wounding/inoculation effects on resin flow (g/day) of loblolly pine (<i>Pinus taeda</i>) after one year.....	26
5. Location of sampling effects at day +90 on the resin flow (g/day) of loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina.....	27

LIST OF FIGURES

	Page
<p>RESIN FLOW RESPONSES TO FERTILIZATION, WOUNDING, AND FUNGAL INOCULATION IN LOBLOLLY PINE (<u>PINUS TAEDA</u>) IN NORTH CAROLINA</p>	
1. The effects of artificial wounding and <i>Ophiostoma minus</i> inoculation treatment on the resin flow (g/day) of 6-year-old loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina during the spring sampling.....	28
2. The effects of artificial wounding and <i>Ophiostoma minus</i> inoculation treatment on the resin flow (g/day) of 12-year-old loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina during the spring sampling.....	29
3. The effects of artificial wounding and <i>Ophiostoma minus</i> inoculation treatment on the resin flow (g/day) of 6-year-old loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina during the summer sampling.....	30
4. The effects of artificial wounding and <i>Ophiostoma minus</i> inoculation treatment on the resin flow (g/day) of 12-year-old loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina during the summer sampling.....	31
5. Temporal effects on the resin flow (g/day) of 6- and 12-year-old loblolly pine (<i>Pinus taeda</i>) in Hill Forest, North Carolina.....	32
<p>SOUTHERN PINE BEETLE AND FIRE IN LINVILLE GORGE WILDERNESS AREA</p>	
1. Plot density and basal area across all species.....	50
2. Plot density and basal area across all pine species.....	51
3. Plot density and basal area of <i>Pinus pungens</i>	52
4. Plot density and basal area of <i>Pinus rigida</i>	53
5. Resin flow of individual burned and/or infested pines.....	54
6. Resin flow of individual burned or unburned pines in 2001 and 2002...	55

Resin flow responses to fertilization, wounding, and fungal inoculation in loblolly pine (*Pinus taeda*) in North Carolina.

ABSTRACT

Southern pine beetle (*Dendroctonus frontalis*) infestations are a major consideration in the management of loblolly pine (*Pinus taeda*) plantations in the southeastern United States. Because resin flow in pines is the primary means of natural defense against southern pine beetle, factors affecting resin flow are of great importance. We examined the influence of fertilization, wounding, and fungal inoculation on resin flow in 6- and 12-year-old stands of loblolly pine and determined the extent of that influence within and above the wounded area and through time. Fertilization increased constitutive resin flow, but only the younger trees were able to sustain increased resin flow after wounding and inoculation treatments. An induced resin flow response occurred between one and thirty days after wounding and inoculation treatments. Wounding with inoculation resulted in greater resin flow than wounding alone, but increasing amounts of inoculum did not result in increasing resin flow. Increased resin flow (relative to controls) lasted at least 90 days after wounding with inoculation treatments. This increase appears to be limited to the area of treatment, at least in younger trees. The lasting effects of fungal inoculation on resin flow in loblolly pines, as well as the response to fertilization, suggest that acquired resistance through induced resin flow could aid in decreasing susceptibility of loblolly pine to southern pine beetle.

INTRODUCTION

The southern pine beetle, *Dendroctonus frontalis*, an indigenous insect in the southern United States, has been reported responsible for widespread damage to southern pine forests since the middle of the eighteenth century (Thatcher 1980). Not only is this damage costly to the ecosystems of which pines are the primary residents, but also to the economy of many Southern states that rely on commercial production from pine plantations (Coulson 1980). Since southern pine beetle will attack and kill even the healthiest trees under favorable conditions, defense against the attack rather than prevention has typically been the theme of southern pine beetle research.

Southern pine beetle females are the pioneers who select host trees, though it is uncertain whether the female beetles are attracted to the trees or land randomly and select host trees only after they have probed the tree. Upon selection of a suitable host, the female beetle begins to bore into the bark of the tree. Both the beetle and the tree release chemicals that serve to attract male beetles (Payne 1980). Pines are able to respond quickly to attack by southern pine beetle through a substantial constitutive supply of resin and an induced resin response following infestation (Phillips and Croteau 1999, Lombardero et al. 2000). Resin, a mixture of turpentine and resin acids formed in the secondary xylem, is a conifer's primary defense against boring insect infestation (Hodges et al. 1979, Ruel et al. 1998, Phillips and Croteau 1999). While the monoterpene compounds in resin can function to chemically attract beetles, and are, paradoxically, toxic to the beetles, the physical properties of the resin are most strongly associated with defense against infestation (Hodges et al. 1979, Phillips and Croteau 1999). The physical effects of resin against southern pine beetle infestation occur as the beetle bores into the bark through the phloem of the tree and begins to sever resin ducts. As the resin ducts are severed, resin begins to flow through the hole bored into the tree, which is still occupied by the beetle. The beetle is thus physically ejected from the tree and most often entombed in the thick resin or 'pitch' (Trapp and Croteau 2001). In addition to limiting the success of beetle entry into the tree, resin soaked tree tissues also limit the success of beetles that have, despite the physical effects of resin flow, successfully established themselves in the tree. These resin-soaked tissues decrease oviposition success and larval survival, and interfere with the establishment of other insects and fungi that

colonize pine trees after beetle attacks (Berryman 1972, Raffa 1991). As long as resin is available in copious amounts, a tree will be able to ward off most subsequent beetle attacks and thus protect itself from a deadly infestation. If the southern pine beetle population densities are low enough, a tree will be able to withstand the attack. If the beetles are in a high-density outbreak, then most trees will not have the available resin needed to withstand repeated attacks (Strom et al. 2002). In this case, females will successfully enter the phloem, feed, attract male beetles, mate, and construct egg galleries in which to leave their offspring. Fungi associated with southern pine beetle will simultaneously infest the tree's vascular system. As large numbers of beetles enter the tree and resin defenses are depleted, the tree will eventually become infested and die due to vascular dysfunction. Generally, a tree either is sufficiently protected to avoid infestation, or it succumbs and is killed.

In association with southern pine beetle infestation, at least three species of fungi may be introduced into the tree by the beetle. Two mycangial species, *Ophiostoma ranaculosum* (J.R. Bridges and T.J. Perry) Hausner (syn. *Ceratocystiopsis ranaculosus*) (Jacobs and Kirisits 2003) and *Entomocorticium sp. A*, exist in a mutualistic symbiotic relationship with the beetle. These two fungal species grow on tree tissue in beetle cavities and serve as a food source for the beetle larvae, primarily through the increased availability of nitrogen in fungal hyphae and spores, and have few if any impacts on the tree directly. In exchange, the beetle provides safe transportation of the fungi to new host trees, (Klepzig et al. 2001, Lombardero et al. 2003). The third species, the phoretic blue-stain fungus, *Ophiostoma minus* (Hedgcock) H. and P. Sydow, is the strongest competitor of the three fungi (Klepzig and Wilkens 1997, Klepzig et al. 2001). *O. minus* also appears to have a more complex relationship with the beetle. Upon initial infestation, *O. minus* too acts in a mutualistic symbiosis with the beetle, aiding in the death of the tree, while the beetle provides safe transport (Paine et al. 1997, Klepzig et al. 2001). However, as the fungus spreads, the relationship becomes antagonistic, resulting in reduced larval development and lower reproductive success for the beetle (Lombardero et al. 2000, Klepzig et al. 2001, Goldhammer et al. 1990, Barras 1970). *O. minus* reduces the carbohydrate concentration of the phloem and competes with the mycangial fungi that provide nitrogen, so the beetles suffer nutritionally in the presence of *O. minus* (Goldhammer et al. 1990). Fewer egg

galleries and fewer eggs are also produced when the tree is infected by *O. minus* (Paine and Stephen 1987b, Goldhammer et al. 1990). *O. minus* is the only of the three fungal species whose presence results in the formation of a necrotic lesion (Paine and Stephen 1987b). This is likely due to the higher level of virulence of *O. minus* to the tree (Klepzig and Walkinshaw 2003). The necrotic lesion induced by *O. minus* is a result of the induced resin response of the tree and includes an increase in monoterpenes and phenolic compounds, accompanied by a decrease in water and sugars, which creates a hostile environment for the fungi (see Popp et al. 1995). The induced hypersensitive response to *O. minus* mirrors a tree's reaction to southern pine beetle infestation. This induced response acts in an attempt to seal off the fungus from further growth and to inhibit the success of the beetle, while allowing for the formation of a periderm (Popp et al. 1995).

Through the physical ejection of beetles from the tree by constitutive resin, as well as the containment of the beetle infestation and/or fungal infection through induced resin defenses and tissue necrosis, a tree may be able to withstand a southern pine beetle attack. Since resin flow is the key to defense of southern pines against southern pine beetle, and *O. minus* infection may be a strong co-factor in tree death (Lieutier 2002, Kopper et al. 2004), any factors that influence resin production and *O. minus* development in pine trees could play significant roles in the ability of a tree to withstand beetle infestation. There has been some indication that older trees have a higher initial resin flow rate and a greater total resin flow than younger trees (Schmitt et al. 1988). However, there has been little study of tree age and size effects in these systems, especially at young ages and small sizes. Several studies have indicated that fertilization of pine stands may affect resin flow (Wilkins et al. 1997, Warren et al. 1999), though effects are not always seen (see Ruel et al. 1998, Lombardero et al. 2000, and Klepzig et al. 2005). Mechanical wounding has been used successfully as a means of altering resin flow in conifers (Ruel et al. 1998, Tisdale and Nebeker 1992, and Lewinsohn et al. 1991), and is often used in studies of fungal impact on resin flow (Klepzig et al. 2005). While each of these factors is thought to affect resin flow, it is unclear to what extent.

Our goal was to gather further information on the effects of stand age and tree size, fertilization, wounding, and fungal inoculation on resin flow in loblolly pines (*Pinus taeda*) and to determine the duration of such effects. This information is increasingly important as

loblolly pine is the most widely planted tree in the world. with about 19 million ha in the U.S. alone (Schultz 1997), and is increasingly managed with intensive silvicultural techniques such as fertilization (Wear and Greis 2002). Intensively managed trees can also attain greater size at younger ages than previously and are thus harvested at younger ages. The influence of these factors on the southern pine beetle – loblolly pine system could have significant economic and ecological impacts on planted forests and perhaps on surrounding natural stands of this species.

METHODS

Two loblolly pine plantation stands were used to evaluate the effects of age and tree size, fertilization, artificial wounding, and fungal inoculation on the resin flow of individual pines. The study was conducted in one 6-year-old stand and one 12-year-old stand, with replication within each stand. The plantations were on the North Carolina State University Department of Forestry and Environmental Resources Hill Research and Demonstration Forest in Durham Co., in the central Piedmont region of North Carolina. The area receives 109 cm precipitation annually and has 200 growing season days (www.erh.noaa.gov, www.ces.ncsu.edu). Both sites are gently sloping with highly eroded soils in the Georgeville series. Both stands were planted (the 6-year-old stand in 1995 and the 12-year-old stand in 1989) at a density of 350-400 trees per acre. Neither weed and competition control, nor fertilization was used prior to the study. None of the trees used in the study showed any obvious signs of disease or previous insect attack. Though fusiform rust galls (*Cronartium quercuum* f. sp. *fusiforme*) were present on several trees within the stand, care was taken to avoid including these trees in the study. At the time of the current study, both stands contained a small percentage of other woody species, such as sweetgum (*Liquidambar styraciflua*), shortleaf pine (*Pinus echinata*) and redbud (*Cercis canadensis*). The 6-year-old stand also contained a prominent early successional component including blackberries (*Rubus spp.*) and catbrier (*Smilax rotundifolia*), among others.

Study trees were located with the restrictions that there was at least one non-study tree or 12 ft. (3.656 m) between the area associated directly with each study tree, and all study trees were as uniform in condition as possible, including straightness, lack of apparent

disease, and healthy appearance, including full crown. Trees used in the 6-year-old stand averaged 11 cm dbh and trees used in the 12-year stand averaged 18 cm dbh. Each study tree was cleared of all competition to a radius of 5 ft. (1.524 m) from the tree by brush removal and application of Garlon™ herbicide (triclopyr triethylamine salt) to remaining and cut stems. Roundup™ herbicide (glyphosate isopropylamine salt) was used several times during the course of the study on any emergent growth. One half of all study trees in each plot received a fertilizer treatment of 1 lb. (0.453 kg) Urea and 0.13 lb. (0.059 kg) TSP dispensed in a circular band at a radius of one foot from the tree (200 lb N/acre as Urea and 25 lb P/acre as TSP), while the remaining trees were left unfertilized. Fertilizer treatments took place on 22 April 2002.

To simulate initial southern pine beetle boring attack and infection with the fungus *O. minus*, two artificial wounding and fungal inoculation experiments were conducted, one in the spring of 2002, and one in the summer of 2002 (referred to hereafter as “trials”). One month after fertilization, on 22 May, the ‘spring’ trial was initiated. A pre-trial resin flow sample, as described below, was collected one day before the wounding and inoculation treatments were applied (day -1). The following day, four wounding/inoculation treatments were applied to study trees. Trees were either mass wounded, mass wounded and half of the wounds inoculated, mass wounded with full inoculation, or were left unwounded and uninoculated as controls. Wounding was carried out in a 2 m high band on each tree beginning at a height of 1 m above the ground, with a 15 cm band left untreated in the center of the wounding area to serve as the location for resin sampling. This area was smoothed prior to wounding/inoculation using a drawshave to remove most of the outer bark, leaving the inner bark unscathed. The diameter at breast height for each tree was used to determine the number of wounds necessary to create a density of 400 wounds/m² in the 2 m high band. The wounds were made using a 4 mm diameter increment hammer to remove the bark and phloem and expose the underlying xylem face. Inoculation of the wound sites with *O. minus* hyphae was conducted by inserting a 4 mm x 3 mm disk of colonized malt extract agar into each wound. Each wound was sealed by insertion of a 4 mm diameter, short (approx. 4 cm) piece of sterilized wooden dowel to limit feral spores from entering the wound site. The fungus was isolated and grown from a single female southern pine beetle collected in

Alabama in the spring of 2000 (Klepzig, pers. comm.). The wounding, inoculation, and fungal culture aspects were nearly identical to those reported in Klepzig et al. (2005).

Inoculation was either conducted on all wounds or on half of the wounds (every other one), depending on the wounding/inoculation treatment category. In addition to the pre-wounding/inoculation (day -1) treatment resin flow, resin flow was also sampled on days +1, +30, +60, +90, and +150 following wounding/inoculation treatments and again at one year after wounding/inoculation treatments. All resin flow samples were taken by removing an 8 mm diameter disk of bark to the xylem/phloem interface from within the 15 cm band at the center of the area where wounding occurred. At each sampling date, a fresh disk of bark was removed. Covered metal trays were used to direct flow from the wound into a pre-weighed collection device (Lorio and summers 1986). After 24 hours, the resin samples were collected and weighed, yielding an index of resin flow in g/day. Previous resin collection was assumed to be of negligible impact to subsequent resin collection due to the paucity of radial connections between resin ducts (Lapasha and Wheeler 1990), an assumption common to studies of this nature (Ruel et al. 1998, Warren et al. 1999, Klepzig et al. 2005).

The resin flow sampling that occurred on day +60 was a sub-sampling of only the wounded and fully inoculated trees, using the same resin flow methods described above. Since this was just a subsampling, and the results did not add to the understanding of system dynamics, the data were not included in the complete statistical analysis.

On day +90, an additional resin flow sample was taken at 1 m above the area of wounding (approx. 4 m above the tree base) to determine if the resin flow response was localized to the 15 cm band within the wounded area or is more systemic. Day +90 also included a foliar and phloem plug collection for analysis of nutrient content to assess the uptake of fertilizer. Phloem plugs were collected from the disk of outer bark and phloem removed from the tree for resin analysis. Foliar samples were collected by taking a sun-exposed branch from the midcrown of each tree and collecting approximately 100 fascicles from the first flush of needles from the previous year's growth. All samples were kept on ice and analyzed in a Carlo-Erba CN analyzer and subsequently analyzed for differences in percent nitrogen between fertilized and unfertilized trees using a t-test.

Since beetle attacks occur seasonally and resin flow varies seasonally, approximately three months after fertilization on 24 July, a second set ('summer' trial) of wounding/inoculation treatments were applied to a previously unwounded and un-inoculated set of study trees. Resin flow was again sampled pre-wounding/inoculation (day -1) treatment and +1, +30, +60, +90, and +150 days post-wounding/inoculation treatment. The procedures followed for wounding, inoculation, and resin sampling were precisely those indicated for the spring trial. The +150 days after wounding/inoculation treatments for the summer trials in both the 6-year-old stand and the 12-year-old stand were not included in the analysis of this study. The timing of the trial was such that 150 days after wounding/inoculation treatment fell in late December. While the methodology was followed, none of the trees displayed any measurable resin flow. This was most likely due to the cold temperatures experienced in the study area in December, when the average high temperature was 50° F (www.erh.noaa.gov). Resin flow from ducts is negligible at temperatures below 65° F (Clements 1974).

Each combination of treatments (2 x fertilizer, 4 x wounding/inoculation, 2 x season, 2 x age) was replicated 6 times, for a total of 192 trees. The study was organized in a completely random design by site (age). ANOVA was used to determine the effects of wounding/inoculation and fertilization treatments, as well as their interactions, on the resin flow of study individuals. A repeated measures ANOVA was used to evaluate the effect of time since wounding/inoculation treatments on the study individuals, and any interaction between time and fertilization or wounding/inoculation treatments. Fisher's LSD ($\alpha = 0.1$) was used to determine significant differences between wounding/inoculation treatments. All statistical analysis was conducted using SAS (version 8.01, SAS Institute, 1999-2000).

RESULTS

Fertilizer effects on trees

Percent nitrogen was significantly higher in the phloem plugs of fertilized trees than in non-fertilized trees for both age classes ($p < 0.05$). Percent nitrogen was also significantly higher in the foliar samples of fertilized trees than in non-fertilized trees ($p < 0.05$) in the 6-year-old trees, but not in the 12-year-old trees (Table 1).

Fertilizer effects on resin flow responses

There was no significant interaction between fertilizer and wounding/inoculation treatments in any of the 4 age by season trials (spring and summer 6- and 12-year-old trees) for resin flow ($p > .10$). Because of this lack of interaction, all analyses of resin flow due to wounding/inoculation treatments were conducted across both fertilization levels.

The day before wounding/inoculation treatments, resin flow was 2-4 times and significantly higher in fertilized trees than in unfertilized control trees in both the 6- and 12-year-old stands, in both the spring and summer trials ($p < 0.05$) (Table 2). In the 6-year old stand, in both the spring and summer trials, at day +1 and at day +30 after wounding/inoculation treatments, resin flow was significantly higher in fertilized trees than in unfertilized controls ($p < 0.10$). In the 6-year-old spring trial, at day +150 after wounding/inoculation treatments, resin flow was significantly higher in fertilized trees than in unfertilized controls ($p < 0.10$), though at day +90, there was no difference. Trees in the 12-year-old stand showed no significant fertilizer effect after wounding/inoculation treatments (Table 2). There were no significant effects of fertilization on resin flow in either stand after one year.

Wounding/inoculation effects on resin flow responses

Since no mechanical manipulation had been conducted, it was expected that constitutive resin flow, as measured on day -1, would be statistically consistent across all wounding/inoculation treatments. However, it is well known that resin flow does vary from tree to tree, and even with 6 replications, we still observed some significant differences in resin flow prior to any wounding/inoculation treatments (day -1) (Table 2). In the 6-year-old spring trial and in both spring and summer 12-year-old trials, one treatment group exhibited resin flow that was significantly different than some of the other treatments even before treatments were applied. We ran tests of linearity to determine if it would be appropriate to use day -1 resin flow as a covariate for subsequent tests, but determined that the data did not meet the necessary criteria and furthermore, that using day -1 resin flow as a covariate did not reduce the error in the data. What was gained in one aspect was lost in another. We also felt that using difference analysis was inappropriate for this dataset. Therefore, the data were analyzed without further statistical consideration of the pre-trial (day -1) differences.

A repeated measures ANOVA analysis of all four age by season trials showed a significant interaction for resin flow between days after wounding/inoculation treatments and wounding/inoculation treatments ($p < 0.10$). This interaction varied by the age and season trial, as well as by days after wounding/inoculation treatments. To more thoroughly evaluate the specific differences in resin flow due to wounding/inoculation treatments, we subjected the data to LSD analysis.

At one day after wounding/inoculation treatments, resin flow of wounded only trees was lower than that of controls in all age by season trials, and significantly lower in 3 of the 4 trials. For the day +30, +90 and +150 samplings, resin flow in wounded only trees was not significantly different than controls, with the exception of two days in the 12-year-old spring trees (Table 3, Fig. 1-4).

At day +30 after wounding/inoculation treatments, significantly higher resin flow occurred in the half and/or full inoculation trees than in the control trees in all four age by season trials (Table 3, Fig. 1-4). Also at day +30 after wounding/inoculation treatments, resin flow was significantly higher in half and/or full inoculation trees than in wounded only trees in three of the four age by season trials.

At day +90 after wounding/inoculation treatments, significantly higher resin flow occurred in the half and/or full inoculated trees than in the control trees in three of the four age by season trials.

There were only two days of the age by season trial that showed any significant difference in the resin flow of half and full inoculation trees. These significant differences occurred at day 90 of the spring 6-year-old trees, and day -1 of the spring 12-year-old trees, though the direction of the difference was not consistent (Table 3, Fig. 1-4). Further differences that were significant in only one or two of the age by season trials varied (Table 3, Fig. 1-4).

At one year after wounding/inoculation treatments, resin flow was significantly higher in the full inoculation trees than in controls (Table 4).

Effects of location of resin sampling in relation to wounded area

Both 12-year-old stand trials (spring and summer) showed a significantly lower resin flow when sampled within the wounded area than above the wounded area at 90 days after

wounding/inoculation treatment (Table 5). Neither 6-year-old stand trials showed any effect of the location of resin sampling.

Age and seasonal effects

Due to lack of replication, no statistical analysis of stand-age effects could be conducted. Observations suggest that fertilizer effects on resin flow lasted longer (up to 30 days after wounding/inoculation treatments) in the 6-year-old stand in both seasons. There was also a significant effect of the location of resin sampling at day +90 after wounding/inoculation treatment in the 12-year-old stands, but not in the 6-year-old stands, as noted above. It was also noted that resin flow in the 12-year-old control trees was at least 1.5 times (and as much as 4.5 times) that of the 6-year-old control trees at every sampling except the final one for each season (Table 3, Fig. 5). No other discernable trends were noted. Due to lack of replication, no statistical analysis of seasonal effects could be carried out. No discernable trends between spring and summer trials were noted.

DISCUSSION

Fertilizer effects

The application of fertilizer resulted in higher percent nitrogen in both the phloem and foliage at 90 days after wounding/inoculation treatments (Table 1). This indicates that the trees were able to acquire nitrogen from the fertilizer. Fertilization of these loblolly pine trees resulted in a higher, but short-lived resin flow across stand age and season, indicating an increase in constitutive resin due to fertilization. Warren et al. (1999) found that fertilized loblolly pine trees had significantly lower resin flow rates than control trees, as did Wilkens et al. (1997), while other studies found that there was no significant effect of fertilization on resin flow in loblolly pine (Klepzig et al. 2005, Lombardero et al. 2000, Ruel et al. 1998, Matson et al. 1987), and in Norway spruce (Kytö et al. 1996). This discrepancy on the influence of fertilizer on resin flow is problematic. Resin flow is widely accepted as a tree's primary defense against bark beetle attack. Elevated resin flow in a tree allows the physical removal of the beetle from the tree. Trees with lower constitutive resin flow have been shown to be less resistant to bark beetle attack. If constitutive resin flow is decreased by fertilization, then managers of loblolly pine plantations across the southeastern United States

are implementing practices that place their trees at greater bark beetle risk in their attempts to increase growth through fertilization. However, if, as found in this study, constitutive resin flow is increased with fertilization, then the loblolly pine industry may be deriving the benefit of increased bark beetle resistance along with increased growth in response to fertilization.

One possible explanation of the discrepancies in the findings of fertilizer effect on resin flow is age. Studies that found no induced resin flow response to fertilizer were on trees that were 15 years old (Ruel et al. 1998), 16 years old (Klepzig et al. 2005), 25 years old (Lombardero et al. 2000), 24 years old (Matson et al. 1987), and 58 years old (Kytö et al. 1996). Studies on 11-year-old trees (Warren et al. 1999) and 12-13 year-old trees (Wilkens et al. 1997), as well as the 6- and 12-year-old trees in the current study all found significant impacts of fertilizer on constitutive resin flow. It is possible that younger trees are able to use fertilizer to increase constitutive resin flow, whereas older trees cannot or do not. What remains problematic is whether these young trees respond to fertilization with a decrease in resin flow (Warren et al. 1999, Wilkens et al. 1997) or with an increase as seen in this study.

Other factors that may influence the effects of fertilization on resin flow include time since fertilization and the type of fertilizer used, though there is insufficient detail in the literature to support a conclusion. Diammonium phosphate has been used in studies that showed no change in resin flow (Klepzig et al. 2005, Ruel et al. 1998, and Lombardero et al. 2000), as well as in a study that showed a decrease in resin flow following fertilization (Wilkens et al. 1997). Studies that performed resin flow analysis in the same year as fertilization (Lombardero et al. 2000) or 4 years after fertilization (Klepzig et al. 2005) have shown no change in resin flow. However, a decrease in resin flow in the same year as fertilization (Warren et al. 1999) or 4 years after fertilization (Wilkens et al. 1997) has also been noted. At the present, it is unclear what effect, if any, age of trees being fertilized, type of fertilizer, or time elapsed between fertilization and measurement may have on resin flow.

The growth-differentiation balance model (GDB) originally presented by Loomis (1932, 1953) states that when there are no limiting factors, photosynthates are directed primarily to growth through cell division and elongation. When plants are under mild stress, the utilization of photosynthates is redirected to cell differentiation through specialization of

cells. Lorio (1986) used GDB to evaluate the growth of southern pines and their production of resin (secondary metabolism, which falls into the category of differentiation) as a means of understanding seasonal behavior by the southern pine beetle. Lorio (1986) contends that water availability is the limiting factor that prompts changes in allocation of photosynthates from growth to resin production. When water is plentiful in early spring, photosynthates are used primarily for growth processes, allowing southern pine beetles the opportunity to attack pines without substantial hindrance from resin. As summer approaches and water is less readily available, photosynthates are directed toward secondary metabolism and the formation of resin ducts and resin, increasing the pine's resistance to attack from southern pine beetle. In the fall, when temperature is unfavorable for both growth and differentiation, the southern pine beetle again exhibits greater success in infestation. Herms and Mattson (1992) also used GDB to hypothesize that herbivory patterns are shaped in large part by the allocation of resources to growth or differentiation, thus supporting Lorio's (1986) application of the model to seasonal patterns of southern pine beetle activity. A recent review of plant defense hypotheses by Stamp (2003) considers GDB as the most mature and well supported of the plant defense hypotheses (including the optimal defense, Carbon:nutrient balance, and growth rate hypotheses).

With regard to fertilization, GDB would indicate that a growth increase in response to fertilization would negatively impact resin production due to allocation of resources to growth rather than production of secondary metabolites. Though growth response to fertilization was not directly measured in this study, fertilization is generally acknowledged to increase growth, thus we wouldn't expect fertilization to increase resin flow. However, in late summer or in drought conditions, when tree growth is limited by water stress, nutrient resources might be allocated to other areas such as plant defense. Our study area began 2002 with a rainfall deficit for the previous year of more than 8 inches below the 30-year average. The area was not able to recover from the drought until October of 2002, when the cumulative annual deficit of more than 6 inches below the 30-year average was eliminated in a single month (www.erh.noaa.gov). Our study trees might have been unable to utilize photosynthates for growth due to the limiting amounts of water and thus allocated them to increased resin production. This is supported by Lombardero et al. (2000), who found that

resin flow was highest during a period of drought when diameter growth had halted. Thus, fertilization may in fact lead to a decrease in constitutive resin production (or leave resin production unchanged) when water availability is plentiful, but would result in an increase in resin production when drought conditions are prevalent and growth is limited. Of course, when drought conditions are extreme, no growth or resin production can occur and in these instances, the trees are attractive targets for southern pine beetles. Despite the easy applicability of the growth/differentiation model to resin based defenses, further investigation is necessary to explicate these interactions (Kytö et al. 1998, Warren et al. 1999).

While constitutive resin flow was significantly higher in fertilized trees, once the trees were wounded and/or inoculated, resin flow decreased sharply in all trials as constitutive resources were drained, as shown in other studies (Klepzig et al. 2005, Ruel et al. 1998, Lombardero et al. 2000, Cook and Hain 1987). At this point, an age effect with respect to fertilizer was seen. The 12-year-old trees showed no significant responses to fertilizer after the wounding/inoculation treatments were carried out, while the 6-year-old trees showed significantly elevated resin flow responses up to 30 days after wounding/inoculation treatments. The younger, smaller trees were able to benefit longer from the elevated constitutive levels brought on by fertilization. DeAngelis et al. (1986) found that older trees have a lower radial duct density than do younger trees. Since the number of ducts present within the area of attack governs the amount of resin produced in that area as well as the amount of resin delivered, a lower density of radial ducts would result in less resin produced and delivered in older trees than in younger trees (DeAngelis et al. 1986). The lower density of resin ducts would result in a lower volume of replenishment to the ducts in the wounded area from other sites of synthesis. Therefore, the increased constitutive resin brought on by fertilization would be less effective in older trees than in younger trees. It may also be that there is a dose-dependent relationship between fertilization and resin flow, and higher amounts of fertilization are required in order for the older and larger trees to sustain resin flow beyond attack.

Wounding/inoculation treatment effects

At 1 day after wounding/inoculation treatments, wounding without inoculation resulted in significantly lower resin flow than seen in controls in three of four age by season

trials. Mass wounding of trees generated resin flow that was less than that in control trees. This response was short-lived, as resin flow differences between wounded only and controls had largely disappeared by 30 days after wounding/inoculation treatments and was only twice seen in all day 30, 90 and 150 samplings.

By 30 days after wounding/inoculation treatments, an induced resin flow response was exhibited. While the response produced by wounding alone at one day after wounding/inoculation treatments was no longer discernable at 30 days, an induced inoculation effect was seen. The control trees produced significantly less resin than the half and/or full inoculation trees. In addition, those trees that were fully inoculated produced significantly more resin than trees that were wounded alone in three of the four trials. There is limited research on the response of resin flow to fungal inoculation. Two studies indicate that the hypersensitive response occurs at 24-72 hours after inoculation (Paine et al. 1988, Popp et al. 1991). Popp et al. (1991) found that two days after fungal infection of loblolly pines, resin flow was significantly higher than in controls. However, this response was no longer present on the third day post-wounding/inoculation treatment. In our study, increased resin flow of inoculated trees over controls began after 24 hours, but before 30 days, and was still present at 90 days after treatments. However, resin flow of wounded/inoculated and wounded only trees was not significantly different at 90 days after treatments. This increase in resin flow in inoculated trees is also consistent with previous findings that fungal inoculation results in increased lesion size over controls (Popp et al. 1995, Paine and Stephen 1987a, Paine and Stephen 1987b, Paine et al. 1988). As lesion formation is a result of resin soaking and necrosis of the infected area, it would follow that fungal presence is associated with elevated resin flow levels.

We did not find any significant differences between the amount of inoculum used and the resin response obtained (Table 3 and 4). This is consistent with the findings of Långström et al. (2001) on Scots pine and Paine and Stephen (1987a) on loblolly pine. However, Solheim et al. (1993) found that inoculation levels of *O. minus*, as well as another blue-stain fungus, did affect survival of Scots pine.

A decrease in resin flow due to wounding alone occurred by one day after treatment, but the same response was not as clear in the inoculated trees. The trees that received

inoculation with *O. minus* were first wounded in the same manner as the wounded only trees. The only difference in the inoculation treatments and the wounded only treatments was that the wounds in the inoculated trees received a disk of colonized agar after wounding. Therefore, we might expect the inoculated trees to show the same response as the wounded trees, though perhaps varying in intensity or longevity. In this manner, we would expect that if wounded trees exhibited suppressed resin flow at one day after treatment, then inoculated trees would as well. Perhaps the fungal inoculum somehow alters the tree's response, so that in its absence, the tree's constitutive resin is quickly depleted, but when the inoculum is present, the response is delayed, giving the fungus some extra time to become established. Age may play some role in the timing of the induced response as both 12-year-old trials showed increase resin flow in inoculated trees at one day after treatments. Additionally, constitutive resin flow appears to be higher in 12-year old controls than in 6-year-old controls. The influence of age on resin flow deserves further study, especially in loblolly plantations where young trees attain greater size more quickly than in natural stands and are harvested at young ages.

Time since wounding/inoculation treatment and seasonal effects

In all four age by season trials, resin flow decreased sharply at one day after wounding/inoculation treatments. In three of the four age by season trials, by 30 days after wounding/inoculation treatments, resin flow had risen to levels up to three times higher than seen in the day before wounding/inoculation treatments, and decreased steadily thereafter, never returning to the pre-wounding/inoculation treatment levels, even 150 days after wounding/inoculation treatments (Fig. 1-4). Cook and Hain (1987) found that resin flow in inoculated loblolly pines decreased within the first two days after inoculations and then returned to near pre-wounding/inoculation treatment rates by 14 days. Though it is tempting to compare resin flow values from the later sampling dates with pre-treatment levels, it would be inappropriate to use this as evidence of response to treatment alone, since resin flow varies with season. Cook and Hain (1987) found that resin flow rates were consistently higher in June than in July and that lesions developed faster in June than in July. Tisdale and Nebeker (1992) found that total resin flow increased from May to August. Our spring study also showed a peak in resin flow levels in late summer, concurrent with higher temperatures

(Fig. 5). However, our repeated measures ANOVA did indicate that time since treatment was a factor, which is to be expected due to seasonal variation, but also that there was a significant interaction between treatment and time since treatment.

While the response to wounding occurred faster in the wounded only trees, it lasted longer in the inoculated trees. Inoculated trees had higher resin flow than controls for at least 90 days after treatment and, in one trial, for as many as 150 days after treatment, and even into the following year. This is consistent with the work of Klepzig et al. (2005) who found an induced resin response as long as 105 days after treatment. However, Popp et al. (1991) found increased resin flow in inoculated loblolly pines lasted only 72 hours after treatment. Krokene et al. (2003) showed that Norway spruce (*Picea abies*), when exposed to pretreatment with the blue-stain fungus, *Ceratocystis polonica*, developed acquired resistance to future exposure with the same fungus or a different species. In their study, the acquired resistance lasted for at least one year. Since resin flow levels remained elevated one full year after inoculation, our results indicate that the same type of acquired resistance to blue-stain fungus may occur in loblolly pine. Further research should be conducted to determine if elevated resin flow responses could consistently be maintained throughout one growing season and even into the next. If resin flow can be maintained, trees that have been previously exposed to these treatments should be challenged with fungal inoculation or southern pine beetle infestations to determine if induced resin flow does indeed confer acquired resistance to subsequent invasion. There are important implications for natural and plantation loblolly pine stands threatened by southern pine beetle infestation. If pre-exposure to pathogens confers resistance, then stands that are in imminent danger could be pre-treated to reduce the threat of infestation rather than losing the stands or conducting early harvest, thus reducing financial and ecological losses due to southern pine beetle.

Literature cited

- Barras, S.J. 1970. Antagonism between *Dendroctonus frontalis* and the fungus *Ceratocystis minor*. *Ann. Entomol. Soc. Am.* 63: 1187-1190.
- Berryman, A.A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. *Bioscience* 22: 598-602.
- Cook, S.P. and F.P. Hain. 1987. Four parameters of the wound response of loblolly and shortleaf pines to inoculation with the blue-staining fungus associated with the southern pine beetle. *Can. J. Bot.* 65: 2403-2409.
- Coulson, R.N. 1980. Populations dynamics. In: *The Southern Pine Beetle*. Eds. R.C. Thatcher, J.L. Searcy, J.E. Coster and G.D. Hertel. USDA For. Ser. Tech. Bull. 1631. pp. 71-105.
- DeAngelis, J.D., T.E. Nebeker, and J.D. Hodges. 1986. Influence of tree age and growth rate on the radial resin duct system in loblolly pine (*Pinus taeda*). *Can. J. Bot.* 64: 1046-1049.
- Goldhammer, D.S., F.M. Stephen, and T.D. Paine. 1990. The effect of the fungi *Ceratocystis minor* (Hedgecock) Hunt, *Ceratocystis minor* (Hedgecock) Hunt var. *barrasii* Taylor, and SJB 122 on reproduction of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). *Can. Ent.* 122: 407-418.
- Herms, D.A., and W.J. Mattson. 1992. The dilemma of plants: To grow or to defend. *Q. Rev. of Biol.* 67: 283-335.
- 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. *Can. Ent.* 111: 889-896.
- Jacobs, K. and T. Kirisits. 2003. *Ophiostoma kryptum* sp. nov. from *Larix decidua* and *Picea abies* in Europe, similar to *O. minus*. *Mycol. Res.* 107: 1231-1242.
- Klepzig, K.D. and R.T. Wilkens. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. *Appl. Environ. Microbiol.* 63: 621-627.
- Klepzig, K.D. and C.H. Walkinshaw. 2003. Cellular response of loblolly pine to wound inoculation with bark beetle associated fungi and chitosan. *Resch. Pap. SRS-30*. Asheville, NC: U.S. Dept. Agric., Forest Service, Southern Research Station. 9 p.
- Klepzig, K.D., J.C. Moser, F.J. Lombardero, R.W. Hofstetter, and M.P. Ayres. 2001. Symbiosis and Competition: Complex interactions among beetles, fungi and mites. *Symbiosis*: 83-96.

- Klepzig, K.D., D.J. Robison, 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 Physiol.* In Press.
- Kopper, B.J., K.D. Klepzig and K.F. Raffa. 2004. Components of antagonism and mutualism in *Ips pini* – fungal interactions: Relationship to a life history of colonizing highly stressed and dead trees. *Environ. Entomol.* 33: 28-34.
- Krokene, R. H. Solheim, T. Krekling and E. Christiansen. 2003. Inducible anatomical defense responses in Norway spruce stems and their possible role in induced resistance. *Tree Physiol.* 23: 191-197.
- Kytö, M., P. Niemelä, and E. Annala. 1996. Vitality and bark beetle resistance of fertilized Norway spruce. *For. Ecol. and Manage.* 84: 149-157.
- Kytö, M., P. Niemelä, and E. Annala. 1998. Effects of vitality fertilization on the resin flow and vigour of Scots pine in Finland. *For. Ecol. and Manage.* 102: 121-130.
- Långström, B., H. Solheim, C. Hellqvist, and P. Krokene. 2001. Host resistance in defoliated pine: Effects of single and mass inoculations using bark beetle-associated blue-stain fungi. *Agric. For. Entomol.* 3: 211-216.
- Lapasha, C.A. and E.A. Wheeler. 1990. Resin canals in *Pinus taeda*: Longitudinal canal lengths and interconnections between longitudinal and radial canals. *Int. Assoc. Wood Anat. Bull.* 11: 227-238.
- Lewinsohn, E., M. Gijzen, T.J. Savage, and R.B. Croteau. 1991. Defense mechanisms of conifers: Differences in constitutive and wound-induced monoterpene biosynthesis among species. *Plant Physiol.* 96: 44-49.
- Lieutier, F. 2002. Mechanisms of resistance in conifers and bark beetle attack strategies. In: *Mechanisms and Deployment of Resistance in Trees to Insects.* Eds. M.R. Wagner, K.M. Clancy, F. Lieutier, and T.D. Paine. Kluwer Academic Press, The Netherlands. pp. 31-77.
- Lombardero, M.J., M.P. Ayres, P.L. Lorio Jr., and J.J. Ruel. 2000. Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. *Ecology Letters* 3: 329-339.
- Lombardero, M.J., M.P. Ayres, R.W. Hofstetter, J.C. Moser and K.D. Klepzig. 2003. Strong indirect interactions of *Tarsonemus* mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Oikos* 102: 243-252.
- Loomis, W.E. 1932. Growth-differentiation balance vs. carbohydrate-nitrogen ratio. *Proc. Am. Soc. Hortic. Sci.* 29: 240-245.

- Loomis, W.E. 1953. Growth and differentiation balance – An introduction and summary. In: Growth and Differentiation in Plants. Ed. W.E. Loomis. Iowa State College Press, Ames. pp. 1-17.
- Lorio, P.L. Jr. 1986. Growth-differentiation balance: A basis for understanding southern pine beetle-tree interactions. For. Ecol. Manage. 14: 259-273.
- Lorio, P.L. and R.A. Summers. 1986. Evidence of competition for photosynthates between growth processes and oleoresin synthesis in *Pinus taeda* L. Tree Physiol. 2: 301-306.
- Matson, P.A. F.P. Hain, and W. Mawby. 1987. Indices of trees susceptibility to bark beetles vary with silvicultural treatment in a loblolly pine plantation. For. Ecol. Manage. 22: 107-118.
- Paine, T.D. and F.M. Stephen. 1987a. Response of loblolly pine to different inoculum doses of *Ceratocystis minor*, a blue-stain fungus associated with *Dendroctonus frontalis*. Can. J. Bot. 65: 2093-2095.
- Paine, T.D. and F.M. Stephen. 1987b. Fungi associated with the southern pine beetle: Avoidance of induced defense response in loblolly pine. Oecologia 74: 377-379.
- Paine, T.D., K.F. Raffa, and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomol. 42: 179-206.
- Paine, T.D., F.M. Stephen, and R.G. Cates. 1988. Phenology of an induced response in loblolly pine following inoculation of fungi associated with the southern pine beetle. Can. J. For. Res. 18: 1556-1562.
- Payne, T.L. 1980. Life history and habits. In: The Southern Pine Beetle. Eds. R.C. Thatcher, J.L. Searcy, J.E. Coster and G.D. Hertel. USDA For. Ser. Tech. Bull. 1631. pp. 7-28.
- Popp, M.P., J.D. Johnson, and T.L. Massey. 1991. Stimulation of resin flow in slash and loblolly pine by bark beetle vectored fungi. Can. J. For. Res. 21: 1124-1126.
- Popp, M.P., J.D. Johnson, and M.S. Lesney. 1995. Characterization of the induced response of slash pine to inoculation with bark beetle vectored fungi. Tree Physiol. 15: 619-623.
- Phillips, M.A. and R.B. Croteau. 1999. Resin-based defenses in conifers. Trends in Plant Science 4: 184-190.

- Raffa, K.F. 1991. Induced defensive reaction in conifer-bark beetle systems. In: Phytochemical Induction by Herbivores. Eds. Tallamy, D.W. & M.J. Raupp. John Wiley and Sons, Inc. New York, NY, USA. pp.245-276.
- Ruel, J.J., M.P. Ayres, and P.L. Lorio Jr. 1998. Loblolly pine responds to mechanical wounding with increased resin flow. *Can. J. For. Res.* 28: 596-602.
- SAS Institute Inc. 1999-2000. SAS Version 8.01. SAS Institute Inc., Cary, North Carolina.
- Schmitt, J.J., T.E. Nebeker, C.A. Blanche, and J.D. Hodges. 1988. Physical properties and monoterpene composition of xylem oleoresin along the bole of *Pinus taeda* in relation to southern pine beetle attack distribution. *Can. J. Bot.* 66: 156-160.
- Schultz, R.P. 1997. Loblolly pine: The ecology and culture of loblolly pine (*Pinus taeda* L.). USDA For. Ser., Southern Forest Expt. Stn., Agric. Handbook No. 713. New Orleans, LA, 493p.
- Solheim, H., B. Långström, and C. Hellqvist. 1993. Pathogenicity of blue-stain fungi *Leptographium windfieldii* and *Ophiostoma minus* to Scots pine: Effect of tree pruning and inoculum density. *Can. J. For. Res.* 23: 1438-1443.
- Stamp, N. 2003. Out of the quagmire of plant defense hypothesis. *Q. Rev. Biol.* 78:23-55.
- 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. *For. Ecol. and Manage.* 158: 169-178.
- Thatcher, R.L. 1980. Introduction. In: The Southern Pine Beetle. Eds. R.C. Thatcher, J.L. Searcy, J.E. Coster and G.D. Hertel. USDA For. Ser. Tech. Bull. 1631. pp. 1-4.
- Tisdale, R.A. and T.E. Nebeker. 1992. Resin flow as a function of height along the bole of loblolly pine. *Can. J. Bot.* 70: 2509-2511.
- Trapp, S. and R. Croteau. 2001. Defensive resin biosynthesis in conifers. *Annu. Rev. Plant Physiol.* 19: 655-663.
- Warren, J.M, H.L. Allen, and F.L. Booker. 1999. Mineral nutrition, resin flow and phloem phytochemistry in loblolly pine. *Tree Physiol.* 19: 655-663.
- Wear, D.N and J.G. Greis, eds. 2002. Southern forest resource assessment. Gen. Tech. Rep. SRS-53. U.S. Dept. Agric., For. Ser., Southern Res. Sta., Asheville, NC 635p.
- Wilkens, R.T., M.P. Ayres, P.L. Lorio Jr., and J.D. Hodges. 1997. Environmental effects on pine tree carbon budgets and resistance to bark beetles. In: Global Change and

Disturbance in Southern Ecosystems. Eds. R.A. Mickler and S. Fox. Springer-Verlag, New York, NY, USA. pp. 591-616.

Table 1. Mean nitrogen (%) in foliage and phloem of fertilized and unfertilized loblolly pine (*Pinus taeda*). Different letters indicate significant differences between fertilized and unfertilized trees within each age class and tissue. Significance was determined using a t-test at $\alpha=0.05$. See Methods for description of sampling and analysis.

	Tissue	Treatment	Mean N (%)
6 year old	Foliage	Fertilized	1.53a
		Unfertilized	1.34b
	Phloem	Fertilized	0.48a
		Unfertilized	0.41b
12 year old	Foliage	Fertilized	1.23a
		Unfertilized	1.24a
	Phloem	Fertilized	0.33a
		Unfertilized	0.29b

Table 2. Effects of fertilization on the resin flow (g/day) of loblolly pines (*Pinus taeda*) in Hill Forest, North Carolina. Values reported are means +/- standard error (n=24). Different letters indicate that the mean of all fertilized trees, including all wounding/inoculation treatments and controls, are significantly different than the mean of all unfertilized trees within each day relative to wounding/inoculation treatment, age class, and season. Significance was determined using repeated measures ANOVA at $\alpha=0.10$. See Methods for further explanation. Fertilization took place on 22 April 2002. Day -1 indicates resin flow rates one day prior to wounding/inoculation treatments; other days are post- wounding/inoculation treatments. Resin flow data for the summer Day +150 sampling were not included due to lack of flow from all sample trees.

			Day -1	Day +1	Day +30	Day +90	Day +150
			20 May 2002	24 May 2002	22 June 2002	21 August 2002	20 October 2002
SPRING	6 year old	Fertilized	0.821 +/- 0.270 a	0.438 +/- 0.085 a	1.982 +/- 0.306 a	1.561 +/- 0.355 a	1.357 +/- 0.362 a
		Unfertilized	0.198 +/- 0.083 b	0.103 +/- 0.035 b	1.334 +/- 0.205 b	1.159 +/- 0.212 a	0.724 +/- 0.208 b
	12 year old	Fertilized	0.727 +/- 0.167 a	0.433 +/- 0.093 a	2.043 +/- 0.276 a	1.879 +/- 0.212 a	0.582 +/- 0.167 a
		Unfertilized	0.372 +/- 0.109 b	0.282 +/- 0.109 a	1.811 +/- 0.249 a	2.085 +/- 0.235 a	0.622 +/- 0.133 a
			22 July 2002	26 July 2002	24 August 2002	23 October 2002	—
SUMMER	6 year old	Fertilized	0.637 +/- 0.126 a	0.281 +/- 0.073 a	1.918 +/- 0.331 a	1.282 +/- 0.266 a	—
		Unfertilized	0.326 +/- 0.049 b	0.089 +/- 0.024 b	1.124 +/- 0.164 b	1.255 +/- 0.264 a	—
	12 year old	Fertilized	3.141 +/- 0.570 a	1.040 +/- 0.171 a	3.073 +/- 0.422 a	1.230 +/- 0.244 a	—
		Unfertilized	1.228 +/- 0.194 b	0.923 +/- 0.164 a	2.508 +/- 0.400 a	1.052 +/- 0.270 a	—

Table 3. Effects of artificial wounding and *Ophiostoma minus* inoculation treatment on the resin flow (g/day) of loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina. Values reported are means +/- standard errors (n=12). Different letters indicate that resin flow values were significantly different within each trial (age/season) and each day of wounding/inoculation treatment. Significance was determined using LSD at $\alpha=0.05$. Day -1 indicates resin flow rates one day prior to wounding/inoculation treatments; other days are post- wounding/inoculation treatments. Resin flow data for the summer Day +150 sampling were not included due to lack of flow from any sample trees.

	Day -1	Day +1	Day +30	Day +90	Day +150
6 year/spring					
Control	0.112 +/- 0.315 b	0.399 +/- 0.109 a	0.897 +/- 0.341 b	0.997 +/- 0.333 b	0.415 +/- 0.118 b
Wounded	0.922 +/- 0.498 a	0.221 +/- 0.115 b	1.084 +/- 0.253 b	0.839 +/- 0.165 b	0.449 +/- 0.125 b
Half	0.404 +/- 0.166 ab	0.222 +/- 0.104 ab	2.524 +/- 0.375 a	2.355 +/- 0.624 a	1.418 +/- 0.355 a
Full	0.598 +/- 0.246 ab	0.240 +/- 0.087 ab	2.128 +/- 0.336 a	1.248 +/- 0.275 b	1.770 +/- 0.688 a
12 year/spring					
Control	0.500 +/- 0.117 ab	0.682 +/- 0.195 a	1.372 +/- 0.343 b	1.492 +/- 0.374 b	0.294 +/- 0.064 b
Wounded	0.345 +/- 0.147 b	0.356 +/- 0.142 b	1.865 +/- 0.340 ab	2.164 +/- 0.369 a	1.008 +/- 0.289 a
Half	0.352 +/- 0.124 b	0.160 +/- 0.073 b	2.227 +/- 0.397 ab	2.218 +/- 0.229 a	0.672 +/- 0.180 ab
Full	0.999 +/- 0.318 a	0.234 +/- 0.103 b	2.244 +/- 0.384 a	2.054 +/- 0.255 ab	0.434 +/- 0.208 b
6 year/summer					
Control	0.546 +/- 0.177 a	0.297 +/- 0.055 a	0.847 +/- 0.156 c	0.966 +/- 0.255 a	—
Wounded	0.561 +/- 0.185 a	0.089 +/- 0.072 b	1.541 +/- 0.607 bc	1.224 +/- 0.501 a	—
Half	0.433 +/- 0.098 a	0.173 +/- 0.078 a	1.601 +/- 0.300 ab	1.362 +/- 0.318 a	—
Full	0.388 +/- 0.088 a	0.269 +/- 0.110 a	2.097 +/- 0.269 a	1.522 +/- 0.397 a	—
12 year/summer					
Control	2.373 +/- 0.901 ab	1.431 +/- 0.246 a	1.800 +/- 0.482 b	0.656 +/- 0.268 b	—
Wounded	2.657 +/- 0.527 a	1.239 +/- 0.193 a	2.083 +/- 0.363 b	0.917 +/- 0.260 ab	—
Half	2.295 +/- 0.793 ab	0.805 +/- 0.241 b	3.482 +/- 0.653 a	1.509 +/- 0.422 a	—
Full	1.394 +/- 0.222 b	0.450 +/- 0.162 b	3.797 +/- 0.618 a	1.484 +/- 0.435 a	—

Table 4. Wounding/inoculation effects on resin flow (g/day) of loblolly pine (*Pinus taeda*) after one year. Values reported are means +/- standard errors (n=12). Different letters indicate that resin flow values were significantly different within rows (age classes). Significance was determined using LSD at $\alpha=0.05$.

	Control	Wounded	Half inoculated	Full inoculated
6 year old	0.5066 +/- 0.1535 b	0.7233 +/-0.3025 b	1.22 +/- 0.2333 b	1.569 +/-0.6594 a
12 year old	0.7239 +/- 0.1166 b	1.3124 +/-0.1938 ab	1.2321 +/- 0.0948 ab	2.1577 +/-0.3309 a

Table 5. Location of sampling effects at day +90 on the resin flow (g/day) of loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina. Resin flow was sampled within the area used for wounding/inoculation treatments and 1m above the area used for wounding/inoculation treatments. Values reported are means +/- standard errors (n=24). Different letters indicate significant differences in resin flow within rows (age/season sample groups). Significance was determined using difference analysis at $\alpha=0.05$.

	Resin flow within wounded area	Resin flow above wounded area
6 year old/spring	1.044 +/- 0.162 a	1.265 +/- 0.277 a
12 year old/spring	2.109 +/- 0.220 b	3.688 +/- 0.444 a
6 year old/summer	1.373 +/- 0.314 a	1.466 +/- 0.342 a
12 year old/summer	1.200 +/- 0.254 b	2.031 +/- 0.434 a

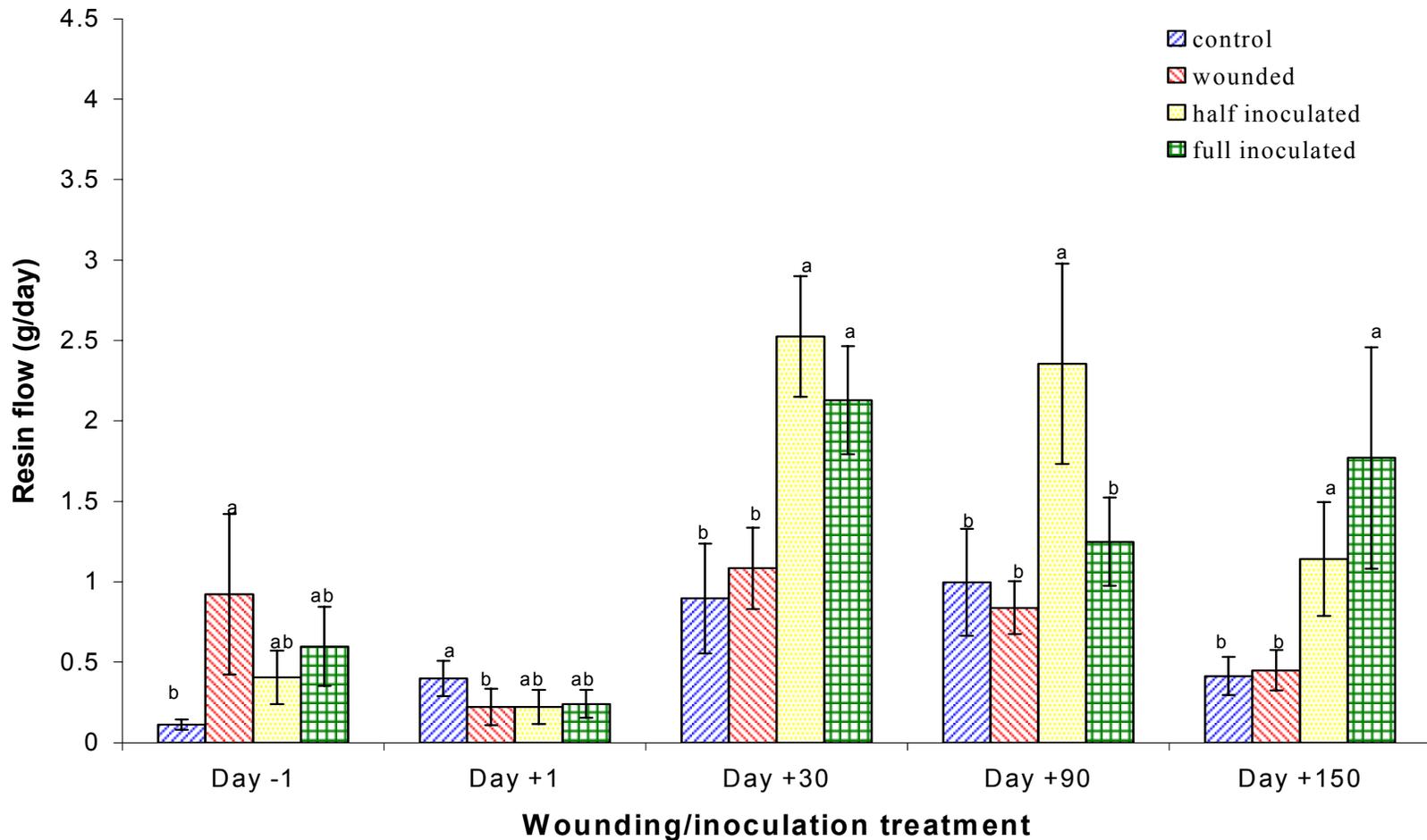


Figure 1. The effects of artificial wounding and *Ophiostoma minus* inoculation treatment on the resin flow (g/day) of 6-year-old loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina during the spring sampling. Values reported are means +/- standard error (n=12). Different letters indicate that resin flow values were significantly different within each day of wounding/inoculation treatment. Significance was determined using LSD at $\alpha=0.05$. Day -1 indicates resin flow rates one day prior to wounding/inoculation treatments; other days are post- wounding/inoculation treatments.

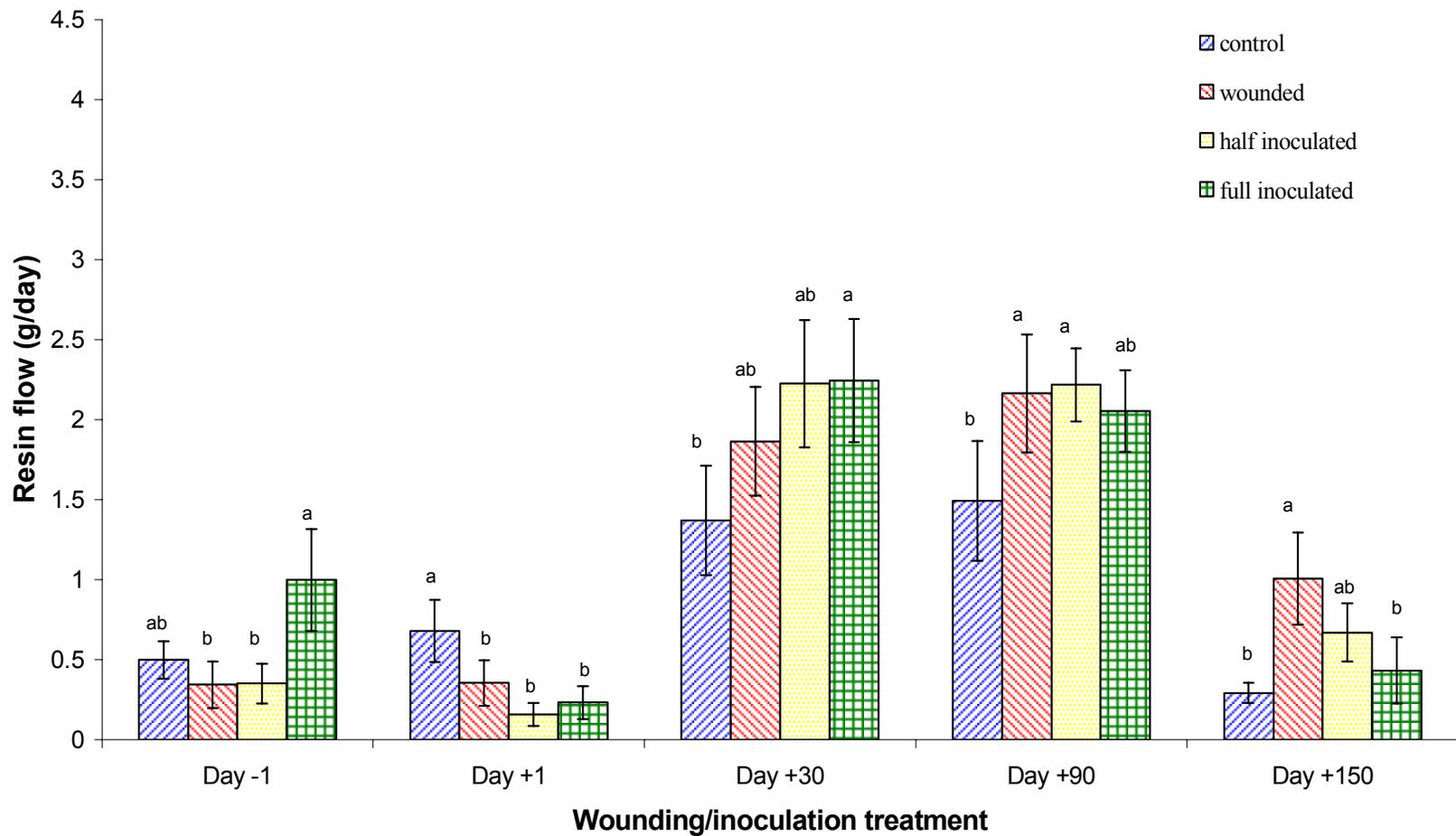


Figure 2. The effects of artificial wounding and *Ophiostoma minus* inoculation treatment on the resin flow (g/day) of 12-year-old loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina during the spring sampling. Values reported are means +/- standard error (n=12). Different letters indicate that resin flow values were significantly different within each day of wounding/inoculation treatment. Significance was determined using LSD at $\alpha=0.05$. Day -1 indicates resin flow rates one day prior to wounding/inoculation treatments; other days are post- wounding/inoculation treatments.

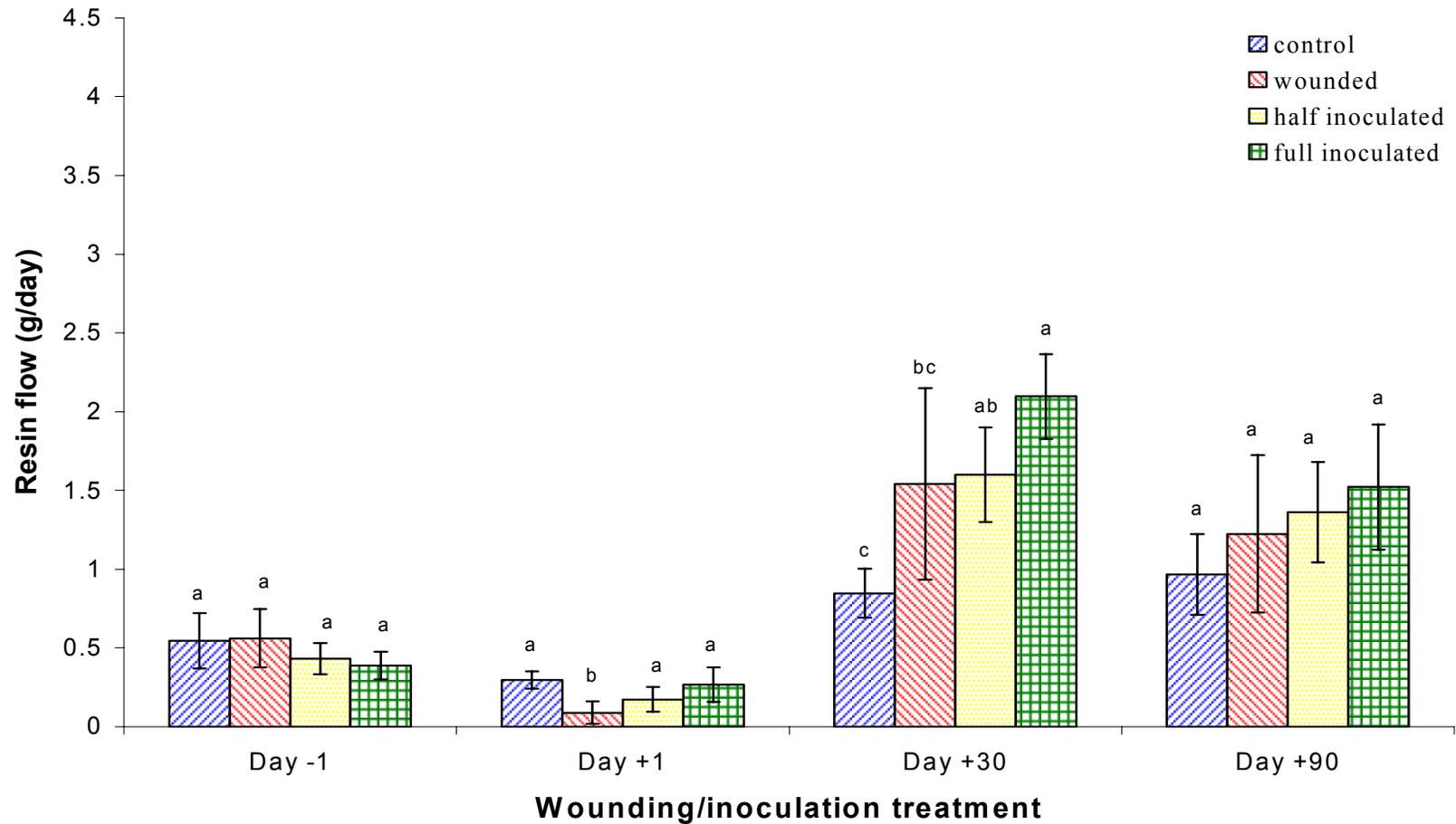


Figure 3. The effects of artificial wounding and *Ophiostoma minus* inoculation treatment on the resin flow (g/day) of 6-year-old loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina during the summer sampling. Values reported are means +/- standard error (n=12). Different letters indicate that resin flow values were significantly different within each day of wounding/inoculation treatment. Significance was determined using LSD at $\alpha=0.05$. Day -1 indicates resin flow rates one day prior to wounding/inoculation treatments; other days are post- wounding/inoculation treatments.

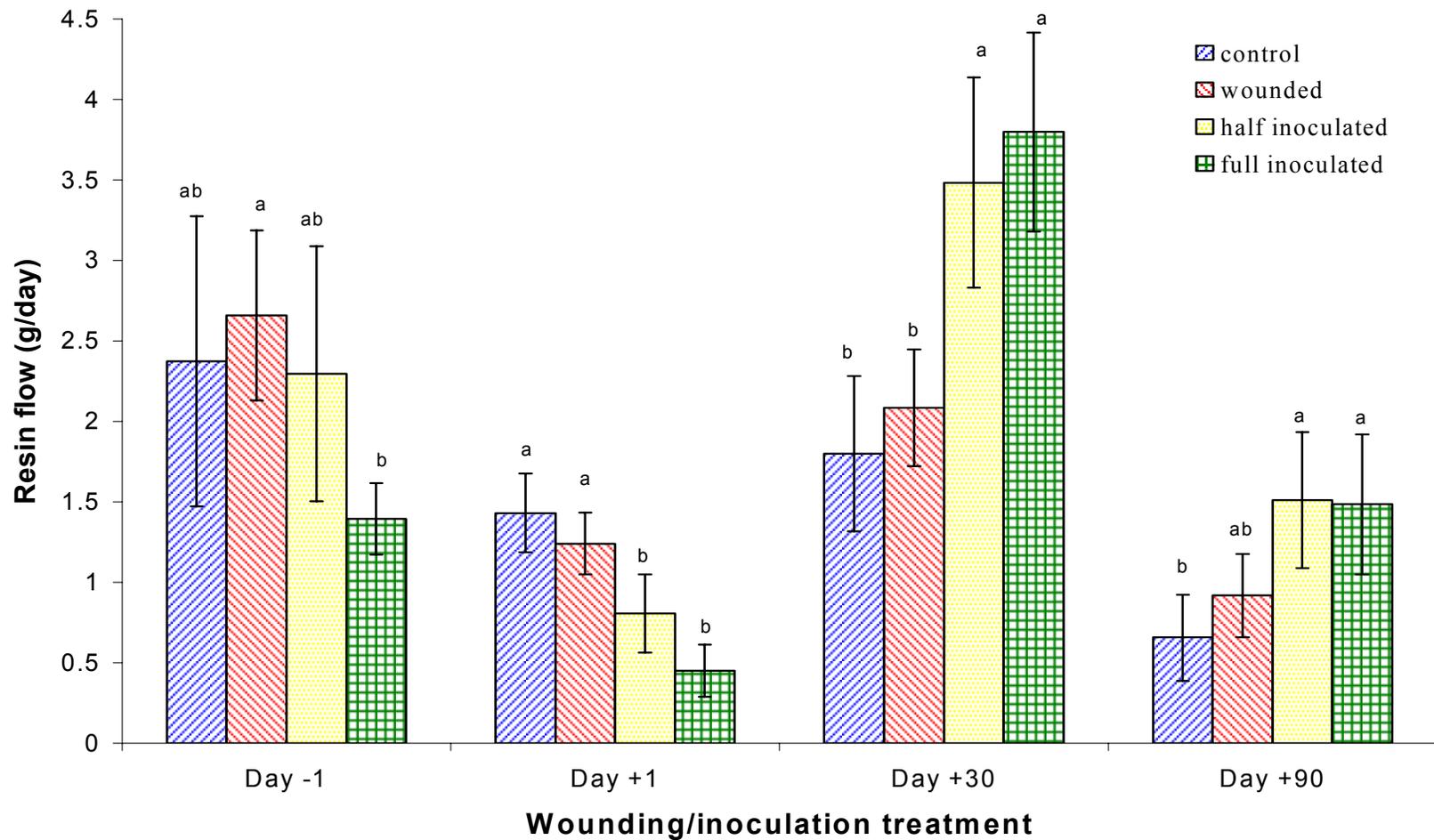


Figure 4. The effects of artificial wounding and *Ophiostoma minus* inoculation treatment on the resin flow (g/day) of 12-year-old loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina during the summer sampling. Values reported are means +/- standard error (n=12). Different letters indicate that resin flow values were significantly different within each day of wounding/inoculation treatment. Significance was determined using LSD at $\alpha=0.05$. Day -1 indicates resin flow rates one day prior to

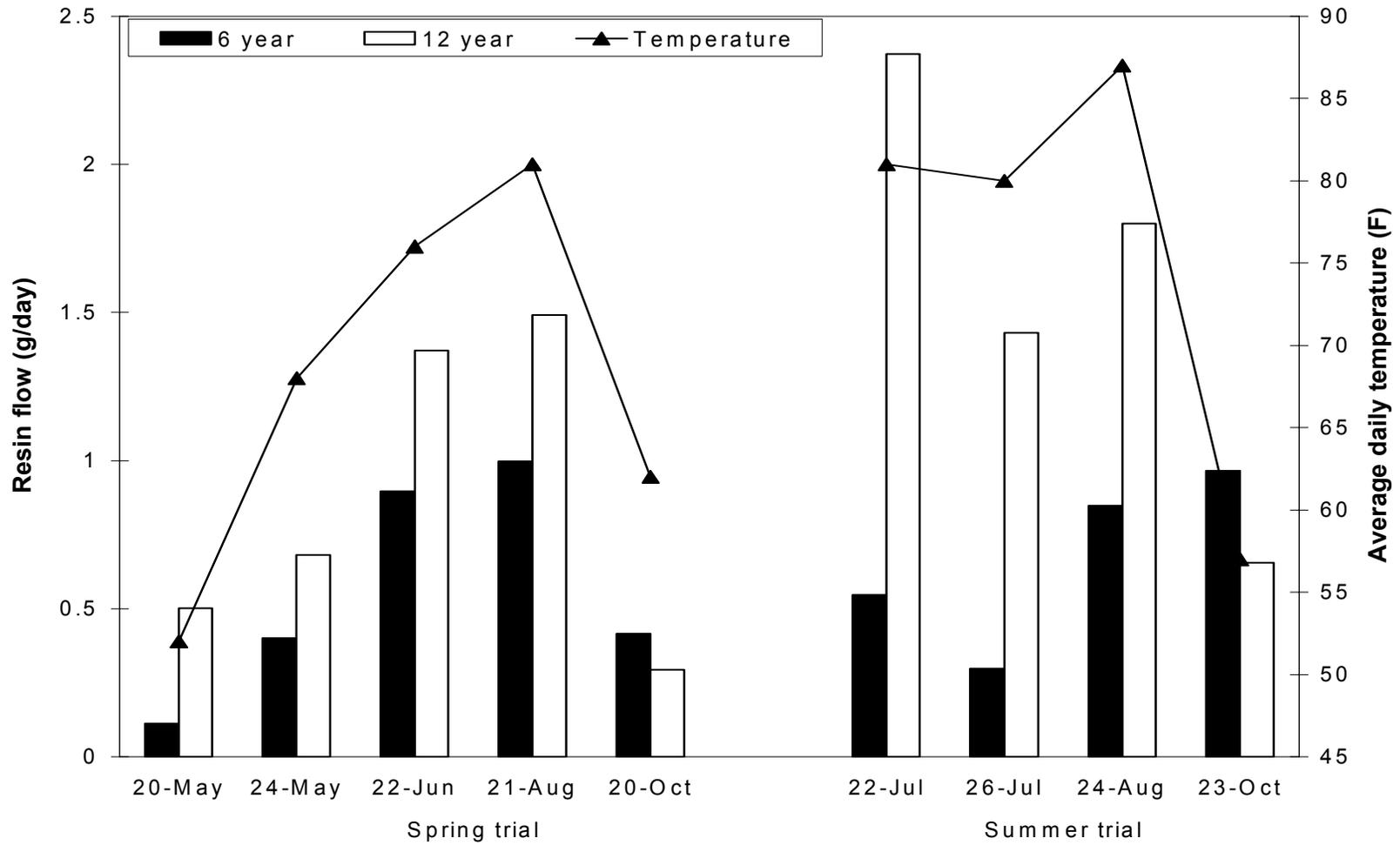


Figure 5. Temporal effects on the resin flow (g/day) of 6- and 12-year-old loblolly pine (*Pinus taeda*) in Hill Forest, North Carolina. Values reported for resin flow are means (n=12). Values reported for temperature are average daily temperatures.

Southern pine beetle and fire in Linville Gorge wilderness area

ABSTRACT

Historically, fire and the southern pine beetle (*Dendroctonus frontalis*) interact in a manner that maintains the stability of pine forests in the southern Appalachians. Fire suppression has recently been implicated in the disruption of pine forest integrity and a shift toward hardwood dominance. This study examined the influences of fire and southern pine beetle infestation on stand composition and resin flow of pines in the Linville Gorge Wilderness of North Carolina. Resin flow in most pine species will show an increase in response to various types of wounding and is a pine tree's primary defense against insect pathogens. Pine-dominated stands showed a decrease in both basal area and density after exposure to fire and/or beetle infestation. Decreases in basal area and density of *Pinus pungens* were strongly associated with infestation, while decreases in *Pinus rigida* were influenced primarily by burning. Resin flow was substantially higher in trees that were burned, but not infested, than in trees experiencing any other combination of burning and infestation. This elevated resin flow was still present 18 months after burning. Since resin flow is the primary means of host defense against southern pine beetle, it is suspected that fire could confer an acquired resistance to future southern pine beetle infestations through elevated resin flow.

INTRODUCTION

Historically, the southern pine beetle (*Dendroctonus frontalis*) and fire are believed to have occurred in an equilibrial relationship that promoted the stability of pine forests (Schowalter et al. 1981). Though the effects of fire on beetle susceptibility are not well understood, the action of fire on beetle-killed trees is responsible for pine regeneration. The southern pine beetle infests mature pine stands, killing trees and leaving high fuel loads on the ground. Historically, high intensity fires have consumed these fuels and thus opened the pathway for re-establishment of pine forests in the southern Appalachians. Without high intensity fires, the beetle-killed pines decay, while shade tolerant hardwoods become established and out-compete the pines for dominance of the stand (Schowalter et al. 1981, White 1987). Similar relationships are also found with western pine species and bark beetles (Geiszler et al. 1980, Geiszler et al. 1984).

The interaction of fire and *D. frontalis* in pine forests maintained widespread pine-dominated communities on upland sites and pine-hardwood communities on lowland sites (Schowalter et al. 1981). The southern Appalachians had mean fire intervals of 7-12 years in the presettlement era (Harmon 1982). Delcourt and Delcourt (1998) found that prehistoric fires used by American Indians increased biological diversity in the southern Appalachians and sharpened the ecotonal boundaries between community types.

More recently, management practices used by many in the southern Appalachians and elsewhere have resulted in fire suppression and mean fire intervals of more than 100 years. Many argue that this suppression is altering the natural equilibrium that occurs in forests among fire, plant communities and the animals that inhabit these communities (Schowalter et al. 1981). As a result, the steady state composition of pine and pine-hardwood communities is no longer intact (Schowalter et al. 1981). In the Great Smoky Mountains National Park, pine forests are no longer considered stable. Pine dominance is decreasing due to interactions among fire suppression, beetle infestation, and drought, while hardwood forests are becoming dominant through succession (White 1987). Older trees are more susceptible to beetle invasion and, without fire to facilitate pine regeneration, pine forests are dwindling. The decline in pine-dominated forests in areas such as the Great Smoky Mountains National Park has led to investigations concerning the stability of pine forests in other areas.

To begin to understand the impacts fire suppression is having on southern pine populations, we must first better understand the role fire plays in the susceptibility of pines to southern pine beetle infestation. When a pine tree is first subjected to southern pine beetle attack, it begins its response by increasing the flow of resin, which is formed in the secondary xylem (Hodges et al. 1979, Ruel et al. 1998, Phillips and Croteau 1999). Since the same reaction has been seen in response to mechanical wounding and fungal infection (Ruel et al. 1998, Klepzig et al. 2005), it is possible that resin also increases in response to fire. The resin response in pines serves as both a chemical and physical barrier to beetle infestation. The monoterpene content of resin has been found to attract beetles but is also toxic to young instar beetles. While the chemical barriers to beetle success are strong, the physical effects of resin are more effective at limiting beetle entry into the tree (Hodges et al. 1979, Phillips and Croteau 1999). As the beetle begins to bore into the tree, resin ducts are severed, and the newly created entry hole, with the beetle inside, begins to fill with resin. As the resin continues to flow, the beetle is either ejected from the tree or becomes entombed in the resin and is killed. As more beetles attack, the response is repeated as long as resin is available. In this manner, low-density southern pine beetle attacks can be thwarted. However, high-density southern pine beetle outbreaks will almost always result in tree death, as the resin resources of the pine are depleted, and the southern pine beetle becomes successfully ensconced in the tree.

While the role of resin in pine defense to southern pine beetle is becoming more widely understood, the response of resin flow to many factors has not been as greatly studied. Because fire has played such an integral role in the maintenance of pine forests and their interactions with southern pine beetle, we were interested in the impact that fire has on the defense systems of pine trees. Our study was designed to evaluate the effects of fire on the resin system of pine trees as well as the effects of interactions between fire and southern pine beetle on resin flow in these trees. We were also able to evaluate change in community composition as a result of fire, southern pine beetle, and their interaction.

STUDY SITE

Linville Gorge, located in Avery County, North Carolina, supports a combination of plant community types that is unusual in the southern Appalachians. Newell and Peet (1998) found that the factors responsible for this uniqueness include low rainfall, infertile soils, complex topography, and protection of the area from human disturbance. Established as a wilderness area in 1951, Linville Gorge's future was secured by the implementation of the Wilderness Act of 1964. Wilderness status designates an area as one that will suffer little from the persistent pressures of human disturbance that have plagued the majority of the natural areas in the United States. Consequently, less than 5% of Linville Gorge has been logged (Newell and Peet 1998).

Consisting of more than 11,500 acres, Linville Gorge Wilderness ranges in elevation from 1650-4120 ft. While generally immune to human disturbance, the area has experienced human-induced fires as well as naturally occurring fires, which are actively managed with suppression despite the designation of Linville Gorge as a wilderness area. The last catastrophic fire was in 1915 and the last surface fire occurred in the 1950's. Since that time, fires have been suppressed (Newell and Peet 1998). In November 2000, a campfire was left unattended and burned approximately 7500 acres of the Wilderness area. The fire was primarily of mid intensity, though at some locations the fire did get into the crowns of trees.

METHODS

In the summer of 1992, Claire Newell established 181 permanent vegetation inventory plots, which she used to develop a plant community classification of the Linville Gorge Wilderness (Newell and Peet 1998). Of her twenty-eight community types, six are either dominated or co-dominated by one of the four pine species occurring there. These pine species are Virginia pine (*Pinus virginiana*), table mountain pine (*P. pungens*), pitch pine (*P. rigida*), and white pine (*P. strobus*). In the summer of 2001, 30 of the plots originally established in 1992 were re-sampled for woody plant composition. The field sampling technique for all 30 plots was based on the protocol developed for the North Carolina Vegetation Survey (Peet et al. 1998). Typically, a 20 X 50 m plot is arranged as ten 10 X 10 m modules, in a 2 X 5 array, resulting in a 0.1 ha plot size. Due to the challenge of

rugged topography in Linville, the original plots often consisted of fewer than ten modules (Newell and Peet 1998). The number of modules used in our survey of each plot equaled the number of modules in the original plot. In all modules, all woody stems greater than 1.4 m in height were identified to species, measured for diameter at breast height, and assigned to diameter classes. Differences in stand density and basal area were analyzed between the two samplings using ANOVA in SAS (version 8.01, SAS Institute, 1999-2000). Changes in stand density and basal area with burning and/or infestation were investigated using ANOVA and Least Squares Means procedures in SAS. Special emphasis was placed on changes in pine density and basal area between the two sampling dates, and changes as a result of burning or southern pine beetle infestation. In addition, logistic regression analysis of 33 stand environmental characteristics from the 1992 sampling was conducted to determine which environmental factors might have served as predictors of the future infestation by southern pine beetle and/or burning. PROC LOGISTIC was run in SAS using forward selection and $\alpha = 0.1$.

A subset of 17 of the 30 plots was used to conduct resin flow analysis on a total of 133 pine trees. We recorded whether each tree was burned or not as determined by the presence or absence of blackened bark and the general condition of the stand. We also recorded whether the tree had been infested by southern pine beetle as determined by the presence of pitch tubes and exit wounds. Resin flow analysis was conducted on each sample tree using a hand drill to remove a 4 mm diameter plug of wood to the xylem/phloem interface. A small metal tray was installed to direct the flow of resin into a pre-weighed collection device. The tree was left for 24 hours, after which the collection device was removed and weighed, yielding a resin flow index for each tree in g/day. All trees were similar in height and diameter and all sampling was conducted within three weeks' time in May and June of 2001. Resin flow with respect to southern pine beetle and burn status was tested for significance using ANOVA in SAS. In the summer of 2002, the same 17 plots were re-surveyed for survivorship and resin flow analysis was conducted on all 133 trees following the same protocols used during the 2001 sampling. Differences in resin flow of survivors were analyzed using a t-test in SAS.

RESULTS

PLANT COMPOSITION

Twelve (40%) of the 30 plots re-sampled in 2001 had burned in the fire of November 2000 and had also been infested by southern pine beetle, 9 plots (30%) were burned but not infested, 2 plots (7%) were not burned but were infested, and 7 plots (23%) were neither burned nor infested with southern pine beetle.

There was a significant decline in density of all species between samplings ($p < 0.0001$); this decline was greater in stands that burned than in stands that did not burn ($p = 0.0078$). Analysis of burning and infestation combinations showed that density decreased significantly in the stands that were burned/uninfested and in the stands that were burned/infested (Fig. 1).

There was also a significant decline in basal area of all species between samplings ($p < 0.0001$); this decline was greater in stands that had been infested with southern pine beetle than in stands that were not infested ($p = 0.0088$). Analysis of burning and infestation combinations showed that basal area decreased significantly in the stands that were unburned/infested and in the stands that were burned/infested (Fig. 1).

There were significant declines in both density and basal area of pines between the sampling dates ($p < 0.0001$); these declines were greater in stands that were infested ($p < 0.0001$ for density and $p = 0.0029$ for basal area). Analysis of burning and infestation combinations showed that density decreased significantly in stands that were infested, whether burned or unburned, and in stands that were burned/uninfested (Fig. 2). Basal area decreased significantly in stands that were unburned/infested and that were burned/infested (Fig. 2).

Density and basal area of *Pinus pungens* decreased significantly between sampling dates ($p = 0.007$ and 0.0021 , respectively). In both cases, the decrease was greater when infestation was present ($p = 0.0144$ and < 0.0001 , respectively). Analysis showed that both density and basal area decreased significantly in stands that were infested, regardless of burning status (Fig. 3). *Pinus rigida* also decreased significantly in density ($p = 0.0011$) and basal area ($p = 0.0336$) between sampling dates. Analysis showed that stands that were burned, regardless of infestation status, decreased significantly in both basal area and density

(Fig. 4). *Pinus rigida* was largely absent from the 2001 study. Neither *Pinus strobus* nor *Pinus virginiana* showed a significant decrease in density or basal area between sampling dates or as a result of burning or infestation.

RESIN FLOW

Of the 133 trees used in the 2001 resin flow study, 22 trees (16%) were burned and also infested with southern pine beetle, 66 trees (50%) were burned but not infested, 9 trees (7%) were not burned but were infested with southern pine beetle, and 36 trees (27%) were neither burned nor infested with southern pine beetle. Additionally, 47 trees (35%) were *Pinus pungens*, 20 trees (15%) were *Pinus rigida*, 47 trees (35%) were *Pinus strobus*, and 19 trees (14%) were *Pinus virginiana*. Despite adequate representation of all four pine species, there were no *Pinus rigida* occurring in stands that were not burned, regardless of the presence of southern pine beetle. This lack of presence prohibited us from statistically investigating the impact of species on resin flow, as well as the interaction between species, burning and southern pine beetle infestation. However, ANOVA analysis of burned stands, regardless of infestation, indicated that species had no significant impact on resin flow ($p=0.275$) and that there was no effect on resin flow due to an interaction between species and southern pine beetle infestation ($p=0.7888$). All resin flow analyses were thus conducted without consideration of species effects.

Both burning and southern pine beetle infestation were found to have a significant effect on the resin flow of pine trees. Resin flow was significantly higher in trees that were burned than in unburned trees ($p=0.008$). Resin flow was significantly lower in trees that were infested with southern pine beetle than in trees that were not infested ($p=0.004$). There was also a significant interaction between infestation and burning ($p=0.012$).

In trees that were infested with southern pine beetle, there was no significant difference in resin flow associated with burn status (0.34 g/day not burned and 0.59 g/day burned). Additionally, in trees that were not burned, there was no significant difference in resin flow regardless of infestation status (0.55 g/day not infested and 0.59 g/day infested). The trees that were burned/uninfested had significantly higher resin flow (1.57 g/day) than trees with any other combination of factors (Fig. 5).

The 2002 survey found that none of the trees that were infested with southern pine beetle survived. Of the 66 trees that were burned/uninfested, 20 survived. These trees were used for resin flow analysis. The 36 unburned/uninfested trees from the 2001 survey were used as 2002 controls. Resin flow in 2001 was not an accurate predictor of survival into 2002. For those trees used in the 2002 survey, trees that burned had significantly higher resin flow than those that did not in both 2001 and 2002 (Fig. 6). There was no significant change in resin flow between 2001 and 2002 in either the burned or the unburned survivors.

DISCUSSION

In both coastal and piedmont stands, environmental factors such as slope, soils and tree density have been found to be the primary influences on susceptibility to southern pine beetle infestation (Hedden and Belanger 1985). Logistic analysis of our data indicated that none of these parameters influenced infestation in our plots. While it is surprising that slope would be a factor in piedmont stands but not in mountainous stands, our results indicate that the factors influencing southern pine beetle susceptibility in mountainous pine stands may not be the same factors at work in coastal and piedmont stands. Our data set was relatively small for this type of analysis with only 30 plots and 33 stand characteristics. Investigations using larger data sets could provide more insight into the characteristics that increase susceptibility of mountainous stands to southern pine beetle.

PLANT COMPOSITION

The density and basal area of the stands in this study decreased significantly in the 9 years between sampling. The decline in density was found to be largely the result of the fire that occurred in 2000, while the decline in basal area was due to southern pine beetle infestation. As the fire was of medium intensity, it can be expected that smaller stems were killed, while most of the larger stems were able to survive, thus explaining the impact of the fire on density between sampling dates. The influence of southern pine beetle infestation on the decline in basal area of the stands would seem out of place in a general study, but as these stands were chosen specifically for the presence of pine species, the major influence of a pine

pest on stands dominated by pines is expected. The decline in basal area as a result of beetle infestation is a reflection of the increased success of southern pine beetle on large pines.

In pine species there was a decrease in both density and basal area between the sampling dates. These decreases were greater in stands infested with southern pine beetle. The density and basal area of *Pinus rigida* and *Pinus pungens* decreased between samplings, but those of *Pinus strobus* and *Pinus virginiana* did not. *Pinus pungens* decline was attributed primarily to infestation by southern pine beetle, while burning was the factor most responsible for the decline of *Pinus rigida*.

Although *Pinus pungens* declined in both density and basal area between the two samplings, there is some hope that this decline might only be temporary, given the occurrence of fire in these pine forests. *P. pungens* is a fire-adapted species, bearing serotinous cones (Williams 1992). When a canopy is opened up by fire and the mineral soil exposed, the newly released seeds are able to germinate and *P. pungens* seedlings can grow to dominate the canopy (Williams and Johnson 1992, Welch et al. 2000). Without fires that open the canopy, reduce the amount of litter on the ground, and eliminate hardwood sprouting, *P. pungens* is not likely to re-establish (Williams and Johnson 1990, Williams and Johnson 1992, Welch et al. 2000). Unfortunately, canopy-opening fires, like the one that occurred in Linville Gorge, are not common in the range of *P. pungens*, due in part to fire suppression. It is thought that *P. pungens* is decreasing in importance throughout its range (Barden and Woods 1976, Harmon 1982, White 1987).

Pinus rigida is also a fire-adapted species, though serotinous cones occur as a polymorphic trait with respect to fire frequency (Givnish 1981). The future of *P. rigida* forests is thought to be bleak in light of fire suppression, with more competitive hardwoods predicted to dominate *P. rigida* forests in the absence of canopy-clearing fires (Waterman et al. 1995). *P. rigida* density and basal area declined significantly between samplings. If fires are not able to clear the forest floor and eliminate hardwood competition, *P. rigida* and *P. pungens* presence may be permanently affected.

While the role of fire in southern Appalachian pine forests is generally known, there is very little information available on the impact of southern pine beetle on these same pine forests. In general, *P. strobus* is thought to be more resistant than yellow pines, and *P.*

virginiana has been categorized as a preferred host (Veysey et al. 2003). Relative susceptibility has not been investigated and the results of our study, beyond indicating that southern pine beetle might have more adversely affected *P. pungens* than the other species, have provided little additional insight. It is somewhat surprising that *Pinus virginiana* showed no decline in basal area or density due to southern pine beetle infestation. Veysey et al. (2003) found that *P. virginiana* was highly susceptible to southern pine beetle infestation, though reproductive success of the beetle was low. However, *P. virginiana* was the least represented pine in our study. *Pinus strobus* has not historically been susceptible to southern pine beetle infestation, as the beetle has gained most of its success with the yellow pines. The ability of *P. strobus* to withstand the southern pine beetle attacks in Linville Gorge thus does not come as a surprise. However, half of the plots in which *P. strobus* were present had been infested with southern pine beetle and the density and basal area of *P. strobus* in these stands was lower than in stands that were not infested by southern pine beetle, though these differences were not significant. Additionally, many dead *P. strobus* were observed in the field with evidence of southern pine beetle infestation. This indicates that southern pine beetle may be more actively utilizing *P. strobus*. This would be especially problematic since the presence of *P. strobus* is increasing as fire suppression favors it and other fire-intolerant species (Welch et al. 2000). While the southern pine beetle may not yet have become fully successful in utilizing *P. strobus* as a host species, its success is increasing and care should be taken in watching their developing relationship.

RESIN FLOW

Resin flow was significantly impacted by southern pine beetle infestation. As in previous studies, resin flow was significantly lower in trees that were infested with southern pine beetle. As the beetle attacks pines, their resin responses react and within days of initial attack, constitutive resin stores are depleted. Induced resin cannot be produced at a rate high enough to overcome the beetle, and resin production ceases as infestation becomes more intense.

Resin flow increased in response to burning in the pine trees used in our study. Burning may be recognized by the tree as a generalized wound. The tree then responds by

increasing resin flow, much as it would in response to insect infestation (Hodges et al. 1979, Ruel et al. 1998, Phillips and Croteau 1999), or mechanical wounding and/or fungal inoculation (Ruel et al. 1998, Klepzig et al. 2005). Santoro et al. (2001) found that resin flow in red pine (*Pinus resinosa*) also increased in response to surface fires. Red pine is viewed as fire-adaptive and similar responses have also been shown in other fire-adaptive species such as longleaf pine (*Pinus palustris*) and ponderosa pine (*Pinus ponderosa*) (Harper 1944, Feeney et al. 1998). Santoro et al. (2001) suggest that the increase in resin flow response might be attributed to fire-adaptation in species that commonly experience surface fires. Our study was not able to determine the importance of species in resin flow responses to southern pine beetle or fire. We did see an increase in resin flow response to fire in white pine (*P. strobus*) from 0.40 g/day to 1.38 g/day. The other three species, Virginia pine (*Pinus virginiana*), table mountain pine (*P. pungens*), and pitch pine (*P. rigida*) remained largely unchanged or were not sampled widely enough for analysis. While table mountain pine and pitch pine have been described as fire-adapted species (Randles et al. 2001), white pine and Virginia pine are generally described as fire-intolerant. The extent of our study was not great enough to refute this hypothesis; however, our results with white pine indicate that further investigation into the relationship between resin flow and fire-adaptation would be of interest.

Our results indicate a strong interaction between burning and southern pine beetle on resin flow. While burning alone increased resin flow to values greater than any other combination of southern pine beetle and burning, the presence of southern pine beetle in burned stands reduced resin flow significantly (Fig. 5). The elevated resin conferred by fire was thus diminished in stands that were also infested.

There are varying hypotheses regarding the relationship between fire and bark beetles. Many believe that trees wounded by fire become weakened and are less resistant to attack. In northern forests, populations of several *Dendroctonus* and *Ips* species have been shown to increase following fire (Santoro et al. 2001, Geiszler et al. 1984, Rasmussen et al. 1996, Amman and Ryan 1991). It is thought that bark beetles accumulate in response to chemical signals originating from burned areas. Even when beetle presence has been established following fire, it is not always clear that the beetles are successful in attacking

burned pines. This may be explained by the increase in resin flow following fire. An increase in resin flow is thought to increase a tree's ability to withstand southern pine beetle infestation (Strom et al. 2002). It may be that the resin flow in burned trees is high enough that the attracted beetles, especially at low levels of aggregation, are unsuccessful in completing infestation.

The intensity of the fire is also hypothesized to play a role in determining insect response (Geiszler et al. 1984, Dixon et al. 1984, Rasmussen et al. 1996, Hanula et al. 1999). McHugh et al. (2003) and Wallin et al. (2003) found that bark beetles preferred trees heavily damaged by fire to those trees affected by low to moderate intensity fires. The burned trees in our study had higher resin flow than unburned trees in both 2001 and 2002. The fact that our pines maintained such high levels of resin flow for at least 17 months after a moderate-intensity fire occurred could be of great relevance to pine survival. These elevated levels more than a year later indicate the possibility of some benefit of low to moderate intensity fires to trees confronted with southern pine beetle infestations.

The development of resistance mechanisms in response to biological or chemical agents is known as systemic acquired resistance (SAR) (Percival 2001). Ross (1961) first demonstrated the effectiveness of SAR in a laboratory setting using tobacco mosaic virus to inoculate a single leaf of tobacco, which conferred resistance to untreated portions of the plant. Since then, many examples of SAR have been discovered in several angiosperms. Research into SAR in gymnosperms is a relatively new topic of research. Christensen et al. (1999) found that Norway spruce (*Picea abies*) trees that were wounded or inoculated with the blue-stain fungus *Ceratocystis polonica* showed acquired resistance to subsequent fungal challenges. Christiansen and Krokene (1999) showed that this resistance was sufficient to increase resistance to attacks by the bark beetle *Ips typographus*. In an early study of acquired resistance, Cook and Hain (1988) found that there was some indication of long-term resistance in loblolly pine following prior attack by southern pine beetle, though results were mixed. In this and other early research that was inconclusive on the possibility of acquired resistance, the application of the fungal challenge was likely insufficient to estimate resistance, given what is now known about threshold levels (Krokene et al. 2000). There are no known inquiries into the effectiveness of fire as a means of inducing SAR, though our

study indicates that induced resin flow responses, present 18 months after burning, may provide some acquired resistance to southern pine beetle since resin flow is the primary means of defense. Subsequent challenges of burned trees with southern pine beetle should be conducted to determine if induced resin flow does confer acquired resistance.

With future research into the effects of fire intensity on resin flow and the duration of those effects, it may be found that fire not only aids in regeneration of pine forests, but may also serve to boost the immunity of pines to southern pine beetle. Upon completion of such work, we may find it possible to begin management of pine stands in the southern Appalachians with an appropriate intensity and frequency of fire conducted in the appropriate season that would help combat the massive damage done by southern pine beetle every year and to preserve the presence of pines in our southern Appalachian montane forests.

Literature cited

- Amman, G.D. and K.C. Ryan. 1991. Insect infestation of fire-injured trees in the greater Yellowstone area. Res. Note INT-398. U.S. Dept. of Agriculture, Forest Service, Intermountain Research Station. 9 p.
- Barden, L.S. and F.W. Woods. 1976. Effects of fire on pine and pine-hardwood forests in the southern Appalachians. For. Sci. 22: 399-403.
- Christiansen, E. and P. Krokene. 1999. Can Norway spruce trees be 'vaccinated' against attack by *Ips typographus*? Agric. For. Entomology 1: 185-187.
- Christiansen, E., P. Krokene, A.A. Berryman, V.R. Franceschi, T. Krekling, F. Lieutier, A. Lönneborg and H. Solheim. 1999. Mechanical injury and fungal infection induce acquire resistance in Norway spruce. Tree Physiol. 19: 399-403.
- Cook, S.P., and F.P. Hain. 1988. Wound response of loblolly and shortleaf pine attacked or reattacked by *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae) or its fungal associate, *Ceratocystis minor* (Hedgecock) Hunt. Can. J. For. Res. 18: 33-37.
- Delcourt, P.A. and H.R. Delcourt. 1998. The influence of prehistoric human-set fires on oak-chestnut forests in the southern Appalachians. Castanea 63: 337-345
- Dixon, W.N., J.A. Corneil, R.C. Wilkinson and J.L. Foltz. 1984. Using stem char to predict mortality and insect infestation of fire-damaged slash pines. South. J. Appl. For. 8: 85-88.
- Feeney, S.R., T.E. Kolb, W.W. Covington, and M.R. Wagner. 1998. Influence of thinning and burning restoration treatments on presettlement ponderosa pines at the Gus Pearson Natural Area. Can. J. For. Res. 28: 1295-1306.
- Geiszler, D.R., R.I. Gara, C.H. Driver, V.F. Gallucci, and R.E. Martin. 1980. Fire, fungi, and beetle influences on a lodgepole pine ecosystem of south-central Oregon. Oecologia 46: 239-243.
- Geiszler, D.R., R.I. Gara, and W.R. Littke. 1984. Bark beetle infestations of lodgepole pine following a fire in south central Oregon. Z. Agnew. Entomol. 98: 389-394.
- Givnish, T.J. 1981. Serotiny, geography, and fire in the pine-barrens of New Jersey. Evolution 35: 101-123.
- Hanula, J.L., J.R. Meeker, D.R. Miller, and E.L. Barnard. 1999. The effects of the 1998 Florida wildfires on pine bark beetles, reproduction weevils, and their associates.

- Study Plan No. FS-SRS-4505-34. U.S. Dept. of Agriculture, Forest Service, Southern Research Station. 20 p.
- Harmon, M.E. 1982. Fire history of the westernmost portion of Great Smoky Mountains National Park. *Bulletin of the Torrey Botanical Club* 109: 74-79.
- Harper, V.L. 1944. Effects of fire on gum yields of longleaf and slash pines. USDA Circular No. 710, Washington, D.C.
- Hedden, R.L. and R.P. Balanger. 1985. Predicting susceptibility to southern pine beetle attack in the coastal plain, piedmont, and southern Appalachians. In: *Integrated Pest Management Research Symposium: The Proceedings*. Eds. S.J. Branham and R.C. Thatcher. USDA For. Ser. Gen. Tech. Rep. S0-56. pp. 233-238.
- 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. *Can. Entomol.* 111: 889-896.
- Klepzig, K.D., D.J. Robison, 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 Physiol.* In Press.
- Krokene, P., H. Solheim, and B. Långström. 2000. Fungal infection and mechanical wounding induce disease resistance in Scots pine. *Eur. J. Plant Pathol.* 106: 537-541.
- McHugh, C.W., T.E. Kolb, and J.L. Wilson. 2003. Bark beetle attacks on ponderosa pine following fire in northern Arizona. *Environ. Entomol.* 32: 510-522.
- Newell, C. L and R.K. Peet. 1998. Vegetation of Linville Gorge Wilderness, North Carolina. *Castanea* 63: 275-322.
- Percival, G.C. 2001. Induction of systemic acquired disease resistance in plants: Potential implications for disease management in urban forestry. *J. Arboric.* 27: 181-191.
- Phillips, M.A. and R.B. Croteau. 1999. Resin-based defenses in conifers. *Trends in Plant Science* 4: 184-190.
- Randles, R.B., D.H. Van Lear, T.A. Waldrop, and D.M. Simon. 2001. Periodic burning in table mountain-pitch pine stands. In: *Proceedings of the Eleventh biennial southern silvicultural research conference*. Ed. Outcalt, K.W. 2001 March 20-22; Knoxville, TN. Gen. Tech. Rep. SRS – XX. Asheville, NC: USDA Forest Service, Southern Research Station. pp. 109-113.

- Rasmussen, L.A., G.D. Amman, J.C. Vandygriff, R.D. Oakes, A.S. Munson, and K.E. Gibson. 1996. Bark beetle and wood borer infestation in the Greater Yellowstone area during four postfire years. Res. Pap. INT-RP-487. Ogden, UT: U.S. Dept. of Agriculture, Forest Service, Intermountain Research Station. 10 p.
- Ross, A.F. 1961. Localized acquired resistance to plant virus infection in hypersensitive hosts. *Virology* 14: 329-339.
- Ruel, J.J., M.P. Ayers, and P.L. Lorio Jr. 1998. Loblolly pine responds to mechanical wounding with increased resin flow. *Can. J. For. Res.* 28: 596-602.
- Santoro, A.E., M.J. Lombardero, M.P. Ayres and J.J. Ruel. 2001. Interactions between fire and bark beetles in an old growth pine forest. *For. Ecol. Manage.* 144: 245-254.
- SAS Institute Inc. 1999-2000. SAS Version 8.01. SAS Institute Inc., Cary, North Carolina.
- Schowalter, T.D., R.N. Coulson, and D.A. Crossley Jr. 1981. Role of the southern pine beetle and fire in maintenance of structure and function of the southeastern coniferous forest. *Environ. Entomol.* 10: 821-825
- 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. *For. Ecol. Manage.* 158: 169-178.
- Veysey, J.S., M.P. Ayres, M.J. Lombardero, R.W. Hofstetter, and K.D. Klepzig. 2003. Relative suitability of Virginia pine and loblolly pine as host species for *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Environ. Entomol.* 32: 668-679.
- Wallin, K.F., T.E. Kolb, K.R. Skov, and M.R. Wagner. 2003. Effects of crown scorch on ponderosa pine resistance to bark beetles in northern Arizona. *Environ. Entomol.* 32: 652-661.
- Waterman, J.R., A.R. Gillespie, J.M. Vose, and W.T. Swank. 1995. The influence of mountain laurel in regeneration of pitch pine canopy gaps of the Coweeta Basin, North Carolina, USA. *Can. J. For. Res.* 25: 1756-1762.
- Welch, N.T., T.A. Waldrop, and E.R. Buckner. 2000. Response of southern Appalachian table mountain pine (*Pinus pungens*) and pitch pine (*P. rigida*) stands to prescribed burning. *For. Ecol. and Manage.* 136: 185-197.
- White, P.S. 1987. Natural disturbance, patch dynamics, and landscape pattern in natural areas. *Natural Areas Journal* 7: 14-22.
- Williams, C.E. 1992. An Appalachian original. *American Forests* 98: 24-26

Williams, C.E. and W.C. Johnson. 1990. Age structure and the maintenance of *Pinus pungens* in Pine-oak forests of southwestern Virginia. *Am. Midl. Nat.* 124: 130-141.

Williams C.E. and W.C. Johnson. 1992. Factors affecting recruitment of *Pinus pungens* in the southern Appalachian mountains. *Can. J. For. Res.* 22: 878-887.

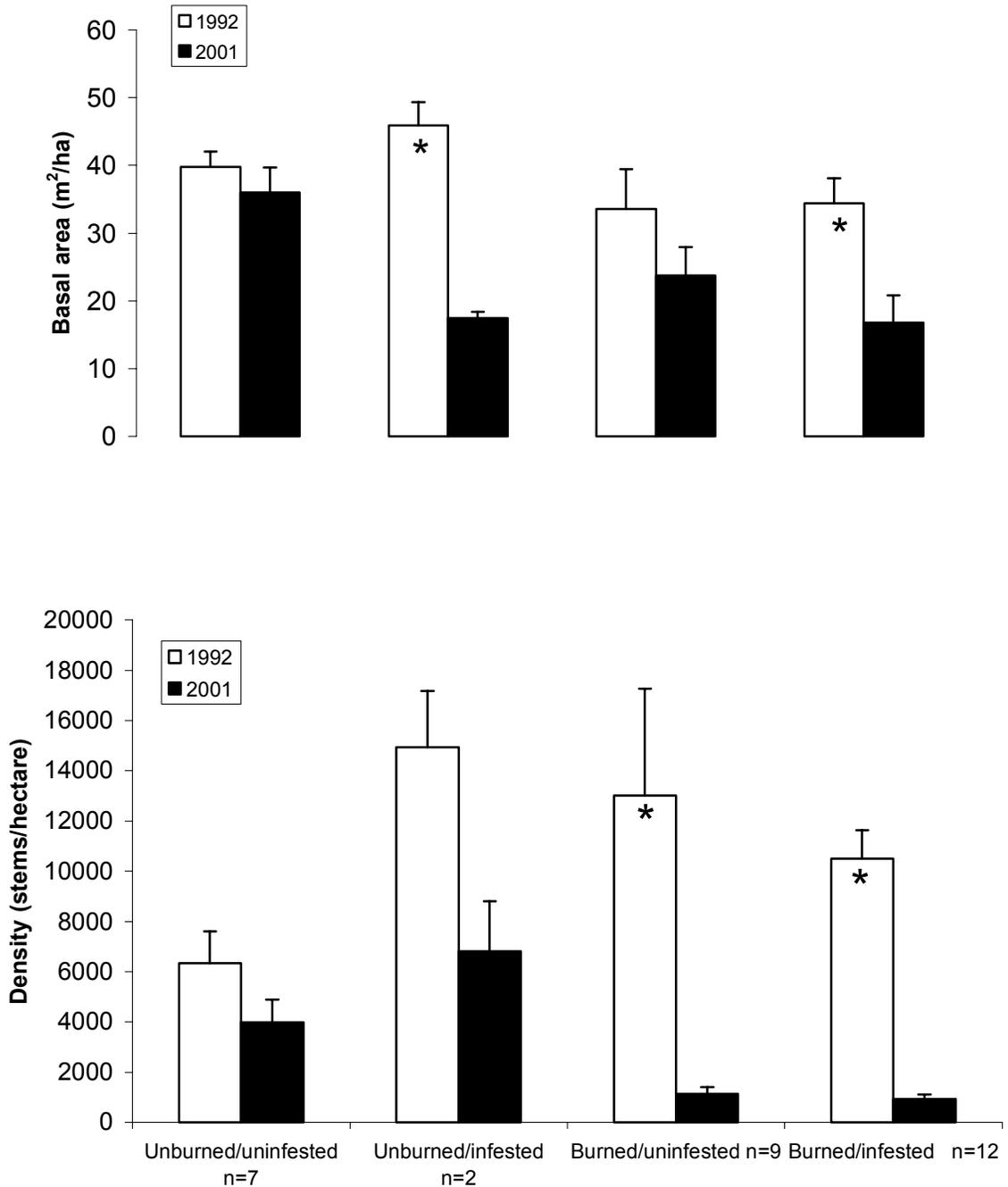


Figure 1. Plot density and basal area across all species. Values reported are means for all plots in each burning/infestation category + standard error. Significant differences between 1992 and 2001 are indicated for each burning/infestation category by an asterisk (*). Significance differences were determined using ANOVA at $\alpha=0.05$.

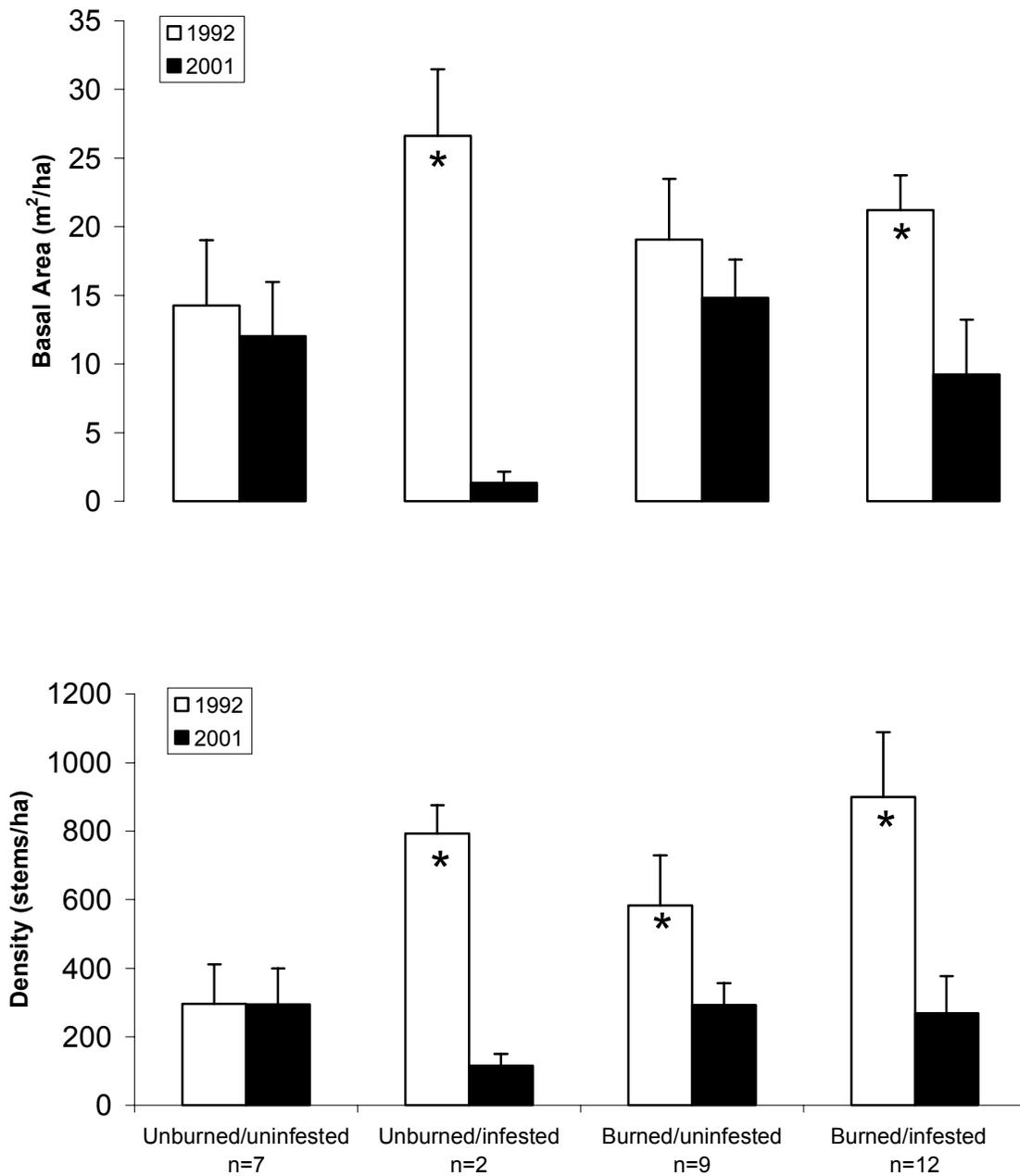


Figure 2. Plot density and basal area across all pine species. Values reported are means for all plots in each burning/infestation category + standard error. Significant differences between 1992 and 2001 are indicated for each burning/infestation category by an asterisk (*). Significance differences were determined using ANOVA at $\alpha=0.05$.

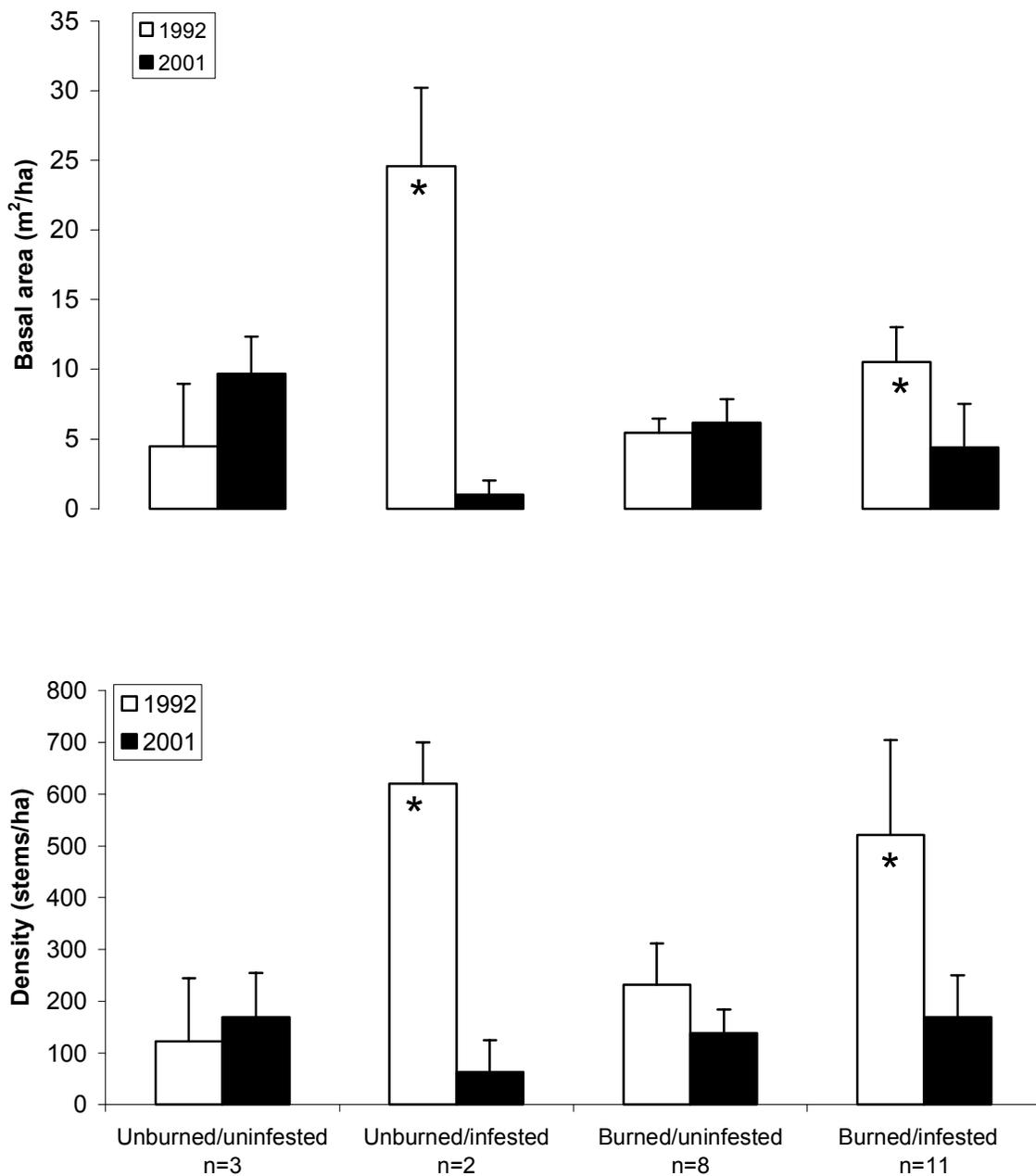


Figure 3. Plot density and basal area of *Pinus pungens*. Values reported are means + standard error for all plots in which *Pinus pungens* was present within each burning/infestation category. Significant differences between 1992 and 2001 are indicated for each burning/infestation category by an asterisk (*). Significance differences were determined using ANOVA at $\alpha=0.05$.

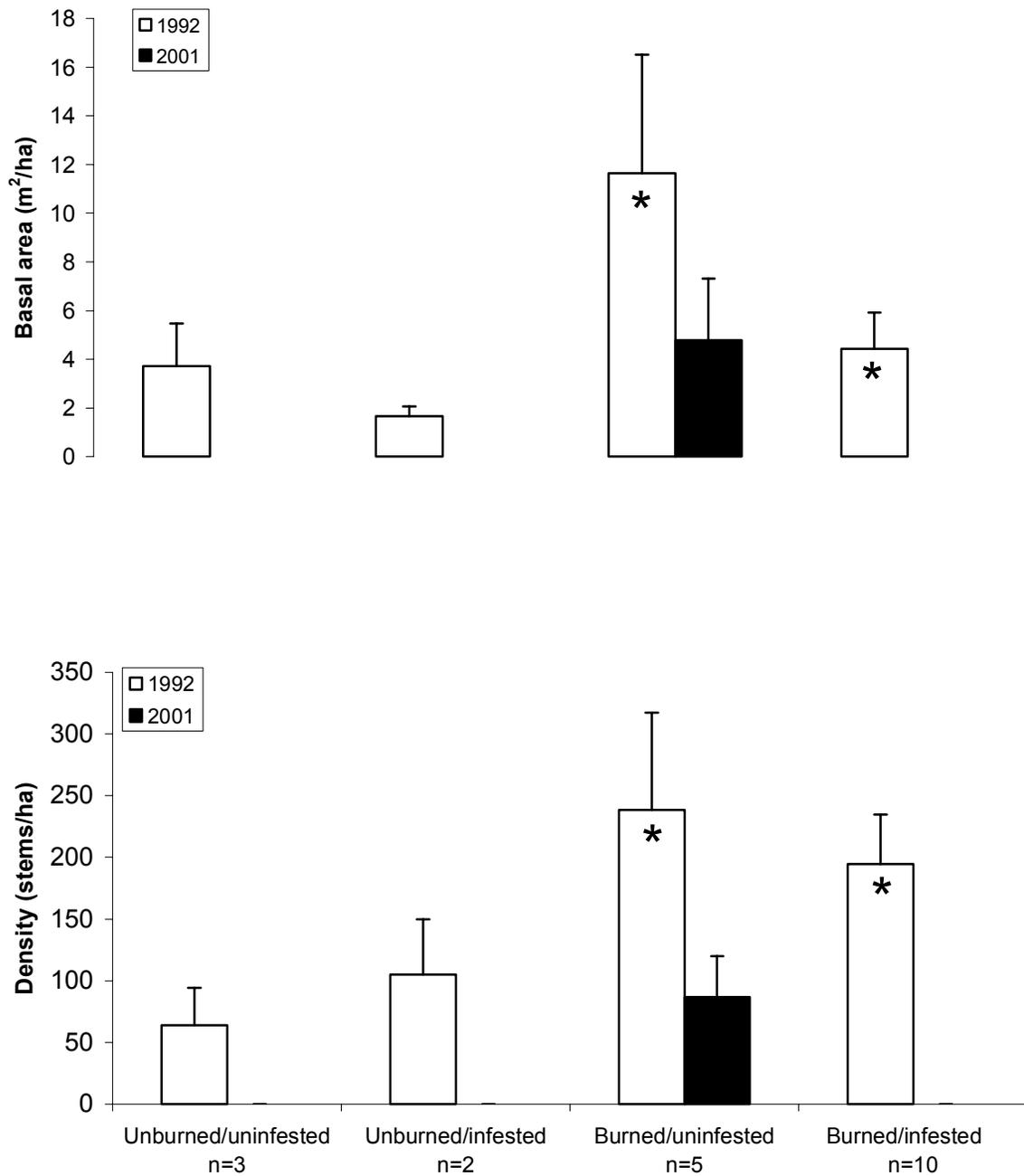


Figure 4. Plot density and basal area of *Pinus rigida*. Values reported are means + standard error for all plots in which *Pinus rigida* was present within each burning/infestation category. Significant differences between 1992 and 2001 are indicated for each burning/infestation category by an asterisk (*). Significance differences were determined using ANOVA at $\alpha=0.05$.

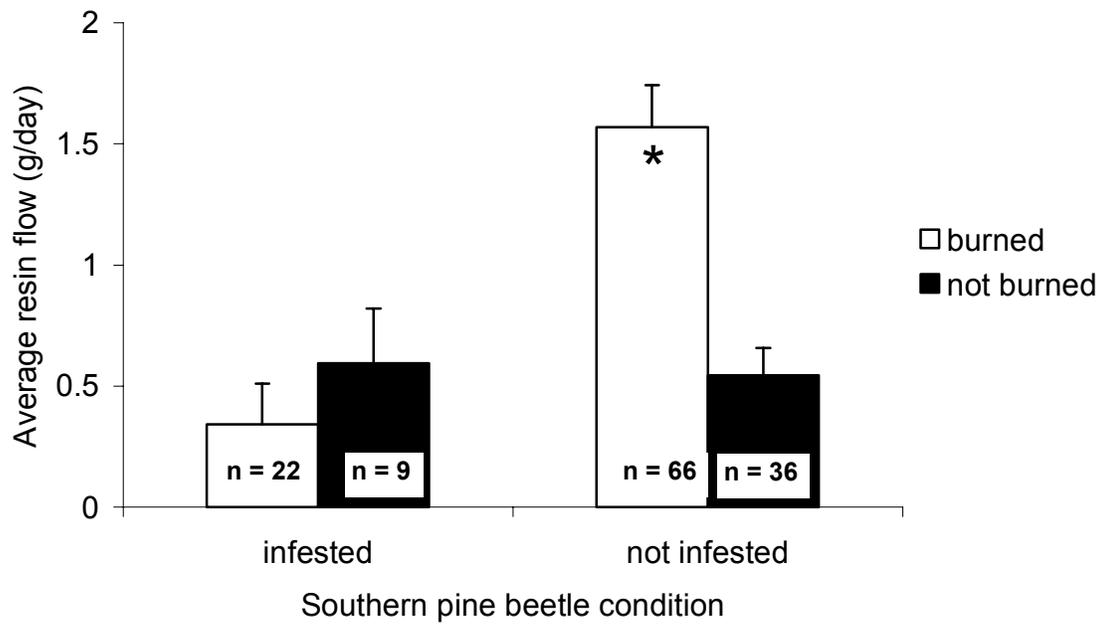


Figure 5. Resin flow of individual burned and/or infested pines. Values reported are means + standard error. Significant differences between burned and unburned trees are indicated for each infestation category by an asterisk (*). Significance differences were determined using ANOVA at $\alpha=0.05$.

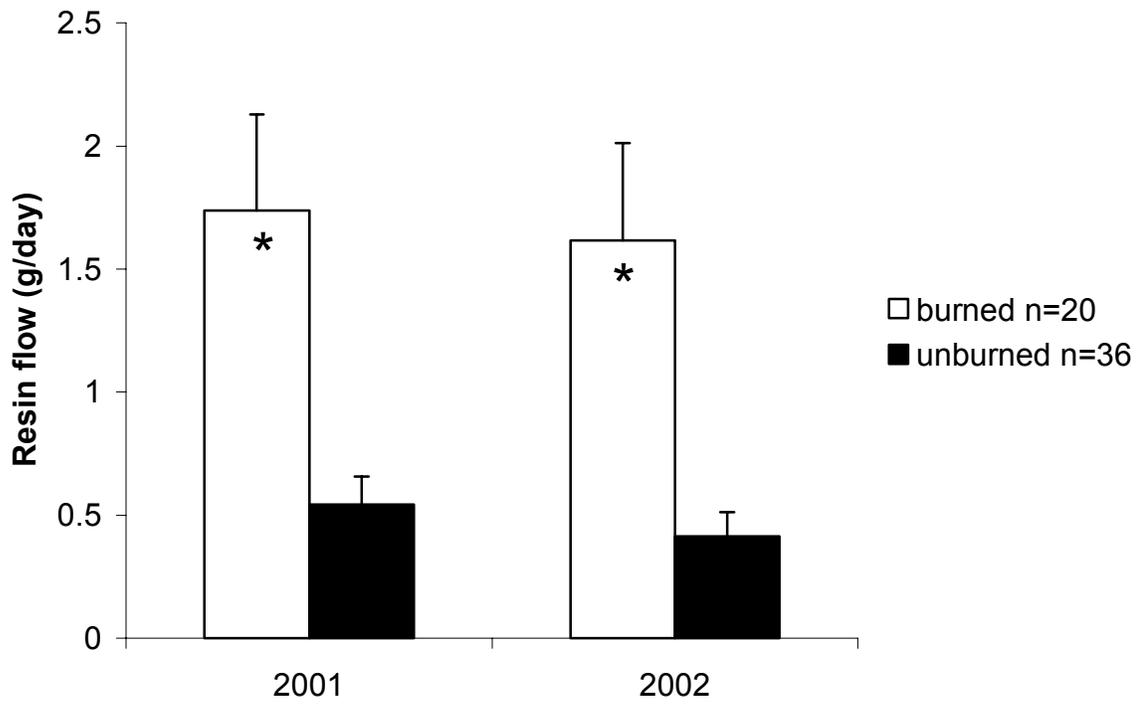


Figure 6. Resin flow of individual burned or unburned pines in 2001 and 2002. The same trees were sampled in each year. Values reported are means \pm standard error. Significant differences between burned and unburned trees are indicated for each year by an asterisk (*). Significance differences were determined using t-test at $\alpha=0.05$.

The potential for acquired resistance
to *Dendroctonus frontalis* in southern pines

ABSTRACT

The interactions among conifers, bark beetles and their common fungal associates have been widely studied, in part due to the severe economic and natural losses that occur annually. Host resistance, involving constitutive and induced defensive measures, and the factors associated with pest success are now widely understood and some losses can be avoided with proper management. Recent research with Norway spruce (*Picea abies*) has provided additional insight into the chemical pathways involved in host resistance, and has determined that trees exposed to mechanical wounding or fungal inoculation show acquired resistance to future pathogen attacks. This aspect of defense in loblolly pine (*Pinus taeda*) has not been adequately investigated. However, our recent studies indicate that fungal inoculation results in elevated resin flow levels that last up to one year after treatment. Further research in southern Appalachian pines showed that elevated resin flow occurs in response to low intensity fire, and that this response is still present at 18 months after burning. These studies indicate that acquired resistance through induced resin flow in southern pines is a possibility. Further research with beetle or fungal challenges could determine new possibilities for management of both natural and planted stands to maximize host defenses against southern pine beetle.

INTRODUCTION

Bark beetles are responsible for extensive damage in coniferous forests worldwide. The relationship between conifers and bark beetles is often heightened by the presence of one or more fungal associates. Economic losses and destruction of natural stands have increased the level of interest in the mechanisms functioning within these systems. Perhaps most alarming is the fact that many species of beetles can successfully attack and kill even the healthiest of trees when environmental conditions, host availability, and beetle numbers are favorable. Consequently, much research has been conducted to determine which factors contribute to host resistance and how that knowledge can be utilized to increase the resistance of host stands now and in the future. Genetic selection of resistant trees for use in plantations and restoration has shown much promise (Nebeker et al. 1992, Strom et al. 2002), but relatively little is currently understood about the mechanisms and chemical pathways involved in resistance of conifers to bark beetle and their associated fungi. Recent aggressive research into these systems has led to several new findings and opened up possibilities beyond genetic selection.

BARK BEETLE / CONIFER / FUNGUS SYSTEMS

The relationship among loblolly pine, the southern pine beetle, and the blue-stain fungus *Ophiostoma minus* is perhaps the most thoroughly studied of the conifer / bark beetle / fungal associate systems. Research has shown that the mechanisms working in the loblolly pine / southern pine beetle / *O. minus* system are much the same as those in other conifer / beetle / fungus systems. Early research found that the reaction of loblolly pine to beetle / fungal invasion consisted of a three-part response, involving both constitutive and induced measures (Berryman 1972, Hain et al. 1983). Constitutive defenses are those that are present before any pathogen attack, while induced defensive measures involve gene activation subsequent to attack, followed by synthesis of defensive compounds (Lieutier 2002). Constitutive defense mechanisms include bark thickness, which is thought to act as a deterrent to beetle success, and resin flow. Constitutive resin flow occurs when resin ducts, filled with preformed resin, are disrupted by the activity of the beetle. The resin is released from the ducts and serves as both a physical and chemical barrier to beetle success (Hodges et al. 1979). Many studies have determined that although the monoterpenes found in

performed resin are inhibitory to both beetles and fungi, the physical properties of the resin are more closely correlated to host resistance than are chemical properties (Hodges et al. 1979, Strom et al. 2002). Nebeker et al. (1992) found that 70-87% of primary resin flow in a typical loblolly pine occurs in the first 8 hours after wounding and resin supply is exhausted within 3 days after invasion (Cook and Hain 1987, Nebeker et al. 1995).

The second and third steps involved are induced defense mechanisms, consisting of infection containment through necrosis and wound healing by periderm formation. Infection containment, or the hypersensitive response, involves several activities, including the formation of traumatic resin ducts and production of secondary resin, which floods the area of impact and leads to necrosis. Secondary resin has increased levels of monoterpenes and phenolics and decreased concentrations of soluble sugars (Cook and Hain 1985, Cook and Hain 1986, Barras and Hodges 1969). Both monoterpenes and phenolics are highly toxic to beetle and fungal invaders (Raffa et al. 1985), and a decrease in soluble sugars is effectively a reduction of available nutrients. The wound area becomes saturated with resin and necrosis occurs around the infection site to isolate the invader (Wong and Berryman 1977, Gambiel et al. 1985, Stephen and Paine 1985). When fungi are present, the area of the necrosis is larger than that formed in response to beetles alone (Lieutier et al. 1996). If these measures are successful, then periderm formation occurs to isolate the infected area from healthy tissues. When environmental conditions are favorable for the host and beetle populations are low, these defensive measures are adequate for resistance to invasion (Paine et al. 1985). When beetle populations are high and colonization is rapid, no defensive measures are adequate to overcome infestation, and even the most resistant trees will die (Paine et al. 1985, Raffa et al. 1993, Strom et al. 2002). Investigations into the specific interactions between fungal species, the host tree, and the beetle, as well as threshold levels of beetles or fungi, and mechanical manipulation of resin flow have formed the bulk of the research into this system through much of the 1980s and early 1990s.

RECENT RESEARCH

An increasing amount of recent research has been carried out with Norway spruce (*Picea abies*) and its interactions with the bark beetle *Ips typographus* and the associated blue-stain fungus *Ceratocystis polonica*. One of the more studied aspects of host resistance

has involved chemical analysis of phenolics in the phloem parenchyma. Axial phloem parenchyma is one of the three major components of the secondary phloem of Norway spruce. The vacuoles of these parenchyma cells contain polyphenolic substances, resulting in the application of the term polyphenolic parenchyma (PP) cells (Franceschi et al. 1998). A single layer of PP cells follows spans of sieve cells, which are ten cells thick. Ray parenchyma cells run perpendicular through the sieve and PP cells (Franceschi et al. 1998). PP cells have been identified as one of the major sources of resistance of Norway spruce to fungal and insect pathogens, providing the phenolics in secondary resin that are toxic to both beetles and fungi. The PP cells of susceptible clones of Norway spruce have more highly condensed polyphenols within and often appear in a single row. The polyphenols of resistant clones show less dense and more evenly dispersed polyphenols within the PP cells, which are formed in double rows, resulting in more PP cells in resistant than susceptible clones (Franceschi et al. 1998). When trees are mechanically wounded, the resistant clones show a change in the phenolic contents as well as an increase in the size of the PP cells. Changes in the susceptible clones are similar, though less dramatic (Franceschi et al. 1998, Brignolas et al. 1998). It is thought that the phenolics in resistant clones are more soluble, making them more easily mobilized in response to disruption of the PP cells. The change in phenolic content of PP cells is of importance because phenolics are known to inactivate fungal enzymes and are deterrents to probing insects (Franceschi et al. 1998). These changes were visible by 6 days after wounding. While the response in PP cells may occur quickly enough to discourage a current beetle or fungal attack, Christiansen et al. (1999b) found that trees previously exposed to mechanical wounding or fungal infection were more resistant to subsequent fungal challenges. They hypothesize that changes in phloem parenchyma cells contribute to this acquired resistance (Franceschi et al. 2000, Nagy et al. 2000). Christiansen et al. (1999b), Krokene et al. (1999) and Christiansen and Krokene (1999) all found that Norway spruce trees pretreated by either wounding or sublethal infections of fungi were more resistant to subsequent, massive challenges of *C. polonica*. The resistance conferred depends on the amount of host tissue destroyed (Krokene et al. 2001) and also follows a dose-response dynamic, with larger doses of inoculum resulting in increased resistance to challenge inoculations (Krokene et al. 1999). Bark beetle attacks on trees previously treated

with mechanical wounding or fungal inoculation are also less successful than attacks on previously unexposed trees (Christiansen and Krokene 1999). Similar changes in PP cells occur in response to fungal inoculation by various species and to beetle attack, which indicates that the defense response is not pathogen specific (Franceschi et al. 2000, Krokene et al. 1999, Krokene et al. 2001).

The formation of traumatic resin ducts in the sapwood has also been directly linked to acquired resistance (Christiansen et al. 1999a). Axial traumatic resin ducts begin formation 6-12 days after wounding or inoculation, but are not fully formed until 36 days later (Franceschi et al. 1998, Christiansen et al. 1999a). These traumatic resin ducts are responsible for secondary resin, which occurs after primary flow has been drained, and contains additional phenolics that are antagonistic to both beetles and fungi (Franceschi et al. 1998). The timing of the formation of resin ducts makes it unlikely that they function solely as a deterrent to current infestation, because they are not fully formed until weeks after beetle attacks have likely subsided, and because they are persistent. These studies were the first to show that acquired resistance in conifers could be gained by prior exposure to pathogens, though there are numerous studies of acquired resistance occurring in angiosperms. Most of the research on acquired resistance in conifers has been conducted with Norway spruce and its associated beetle and fungus, but there is some evidence that acquired resistance may also play a role in the dynamics of the loblolly pine / southern pine beetle / *O. minus* system.

ACQUIRED RESISTANCE IN LOBLOLLY PINE

Early research in loblolly pine showed some indications of acquired resistance. Cook and Hain (1986) found that there was increased presence of 6 monoterpenes and changes in the relative amounts of those monoterpenes, along with a decrease in the soluble sugars, in necrotic lesions when compared to unwounded tissues. Their follow-up study (Cook and Hain 1988) showed that those monoterpene changes were still present when previously inoculated or beetle attacked trees were challenged with fungal inoculation one year later. While their findings were not statistically significant, they were promising, especially given the small sample size and the relatively low density of fungal challenge (Krokene et al. 2000). Paine and Stephen (1987) also examined the effects of previous exposure on subsequent resistance as indicated by lesion size following fungal challenge. Their findings

indicated that there was no benefit from previous exposure, but their fungal challenge dosage was also low relative to current practices. After this early work, not much research has been directly toward the possibility of acquired resistance in loblolly pine.

Our recent investigations and those of others indicate that loblolly pine, as well as other southern U.S. pines, may also have the ability to acquire long-term resistance to subsequent beetle/fungal invasions. Our research with loblolly pines showed that resin flow was significantly higher in trees that were mass wounded or wounded with fungal inoculation than in controls. This elevated resin flow lasted at least 150 days after treatments and was still present one year later in inoculated pines. Klepzig et al. (2005) found that elevated resin flow was present at 105 days after wounding and inoculation treatments, though the effects were not present one year later. Our research with four species of pines in the Linville Gorge wilderness area showed that exposure to a low intensity fire resulted in significantly higher resin flow, relative to controls. This elevated resin response was still present 18 months after the fire occurred. This shows that it is possible for induced resin flow to remain elevated over controls for an extended amount of time, and even into the next southern pine beetle flight season.

Another question that deserves further study is the extent of acquired resistance through the tree. It appears that the acquired resistance gained by sub-lethal exposure to wounding, fungal pathogens, or beetle attacks is limited to the area of influence in Norway spruce (Krokene et al. 1999). Klepzig et al. (2005) support this finding in loblolly pine. Our study found that in 12-year-old loblolly pines, resin flow was significantly lower within the area of wounding/inoculation than at 1 m higher along the bole, but there were no significant locational differences in 6-year-old loblolly pines. While the indications are that resin flow response to wounding and fungal inoculation seems to be isolated to the area of infection, this may not be the case with response to fire. The fire that occurred in Linville Gorge was of low intensity and visual evidence of fire was not usually present at heights much greater than 1.5 meters. Resin flow measurements were taken at a height of 2.4 meters, indicating that the influence of the fire on resin flow was present at a distance of almost 1 meter from the area of visual influence, though elevated temperatures occur well above that area, and no measures of tissue damage were taken. However, if a low intensity fire can confer acquired

resistance at a height of 2.4 m on the bole, and this corresponds with the area of attack of southern pine beetle, the potential for southern pine beetle defense is high, and further research into the effects of fire on southern pine beetle resistance would be worthwhile.

Obviously, mechanical wounding, fungal inoculation, and fire are not the only factors that may influence resin flow in conifers. One of the most studied elicitors of resin flow is fertilization. It has generally been found that fertilization has a negative impact on resin flow or none at all (Knebel et al. 2006). Our study found that fertilized trees had higher resin flow than unfertilized controls at one month after fertilization. This area requires some further research into the impact of age, fertilization rate, and environmental conditions such as drought to determine if fertilization has any impact on resin flow.

Even more promising are recent findings in the chemical pathway involved in inducing resin flow in conifers. In addition to occurring in response to wounding, fungal inoculation, or beetle attack, acquired resistance also occurs in Norway spruce in response to exogenous application of methyl jasmonate, which may be a more realistic field approach to acquired resistance than mechanical manipulation (Franceschi et al. 2002). Methyl jasmonate application has shown similar responses in various conifers (Hudgins et al. 2003, Hudgins et al. 2004, Hudgins and Franceschi 2004, Miller et al. 2005). Ethylene has also been identified as an elicitor of the resin flow response. This area of research has only very recently begun to be investigated and is certain to become more prevalent in the literature as knowledge into pathogen resistance is gained.

RECOMMENDATIONS AND CONCLUSIONS

While research into conifer host resistance is continually increasing worldwide, special emphasis needs to be placed on the possibility of acquired resistance in southern pines. Southern pine beetle damage results in severe economic losses, and there are recent indications that abundance of natural stands is decreasing due to the interactions of southern pine beetle and fire suppression (Schowalter et al. 1981). While our research with loblolly and Appalachian pines was limited to the investigation of resin flow, this response is the primary means of defense against attack, and trees with naturally higher constitutive levels of resin flow have repeatedly been shown to have greater resistance to southern pine beetle attacks. There are now several known methods of artificially inducing resin in southern

pinus, and we may assume that this induced defensive mechanism confers acquired resistance against southern pine beetle. Southern pines with mechanically elevated resin flow levels should be challenged with bark beetle attacks and fungal inoculations to determine if acquired resistance is present. Optimal levels of mechanical manipulation required to induce a favorable response as well as the duration of the effects of elicitors are also of importance. Research at the cellular level to examine changes in the PP cells and the formation of TD in southern pines should also be conducted in an effort to determine the chemical pathways involved in resistance. With increased knowledge about the specific mechanisms involved in host resistance to southern pine beetle and *O. minus*, as well as the potential for acquired resistance in southern pines, more effective decisions can be made when planting forests for harvest as well as for managing and maintaining natural pine forests.

Literature cited

- Barras, S.J. and J.D. Hodges. 1969. Carbohydrates of inner bark of *Pinus taeda* as affected by *Dendroctonus frontalis* and associated microorganisms. *Can. Entomol.* 101: 489-493.
- Berryman, A.A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. *Bioscience* 22: 598-602
- Brignolas F., F. Lieutier, D. Sauvard, E. Christiansen, and A.A. Berryman. 1998. Phenolic predictors for Norway spruce resistance to the bark beetle *Ips typographus* (Coleoptera: Scolytidae) and an associated fungus, *Ceratocystis polonica*. *Can. J. For. Res.* 28: 720-728.
- Christiansen, E. and P. Krokene. 1999. Can Norway spruce trees be 'vaccinated' against attack by *Ips typographus*? *Agric. For. Entomol.* 1: 185-187.
- Christiansen, E., V.R. Franceschi, N.E. Nagy, T. Krekling, A.A. Berryman, P. Krokene and H. Solheim. 1999b. Traumatic resin duct formation in Norway spruce (*Picea abies* (L.) Karst.) after wounding or infection with a bark beetle-associated blue-stain fungus, *Ceratocystis polonica*. In: *Physiology and Genetics of Tree-Phytophage Interactions*. Eds. F. Lieutier, W.J. Mattson and M.R. Wagner. Les Colloques de l'INRA, INRA Editions, Versailles, France.
- Christiansen, E., P. Krokene, A.A. Berryman, V.R. Franceschi, T. Krekling, F. Lieutier, A. Lönneborg, and H. Solheim. 1999a. Mechanical injury and fungal infection induce acquired resistance in Norway spruce. *Tree Physiol.* 19: 399-403.
- Cook, S.P. and F.P. Hain. 1985. Qualitative examination of the hypersensitive response of loblolly pine, *Pinus taeda* L., inoculated with two fungal associates of the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae). *Environ. Entomol.* 14: 396-400.
- Cook, S.P. and F.P. Hain. 1986. Defensive mechanisms of loblolly and shortleaf pine against attack by the southern pine beetle, *Dendroctonus frontalis* Zimmermann, and its fungal associate *Ceratocystis minor* (Hedgecock) Hunt. *J. Chem. Ecol.* 12: 1397-1406.
- Cook, S.P. and F.P. Hain. 1987. Four parameters of the wound response of loblolly and shortleaf pines to inoculation with the blue-staining fungus associated with the southern pine beetle. *Can. J. Bot.* 65: 2403-2409

- Cook, S.P., and F.P. Hain. 1988. Wound response of loblolly and shortleaf pine attacked or reattacked by *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae) or its fungal associate, *Ceratocystis minor* (Hedgecock) Hunt. *Can. J. For. Res.* 18: 33-37.
- Franceschi V.R., T. Krekling, A.A. Berryman, and E. Christiansen. 1998. Specialized phloem parenchyma cells in Norway spruce (Pinaceae) bark are an important site of defense reactions. *Am. J. Bot.* 85: 601-615.
- Franceschi V.R., P. Krokene, T. Krekling, and E. Christiansen. 2000. Phloem parenchyma cells are involved in local and distant defense responses to fungal inoculation or bark-beetle attack in Norway spruce (Pinaceae). *Am. J. Bot.* 87: 314-326.
- Franceschi, V.R., T. Krekling, and E. Christiansen. 2002. Application of methyl jasmonate on *Picea abies* (Pinaceae) stems induces defense-related responses in phloem and xylem. *Am. J. Bot.* 89: 578-586.
- Gambiel, H.A., R.G. Cates, M.K. Caffey-Moquin, and T.D. Paine. 1985. Variation in the chemistry of loblolly pine in relation to infection by the blue-stain fungus. In: *Integrated Pest Management Research Symposium*. Eds. S.J. Branham and R.C. Thatcher. USDA For. Sev. Gen. Tech. Rep. SO-56. pp. 177-184.
- Hain, F.P., W.D. Mawby, S.P. Cook, and F.H. Arthur. 1983. Host conifer reaction to stem invasion. *Z. Angew. Entomol.* 96: 247-256.
- 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. *Can. Ent.* 111: 889-896.
- Hudgins, J.W., E. Christiansen, and V.R. Franceschi. 2003. Methyl jasmonate induces changes mimicking anatomical defenses in diverse members of the Pinaceae. *Tree Physiol.* 23: 361-371.
- Hudgins, J.W., and V.R. Franceschi. 2004. Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiol.* 135: 2134-2149.
- Hudgins J.W., E. Christiansen, and V.R. Franceschi. 2004. Induction of anatomically based defense responses in stems of diverse conifers by methyl jasmonate: A phylogenetic perspective. *Tree Physiol.* 24: 251-264.
- Klepzig, K.D., D.J. Robison, 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 Physiol.* In Press.

- Knebel, L., D.J. Robison, T.R. Wentworth, and K.D. Klepzig. 2006. Resin flow responses to fertilization, wounding, and fungal inoculation in loblolly pine (*Pinus taeda*) in North Carolina. In Prep.
- Krokene P., E. Christiansen, H. Solheim, V.R. Franceschi, and A.A. Berryman. 1999. Induced resistance to pathogenic fungi in Norway spruce. *Plant Physiol.* 121: 565-570.
- Krokene, P., H. Solheim, and B. Långström. 2000. Fungal infection and mechanical wounding induce disease resistance in Scots pine. *Eur. J. Plant Pathol.* 106: 537-541.
- Krokene, P., H. Solheim, and E. Christiansen. 2001. Induction of disease resistance in Norway spruce (*Picea abies*) by necrotizing fungi. *Plant Pathol.* 50: 230-233.
- Lieutier, F. 2002. Mechanisms of resistance in conifers and bark beetle attack strategies. In: *Mechanisms and deployment of resistance in trees to insects*. Eds. M.R. Wagner, K.M. Clancy, F. Lieutier, and T.D. Paine. Kluwer Academic Press, The Netherlands. pp. 31-77.
- Lieutier, F, F. Brignolas, V. Picron, A. Yart, and C. Bastien. 1996. Can phloem phenols be used as markers of Scots pine resistance to bark beetles? In: *Dynamics of forest herbivory: Quest for pattern and principle*. Eds. W.J. Mattson, P. Niemela, and M. Rousi. USDA For. Ser. Tech. Rep. NC-183, North Central For. Exp. Sta., St. Paul, Minn. pp. 178-196.
- Miller, B. L.L. Madilao, S. Ralph, and J. Bohlmann. 2005. Insect-induced conifer defense. White pine weevil and methyl jasmonate induce traumatic resinosis, de novo formed volatile emissions, and accumulation of terpenoid synthase and putative octadecanoid pathway transcripts in Sitka spruce. *Plant Physiology* 137: 369-382.
- Nagy N.E., V.R. Franceschi, H. Solheim, T. Krekling, and E. Christiansen. 2000. Wound-induced traumatic resin duct formation in stems of Norway spruce (Pinaceae): Anatomy and cytochemical traits. *American Journal of Botany* 87: 302-313.
- 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. *For. Sci.* 38: 457-466.
- Nebeker, T.E., R. F. Schmitz, and R.A. Tisdale. 1995. Comparison of oleoresin flow in relation to wound size, growth rates, and disease status of lodgepole pine. *Can. J. Bot.* 73: 370-375.
- Paine, T.D., F.M. Stephen, and R.G. Cates. 1985. Induced defenses against *Dendroctonus frontalis* and associated fungi: Variation in loblolly pine resistance. In: *Integrated*

- Pest Management Research Symposium. Eds. S.J. Branham and R.C. Thatcher. USDA For. Sev. Gen. Tech. Rep. SO-56. pp. 169-176.
- Paine, T.D., and F.M. Stephen. 1987. Influence of tree stress and site quality on the induced defense system of loblolly pine. *Can. J. For. Res.* 17: 569-571.
- Raffa, K.F., A.A. Berryman, J. Simasko, W. Teal, and G.L. Wong. 1985. Effects of grand fir monoterpenes on the fir engraver, *Scolytus ventralis* (Coleoptera: Scolytidae), and its symbiotic fungus. *Environ. Entomol.* 14: 552-556.
- Raffa, K.F., T.W. Phillips, and S.M. Salmon. 1993. Strategies and mechanisms of host colonization by bark beetles. In: *Beetle-Pathogen Interactions in Conifer Forests*. Eds. T.D. Schowalter and P.W. Price. Academic Press, New York. pp. 339-357.
- Schowalter, T.D., R.N. Coulson, and D.A. Crossley Jr. 1981. Role of the southern pine beetle and fire in maintenance of structure and function of the southeastern coniferous forest. *Environ. Entomol.* 10: 821-825.
- Steven, F.M. and T.D. Paine. 1985. Seasonal patterns of host tree resistance to fungal associates of the southern pine beetle. *Z. Agnew. Entomol.* 99: 113-122.
- 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. *For. Ecol. and Manage.* 158: 169-178.
- Wong, B.L. and A.A. Berryman. 1977. Host resistance to the fir engraver beetle. Lesion development and containment of infection by resistant *Abies grandis* inoculated with *Trichosporium symbioticum*. *Can. J. Bot.* 55: 2358-2365.