

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

LARISSA KNEBEL,^{1,2} DANIEL J. ROBISON,³ THOMAS R. WENTWORTH¹ and KIER D. KLEPZIG⁴

¹ Department of Plant Biology, North Carolina State University, Raleigh, NC 27695, USA

² Corresponding author (lknebel@ves.org)

³ Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA

⁴ USDA Forest Service, Southern Research Station, Pineville, LA 71360, USA

Received August 21, 2007; accepted December 4, 2007; published online April 1, 2008

Summary Resin flow is the primary means of natural defense against southern pine beetle (*Dendroctonus frontalis* Zimm.), the most important insect pest of *Pinus* spp. in the southern United States. As a result, factors affecting resin flow are of interest to researchers and forest managers. We examined the influence of fertilization, artificial wounding and fungal inoculation on resin flow in 6- and 12-year-old stands of loblolly pine (*Pinus taeda* L.) and determined the extent of that influence within and above the wounded stem area and through time. Fertilization increased constitutive resin flow, but only the younger trees sustained increased resin flow after wounding and inoculation treatments. An induced resin flow response occurred between 1 and 30 days after wounding and inoculation treatments. Wounding with inoculation resulted in greater resin flow than wounding alone, but increasing amounts of inoculum did not increase resin flow. Increased resin flow (relative to controls) lasted for at least 90 days after wounding and inoculation. This increase appeared to be limited to the area of treatment, at least in younger trees. The long-lasting effects of fungal inoculation on resin flow, as well as the response to fertilization, suggest that acquired resistance through induced resin flow aids in decreasing susceptibility of loblolly pine to southern pine beetle.

Keywords: *Dendroctonus frontalis*, hypersensitive response, *Ophiostoma minus*, southern pine beetle.

Introduction

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimm. (Coleoptera: Curculionidae: Scolytinae), has been responsible for more widespread damage to southern pine (*Pinus* spp.) than any other insect (Coulson 1980, Pye et al. 2004). Because SPBs can attack and kill even the healthiest trees, tree defense against attack has been a major emphasis of SPB research.

Female SPBs select suitable host trees, bore into the inner bark and both the beetle and the tree release chemicals that attract male beetles (Payne 1980). Pines respond quickly to at-

tack through release of a substantial constitutive supply of resin and by an induced resin response (Phillips and Croteau 1999, Lombardero et al. 2000). The physical, rather than chemical, properties of resin are the primary defense of conifers against boring insect infestation (Hodges et al. 1979, Ruel et al. 1998, Phillips and Croteau 1999). As the beetle bores through the phloem and severs resin ducts, resin flows into the hole occupied by the beetle. In a resistant tree, the beetle is thus physically ejected or entombed in the resin (Trapp and Croteau 2001). Should beetles become successfully established, resin-soaked tree tissues may decrease oviposition success and larval survival (Berryman 1972, Raffa 1991). At low SPB population densities, resin flow is often sufficient to allow trees to withstand attack. In a high-density SPB outbreak, most trees will have insufficient resin to withstand repeated attacks (Strom et al. 2002). As large numbers of beetles enter the tree and resin defenses are depleted, the tree may die as a result of vascular dysfunction.

There are at least three species of fungus that may enter the tree in association with SPBs and infect the tree's vascular system. The phoretic blue-stain fungus, *Ophiostoma minus* (Hedgcock) H. and P. Sydow, is the strongest competitor and the most virulent of the three associates (Klepzig and Wilkens 1997, Klepzig et al. 2001, Klepzig and Walkinshaw 2003). This fungus is the only one of the three to cause extensive necrotic lesions within trees (Paine and Stephen 1987a). This induced hypersensitive tree response seals off the fungus and allows the formation of a wound periderm (Popp et al. 1995). The presence of *O. minus* in larval galleries also inhibits SPB larval development and lowers reproductive success (Barras 1970, Goldammer et al. 1990, Lombardero et al. 2000, Klepzig et al. 2001).

Through the physical ejection or entombment of beetles by constitutive resin flow, and the containment of beetle infestation or fungal infection, or both, through induced resin defenses, trees can withstand low density beetle attack. Because resin flow is the key to tree defense, and *O. minus* infection may be a strong co-factor in tree death (Lieutier 2002, Kopper

et al. 2004), factors that influence resin production and *O. minus* infection could play significant roles in the ability of trees to withstand infestation.

Some reports indicate relatively higher resin flow in older trees (Schmitt et al. 1988), but little is known of such responses in younger smaller trees. Several studies have indicated that fertilization of pines may affect resin flow (Wilkens et al. 1997, Warren et al. 1999), though the effects are inconsistent (see Ruel et al. 1998, Lombardero et al. 2000, Klepzig et al. 2005). Mechanical wounding has been used to alter resin flow in conifers (Lewinsohn et al. 1991, Tisdale and Nebeker 1992, Ruel et al. 1998) and is often used in studies of fungal impact on resin flow (Klepzig et al. 2005). Each of these factors is thought to affect resin flow, but it is unclear to what extent.

We examined the effects of stand age, fertilization, wounding and fungal inoculation on the extent and duration of resin flow in loblolly pine (*Pinus taeda* L.). This information is increasingly important because loblolly pine is the most widely planted tree worldwide, with about 19 million ha in the USA alone (Schultz 1997). It is increasingly subjected to intensive silvicultural techniques such as fertilization (Wear and Greis 2002) to increase yield. The influence of our study variables on the SPB–loblolly pine system could have significant economic and ecological effects on planted forests and perhaps on surrounding natural forests containing loblolly pine.

Materials and methods

Study site

Effects of age, fertilization, artificial wounding and fungal inoculation on resin flow of individual trees were evaluated in two loblolly pine plantations. The study was conducted in one 6-year-old stand and one 12-year-old stand, with replication within each stand. The plantations were in 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 (36°12'4" N, 78°53'17" W, 134 m a.s.l.). The area receives 1090 mm precipitation annually and has 200 growing season days in which the air temperature is above 0 °C (www.erh.noaa.gov, www.ces.ncsu.edu). Both sites are gently sloping with highly eroded soils in the Georgeville series (Kirby 1976). Both stands were planted at a density of 350–400 trees per 0.4 ha (the 6-year-old stand in 1995 and the 12-year-old stand in 1989). Neither weed nor competition control, nor fertilization was used prior to the study. No trees used in the study showed obvious signs of disease or previous insect attack. Though fusiform rust galls (*Cronartium quercuum* f. sp. *fusiforme*) were present on several trees in the stands, these trees were excluded from the study. At the time of the study, both stands contained a small percentage of other woody species, such as sweetgum (*Liquidambar styraciflua* L.), shortleaf pine (*Pinus echinata* Miller) and redbud (*Cercis canadensis* L.). The 6-year-old stand also contained a prominent early successional component including blackberries (*Rubus* spp.) and catbrier (*Smilax rotundifolia* L.), among other species.

Tree selection and fertilization treatment

Study trees were selected with the restriction that there was at least one non-study tree or 3.7 m between study trees. The study trees were selected for uniformity (i.e., straightness, crown condition, overall appearance of health). Study trees in the 6-year-old stand averaged 11 cm diameter at breast height (DBH) and study trees in the 12-year stand averaged 18 cm DBH. Each study tree was cleared of competing vegetation to a radius of 1.5 m from the tree by mechanical 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 study on emergent growth within that brushed cleared circle. Half the study trees in each plot received a fertilizer treatment of 0.45 kg urea and 0.06 kg triple superphosphate (TSP) dispensed in a circle at a radius of 30 cm from the tree (225 kg N ha⁻¹ as urea and 30 kg P ha⁻¹ as TSP), while the remaining trees were unfertilized. Fertilizer was applied on April 22, 2002. At 90 days after wounding/inoculation treatments (described below), phloem and foliage samples were collected for analysis of nutrient content to assess the uptake of fertilizer. Phloem plugs were collected from the disk removed from the tree for resin analysis (Warren et al. 1999). Foliar samples were collected by taking a sun-exposed branch from the mid-crown of each tree and collecting about 100 fascicles from the first flush of needles from the previous year's growth (Vose and Allen 1988). All samples were kept on ice and analyzed in a Carlo-Erba CN analyzer (e.g., Hooker and Compton 2003). Differences in percent nitrogen between fertilized and unfertilized trees were analyzed by a *t*-test (*P* = 0.05).

Wounding and inoculation treatments

To simulate initial SPB 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 "spring" and "summer" trials). One month after fertilization, on May 22, 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 the study trees. Trees were either mass wounded, mass wounded and half of the wounds inoculated, mass wounded with full inoculation, or left unwounded and uninoculated as controls. Wounding was carried out in a 2-m high band on each tree beginning 1 m above ground, with a central 15-cm band untreated where resin sampling took place. The wounded area was smoothed before wounding/inoculation with a drawshave to remove most of the outer bark, leaving the inner bark unscathed. The DBH for each tree was used to determine the number of wounds necessary to create a density of 400 wounds m⁻² in the 2-m high band. The wounds were made with a 4-mm diameter increment hammer to remove the bark and phloem and expose the underlying xylem surface. Inoculation of the wound sites with *O. minus* mycelium was conducted by inserting a 4-mm diameter × 3 mm disk of colonized

malt extract agar into each wound. Each wound was sealed by insertion of a 4-mm-diameter $\times$ 4-cm-long, sterilized wooden dowel to limit feral spores from entering the wound site. The fungus was isolated and grown from a single female SPB collected in Alabama in the spring of 2000. The methods of wounding, inoculation and fungal culture have been described by Klepzig et al. (2005).

Resin flow sampling

In addition to the Day -1 (pre-treatment) sampling, resin flow was also analyzed on Days $+1$, $+30$, $+60$, $+90$ and $+150$ and $+1$ year following 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 to a pre-weighed collection device (Lorio and Summers 1986). After 24 h, the resin samples were collected and weighed, yielding an index of resin flow in g day^{-1} . Previous resin collection was assumed to have a negligible effect on subsequent resin collection because of the paucity of radial connections between resin ducts (Lapasha and Wheeler 1990), an assumption commonly made for studies of this nature (Ruel et al. 1998, Warren et al. 1999, Klepzig et al. 2005).

On Day $+60$, resin flow was measured only in wounded and fully inoculated trees. Because the sampling was limited to two treatments and the results did not add to the understanding of system dynamics, these data were omitted from the complete statistical analysis.

On Day $+90$, an additional resin flow sample was collected at 1 m above the area of wounding (about 4 m above the tree base) in all wounded-only and full-inoculation trees to determine if the resin flow response was localized to the band within the wounded area or was more systemic.

Because beetle attacks occur seasonally and resin flow varies seasonally, about three months after fertilization (July 24), a second set of wounding/inoculation treatments were applied to a new set of previously unwounded and un-inoculated trees (summer trial). Resin flow was again sampled pre-wounding/inoculation (Day -1) treatment and on Days $+1$, $+30$, $+60$, $+90$ and $+150$ after wounding/inoculation. The trees in the summer trial were not sampled one year after treatment. The procedures followed for wounding, inoculation and resin sampling were as described for the spring trial. The data for the Day $+150$ after wounding/inoculation for the summer trials in both the 6-year-old stand and the 12-year-old stand were also omitted from the analysis. The timing of the summer trial was such that Day $+150$ fell in late December when none of the trees displayed measurable resin flow. This was most likely because of the low temperatures in the study area in December, when the mean maximum temperature was 10°C (www.erh.noaa.gov). Resin flow from ducts is negligible at temperatures below 18.3°C (Clements 1974).

Each combination of treatments ($2 \times$ fertilizer, $4 \times$ wounding/inoculation, $2 \times$ season, $2 \times$ age) was replicated six times,

for a total of 192 trees. The study was organized in a completely random design by site (age). Analysis of variance (ANOVA; $\alpha = 0.05$) was used to determine the effects of wounding/inoculation and fertilization treatments, as well as their interactions, on the resin flow of study individuals. The effect of time since wounding/inoculation treatments on the study trees, and any interaction between time and fertilization or wounding/inoculation treatments, was evaluated by a repeated measures ANOVA ($\alpha = 0.05$). Significant differences between wounding/inoculation treatments were determined by Fisher's LSD ($\alpha = 0.05$).

Results

Fertilizer effects on trees

Percent nitrogen was significantly higher ($P < 0.05$) in the phloem plugs of fertilized trees than in non-fertilized trees in both the 6-year-old stand (0.48 and 0.41%, respectively) and the 12-year-old stand (0.33 and 0.29%, respectively). Percent nitrogen was also significantly higher ($P < 0.05$) in the foliar samples of fertilized trees (1.53%) than of non-fertilized trees (1.34%) in the 6-year-old, but not in the 12-year-old stand (1.23 and 1.24%, respectively).

Fertilizer effects on resin flow responses

The day before wounding/inoculation treatments, resin flow was 2 to 4 times higher in fertilized trees than in unfertilized control trees in all four age by season trials ($P < 0.05$). For the spring trial, resin flow in the 6-year-old fertilized and unfertilized trees was 0.82 and 0.20 g day^{-1} , respectively, and the corresponding values in the 12-year-old trees were 0.73 and 0.37 g day^{-1} , respectively. For the summer trial, resin flow in the 6-year-old fertilized and unfertilized trees was 0.64 and 0.33 g day^{-1} , respectively, and the corresponding values in the 12-year-old fertilized and unfertilized trees were 3.14 and 1.23 g day^{-1} , respectively.

In the 6-year-old stand, resin flow in response to wounding/inoculation was significantly higher in fertilized trees than in unfertilized trees for up to 30 days after wounding/inoculation (1.92 and 1.12 g day^{-1} , respectively). Trees in the 12-year-old stand showed no significant fertilizer effect on resin flow after wounding/inoculation. There were no significant effects of fertilization on resin flow in either stand after one year in the spring trial.

There was no significant interaction between fertilizer and wounding/inoculation treatments on resin flow in any of the four age by season trials (spring and summer, 6- and 12-year-old trees) ($P > 0.05$). Because of the absence of an interaction, all analyses of resin flow in response to wounding/inoculation treatments were conducted across both fertilization regimes.

Wounding/inoculation effects on resin flow responses

Because the trees had not been subjected to wounding or inoculation on Day -1 , it was expected that constitutive resin flow, as measured on Day -1 , would be consistent across all wounding/inoculation treatments. However, resin flow can

vary among trees, and we found significant differences in resin flow among trees before the wounding/inoculation treatments (Day -1; Figure 1). In the spring trial in the 6-year-old stand, and in both spring and summer trials in the 12-year-old stand, one pre-treatment group exhibited resin flow that differed significantly from that in some of the other groups. Tests of linearity were used to determine if it were appropriate to use Day -1 resin flow as a covariate for subsequent tests, but it was determined that the data did not meet the necessary criteria and, furthermore, using Day -1 resin flow as a covariate did not reduce the error in the data. Therefore, the data were analyzed without further statistical consideration of the pre-trial (Day -1) differences (Figure 1).

Repeated measures ANOVA of the four age by season trials indicated a significant interaction between wounding/inoculation treatments and days after treatment on resin flow in three of the trials ($P < 0.05$). This interaction varied by trial, as well as by days after wounding/inoculation treatments. To evaluate in detail the specific differences in resin flow in response to the treatments, we subjected the data to LSD analysis ($\alpha = 0.05$).

At Day +1 after treatments, resin flow of wounded-only trees was lower than that of controls in all age by season trials, and significantly lower in three of the four trials. For subsequent days, resin flow in wounded-only trees did not differ significantly from that of controls, with the exception of Days +90 and +150 in the 12-year-old trees in the spring trial (Figure 1).

Resin flow in inoculated trees (half and full inoculation) was significantly higher than in controls in all four age by season trials at Day +30 after treatments, and in three of four trials at Day +90. Resin flow in inoculated trees (half and full inoculation) was also significantly higher than in wounded-only trees in three of four age by season trials at Day +30 and in both spring trials at Day +150 (Figure 1).

There were only two days overall that showed a significant

difference in resin flow between the half- and full-inoculation trees. These differences occurred at Day +90 in the spring trial of 6-year-old trees, and Day -1 in the spring trial of 12-year-old trees, though the differences were in opposite directions (Figure 1). Additional significant differences occurred in only one or two of the age by season trials (Figure 1).

One year after wounding/inoculation treatments, resin flow in full-inoculation trees was significantly higher than in control trees in both the 6-year-old stand (1.57 and 0.51 g day⁻¹, respectively) and the 12-year-old stand (2.16 and 0.72 g day⁻¹, respectively).

Effects of location of resin sampling in relation to wounded/inoculated area

Resin flow at Day +90 was significantly lower within the wounded/inoculated area than at 1 m above the wounded/inoculated area in both the 12-year-old trees in the spring trial (2.11 and 3.69 g day⁻¹, respectively) and the 12-year-old trees in the summer trial (1.20 and 2.03 g day⁻¹, respectively). Neither of the 6-year-old stand trials showed any significant effect of the location of resin sampling on resin flow.

Age and seasonal effects

Statistical analysis of stand-age effects was impossible because of a lack of stand replication. Our 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 treatments in the 12-year-old stands, but not in the 6-year-old stands, as previously noted. We also observed that resin flow in the 12-year-old control trees was at least 1.5 times (and as much as 4.5 times) greater than in the 6-year-old control trees at every sampling date except the final one for each season (Figure 2). No other discernable trends were

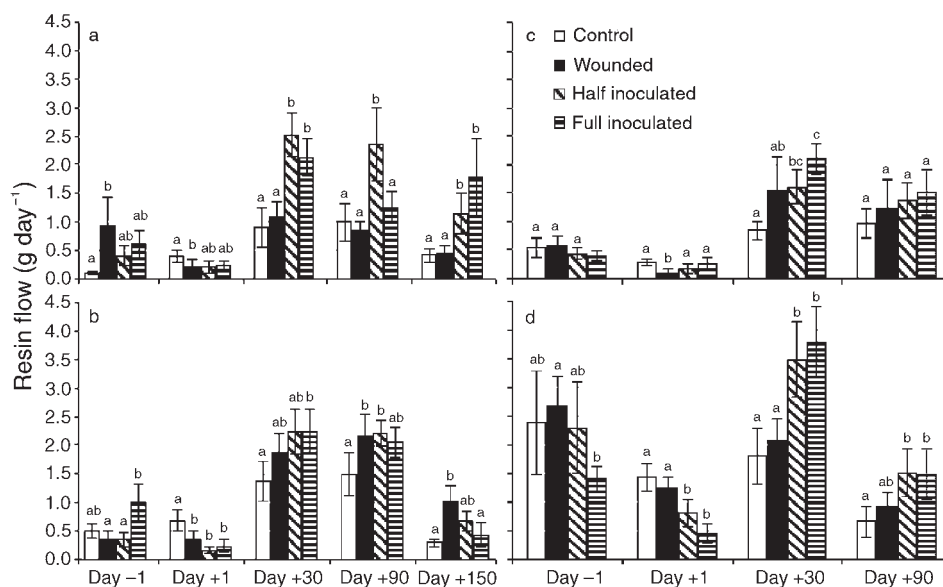


Figure 1. Effects of artificial wounding and *Ophiostoma minus* inoculation treatment on mean ($\pm$ SE, $n = 12$) resin flow of loblolly pine (*Pinus taeda*) trees in Hill Forest, NC. Different letters indicate that resin flow values differed significantly within each day after wounding/inoculation treatment. Significance was determined by LSD at $\alpha = 0.05$. Day -1 indicates resin flow 1 day before wounding/inoculation; other days are post-wounding/inoculation.

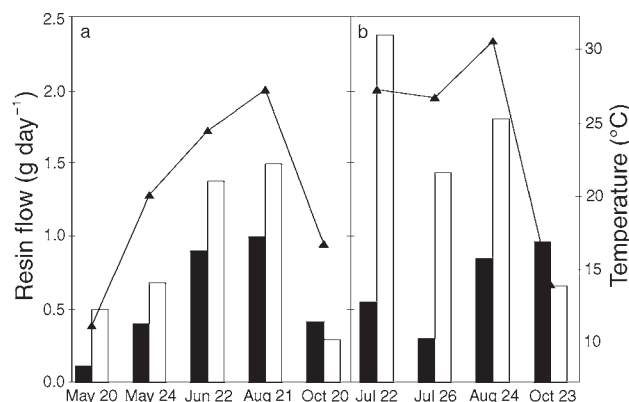


Figure 2. Temporal effects on mean ($n = 12$) resin flow of 6-year-old (filled bars) and 12-year-old (open bars) loblolly pine (*Pinus taeda*) control trees in Hill Forest, NC in the (a) spring and (b) summer trials. Corresponding mean daily temperatures ($\blacktriangle$) are also shown.

noted. No statistical analysis of seasonal effects was possible because of a lack of replication. No discernable trends between spring and summer trials were noted.

Discussion

Fertilizer effects

Fertilization resulted in a short-lived but significant increase in resin flow across stand ages and seasons, indicating an increase in constitutive resin in response to fertilization. This finding is consistent with some previous reports (Wilkens et al. 1997, Warren et al. 1999) though not with others (Matson et al. 1987, Ruel et al. 1998, Lombardero et al. 2000, Klepzig et al. 2005). Resin flow is widely accepted as the primary defense of southern pines against bark beetle attack (Strom et al. 2002). Trees with lower constitutive resin flow have been shown to be less resistant to bark beetle attack. If constitutive resin flow is reduced by fertilization, then attempts to increase growth through fertilization may place southern pines at greater risk from SPB. However, if, as found in this study, constitutive resin flow is increased with fertilization, then the converse is true. At present, it is unclear what effect, if any, age of trees being fertilized, type of fertilizer or time elapsed between fertilization may have on resin flow.

The growth-differentiation balance model (GDB) (Loomis 1932, 1953, Lorio 1986), as expanded on by Herms and Mattson (1992), states that, in the absence of limiting factors, photosynthates are directed primarily to growth through cell division and elongation. When plants are grown under conditions of mild stress, photosynthates are redirected to cell differentiation through specialization of cells. Therefore, growth in response to fertilization may negatively impact resin production. However, in late summer or during drought, when tree growth is limited by water stress, the GDB predicts that nutrient resources are allocated to other areas like plant defense, such as resin production (Lombardero et al. 2000). Our study began in 2002 with a rainfall deficit from the previous

year of more than 20 cm below the 30-year mean. This area did not recover from the 2001 drought until October of 2002 (www.erh.noaa.gov). Although we did not measure water potential, restricted water availability may have limited growth of our study trees, while allowing for increased resin production.

Although constitutive resin flow was significantly higher in fertilized trees than in unfertilized trees, once the trees were wounded or inoculated, or both, resin flow decreased sharply in all trials as constitutive resources were drained, as shown in other studies (Cook and Hain 1987, Ruel et al. 1998, Lombardero et al. 2000, Klepzig et al. 2005). At this point, we noted an age by fertilization interaction. Although 12-year-old trees showed no significant responses to fertilizer post wounding/inoculation, 6-year-old fertilized trees showed significantly elevated resin flow up to 30 days later. The younger smaller trees apparently benefited longer from the elevated constitutive resin flows than the 12-year-old trees. This age effect may be associated with differences in radial duct density (DeAngelis et al. 1986). The lower density of resin ducts in older trees would have limited replenishment from other sites of synthesis resulting in a less effective response. The possibility also exists of a dose-dependent relationship between fertilization and resin flow, requiring heavier fertilization of the older and larger trees to sustain resin flow after attack.

Wounding/inoculation treatment effects

At Day +1 after wounding/inoculation treatments, wounding without inoculation resulted in significantly lower resin flow than in controls in three of four age by season trials. This response was short-lived, and resin flow differences between wounded only and controls largely disappeared by 30 days after wounding/inoculation treatments and was seen only twice across the Day +30, +90 and +150 samplings (Figure 1).

By Day +30 after wounding/inoculation treatments, an induced resin flow response was recorded. The induced effect appears to have been due to inoculation, because the response produced by wounding alone was no longer discernable at 30 days. The half- and full-inoculation trees produced significantly more resin than control trees. In addition, fully inoculated trees produced significantly more resin than wounded-only trees in three of four trials. There is limited research on the response of pine resin flow to fungal inoculation (but see Luchi et al. 2005). Two previous studies on southern pines indicated that the hypersensitive response occurs between 24 and 72 h after inoculation (Popp et al. 1991, Paine et al. 1988). In our study, increased resin flow in inoculated trees began after 1 day, but before 30 days, and was still present at 90 days after treatments. This increase in resin flow that we observed in our inoculated trees is also consistent with previous conclusions that fungal presence leads to resin soaking and necrosis, whereas mechanical wounding does not (Paine and Stephen 1987a, 1987b, Paine et al. 1988, Popp et al. 1995).

The amount of inoculum used did not correlate with the resin flow response (Figure 1) (Paine and Stephen 1987b, Långström et al. 2001). A decrease in resin flow in response to

wounding alone occurred by 1 day after treatment, but this response was less clear in the inoculated trees. Age may play some role in the timing of the induced response and deserves further study, especially in loblolly pine plantations where young trees attain greater size (and are harvested) more quickly than in natural stands.

Time since wounding/inoculation treatment and seasonal effects

Our findings of rapid increases in resin flow after wounding, followed by declines within 30 days are consistent with earlier work (Cook and Hain 1987). We also found time since treatment to be a factor, which is to be expected because of seasonal variation (Figures 1 and 2) (Cook and Hain 1987, Tisdale and Nebeker 1992). There was a strong relationship between resin flow in control trees and mean air temperature on the specific days when resin sampling was conducted for 6- and 12-year-old trees (Figure 2), though the smaller 6-year-old trees showed a more constrained response to air temperature than the 12-year-old trees. Overall, this suggests that the relative differences in resin flow were a response to the treatments applied, whereas the magnitude of resin flow was also strongly influenced by air temperature (Figure 2 and Clements 1974).

Wound-induced resin flow increases were faster in wounded-only trees, but lasted longer in inoculated trees, suggesting that fungal presence acted as a sustained elicitor of the induced response. This is consistent with findings of induced resin responses lasting as long as 105 days (Klepzig et al. 2005) and localized induced resistance lasting as long as a year (Krokene et al. 2003). Others have also found induced resistance in pines (Bonello et al. 2001, Luchi et al. 2005, Krokene et al. 2000), though Popp et al. (1991) reported that the effects lasted only 72 h. Although induced responses to plant defense elicitors have been reported previously (e.g., Francheschi et al. 2002), it is not known if elevated resin flow can be maintained consistently throughout a growing season and even into the next by the application of potent elicitors of the resin flow response. Such an effect might protect trees from repeated SPB attack. If resin flow can be consistently enhanced, trees that have been exposed to these treatments could be challenged with fungal inoculation or SPB infestations to determine if induced resin flow confers acquired resistance to subsequent invasion. There are important implications for natural and plantation loblolly pine stands threatened by SPB infestation. If pre-exposure to pathogens or chemical elicitors (Zeneli et al. 2006) confers resistance, then stands that are in imminent danger could be pretreated to reduce susceptibility to infestation. Furthermore, understanding the relationships between tree growth rate, site quality, fertilization and bark beetle success will provide insight into where and when new SPB spots (localized initial attack) might occur on the landscape. All of these factors have important implications for the silvicultural management of pines.

Acknowledgments

Funding for this work was provided through a cooperative agreement with the USDA Forest Service, Southern Research Station, and the

NC State University Department of Forestry and Natural Resources. Additional support was provided by the NC State University Department of Plant Biology. The authors thank Jamie Schuler for his invaluable help with all aspects of logistics and field work. We also thank Steven Walters for field assistance and Cavell Brownie for statistical support.

References

- 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.
- Bonello, P., T.R. Gordon and A.J. Storer. 2001. Systemic induced resistance in Monterey pine. For. Pathol. 31:99–106.
- Clements, R. 1974. Modern gum naval stores methods. USDA For. Serv. Gen. Tech. Rep. SE-7, pp 1–29.
- 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. Serv. 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.
- 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.
- Goldammer, 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. Entomol. 122: 407–418.
- Harms, D.A. and W.J. Mattson. 1992. The dilemma of plants: to grow or to defend. Q. Rev. 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. Entomol. 111:889–896.
- Hooker, T.D. and J.E. Compton. 2003. Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. Ecol. Appl. 13:299–313.
- Kirby, R.M. 1976. Soil survey of Durham Co., NC. USDA Soil Cons. Serv., NC Agric. Exp. Stn., 74 p.
- Klepzig, K.D. and C.H. Walkinshaw. 2003. Cellular response of loblolly pine to wound inoculation with bark beetle associated fungi and chitosan. Res. Pap. SRS-30. USDA For. Serv., Southern Res. Stn., Asheville, NC, 9 p.
- 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., 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 30:83–96.
- 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. 25: 681–688.

- 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, 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, 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.
- 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. *IAWA 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, Dordrecht, 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*. *Ecol. Lett.* 3:329–339.
- 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, Jr., 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.
- Luchi, N., R. Ma, P. Capretti and P. Bonello. 2005. Systemic induction of traumatic resin ducts and resin flow in Austrian pine by wounding and inoculation with *Sphaeropsis sapinea* and *Diplodia scrobiculata*. *Planta* 221:75–84.
- Matson, P.A., F.P. Hain and W. Mawby. 1987. Indices of tree 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. Fungi associated with the southern pine beetle: avoidance of induced defense response in loblolly pine. *Oecologia* 74:377–379.
- Paine, T.D. and F.M. Stephen. 1987b. 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., 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. Serv. Tech. Bull. 1631, pp 7–28.
- Phillips, M.A. and R.B. Croteau. 1999. Resin-based defenses in conifers. *Trends Plant Sci.* 4:184–190.
- 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.
- Pye, J.M., T.S. Price, S.R. Clarke and R.J. Huggett, Jr. 2004. A history of southern pine beetle outbreaks in the Southeastern United States through 2004. <http://www.srs.fs.usda.gov/econ/data/spb/index.htm>.
- Raffa, K.F. 1991. Induced defensive reaction in conifer–bark beetle systems. In *Phytochemical Induction by Herbivores*. Eds. D.W. Tallamy and M.J. Raupp. John Wiley and Sons, New York, 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.
- 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. Serv., Southern For. Expt. Stn., Agric. Handbook No. 713., New Orleans, LA, 493 p.
- 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.
- 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.
- Vose, J.M. and H.L. Allen. 1988. Leaf area, stemwood growth, and nutrient relationships in loblolly pine. *For. Sci.* 34:547–563.
- 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. 2002. Southern forest resource assessment. Gen. Tech. Rep. SRS-53. USDA For. Serv., Southern Res. Stn., Asheville, NC, 635p.
- Wilkins, 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, pp 591–616.
- Zeneli, G., P. Krokene, E. Christiansen, T. Krekling and J. Gershenson. 2006. Methyl jasmonate treatment of large Norway spruce (*Picea abies*) trees increases the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. *Tree Physiol.* 26:977–988.