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# Loblolly pine responds to mechanical wounding with increased resin flow

Jonathan J. Ruel, Matthew P. Ayres, and Peter L. Lorio, Jr.

**Abstract:** The oleoresin produced by many conifers has a deleterious effect on numerous associated herbivores, including bark beetles (Coleoptera: Scolytidae), and may have evolved as a plant defense mechanism. Three experiments with juvenile loblolly pine (*Pinus taeda* L.) used mechanical wounding to drain resin reserves and assess the effects of prior bark wounding on subsequent resin flow up to 7 days post-treatment. Resin flow returned to pre-treatment values within 2 days after wounding began and, in nearly every tree in each experiment, continued to increase on subsequent days. On average, resin flow reached double the pre-treatment values (mean ± SE: 2.10 ± 0.10 vs. 1.13 ± 0.10 g/3 h and 2.28 ± 0.09 vs. 0.90 ± 0.09 g/3 h for wounded vs. pre-treatment in experiments 1 and 2, respectively). Considering its timing and magnitude, this previously undescribed response may be important in modulating interactions between pine trees and bark beetles. In addition, resin flow following treatment was greater in trees in larger crown size classes (thinned > edge of stand = unthinned in experiment I ; edge of stand > thinned > unthinned in experiment 2). This may help explain why trees in thinned stands are less susceptible to southern pine beetle (*Dendroctonus frontalis* Zimm.) infestation than trees in unthinned stands.

**Résumé** : L'oléorésine produite par plusieurs conifères a un effet délétère sur de nombreux herbivores qui leurs sont associés, incluant les rongeurs de l'écorce (Coleoptera : Scolytidae), et serait devenue un mécanisme de défense chez les plantes au fil de I'tvolution. Dans le cadre de trois experiences, des blessures mécaniques ont été infligées à I'tcorce de pins à encens (*Pinus taeda* L.) juvéniles, afin de drainer leurs réserves de résine et d'tvaluer les effets de ces blessures sur l'écoulement de résine durant les 7 jours suivant le traitement. Moins de 2 jours après la blessure, l'écoulement de rtsine ttait revenu à des valeurs prétraitement et continuait à augmenter chez presque tous les arbres de chacune des experiences au cours des jours suivants. En moyenne, l'écoulement de résine atteignait le double des valeurs prétraitement (moyenne ± erreur type: 2,10 ± 0, IO versus 1,13 ± 0, IO g/3 h et 2.28 ± 0.09 versus 0.90 ± 0.09 g/3 h après blessure versus prétraitement lors des expériences 1 et 2, respectivement). Compte tenu de son délai et de son ampleur, cette réponse, qui jusqu'à présent n'avait pas été décrite, pourrait s'avérer importante pour moduler les interactions entre les pins et les rongeurs de l'écorce. De plus, l'écoulement de résine après traitement était plus important chez les individus des classes de cime supérieures (éclairci > bordure de peuplement = non Cclairci dans l'expérience I; bordure de peuplement > tclairci > non Cclairci dans l'expérience 2). Ceci pourrait contribuer à expliquer pourquoi les arbres des peuplements éclaircis sont moins susceptibles au dendroctone meridional du pin (*Dendroctonus frontalis* Zimm.) que les arbres des peuplements non Cclaircis.
[Traduit par la Rédaction]

## Introduction

The oleoresin produced by many conifers (a mixture of monoterpenes, resin acids, and neutral compounds) may have evolved as a defense against herbivores. The oleoresin in the boles of pines is synthesized primarily by epithelial cells that form the membrane walls of resin ducts in the xylem and is stored within the resin ducts at positive pressure (Esau 1965; Barbosa and Wagner 1989). Animals from squirrels to bark beetles prefer and (or) attain higher reproductive success on trees with less resin (Snyder 1992; Reeve et al. 1995). While some conifers also depend on an induced response in the phloem tissue at the site of invasion, it has been suggested that species with well-developed resin ducts (Pinus spp.) do not depend heavily on this response and instead rely more on the flow of resin from ducts in the xylem (Lewinsohn et al. 1991*b*; Nebeker et al. 1993).

Pine bark beetles (Coleoptera: Scolytidae) oviposit and develop in the inner bark of trees and can cause extensive mortality in pine forests (Thatcher et al. 1980; Price et al. 1992). When beetles penetrate the bark of living pine trees, they expose the xylem surface and release a flow of resin that acts as a physical and chemical barrier to the excavation of oviposition galleries (Thatcher et al. 1980). Lower resin flow and rapid crystallization may explain why loblolly pines (*Pinus taeda* L.) and shortleaf pines (*Pinus echinata* Mill.) are more vulnerable to attack by southern pine beetles (*Dendroctonus frontalis* Zimm.) than longleaf (*Pinus palustris* Mill.) or slash pines (*Pinus elliottii* Engelm.) (Hodges et al. 1979).

Resin flow in previously unwounded trees (constitutive resin flow) varies not only among conifer species but also among populations and individuals (Hodges et al. 1977, 1979; Barbosa and Wagner 1989). Water and mineral nutrient availability exert strong effects on constitutive resin flow in loblolly pine. Under moderate water deficits, resin flow is increased and beetle attack success is reduced, apparently because a greater proportion of assimilated carbon is available to support

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secondary metabolism when water availability limits growth (see Loomis 1932, 1953; Lorio and Hodges 1985; Lorio 1986; Lorio et al. 1990, 1995; Dunn and Lorio 1993; Reeve et al. 1995). However, it is generally recognized that extreme water deficits that constrain photosynthesis as well as growth lead to reduced resin flow. Fertilization, like irrigation, tends to increase growth but reduce constitutive resin flow in juvenile loblolly pine (Wilkens et al. 1998; but maybe not in mature trees, see Matson et al. 1987).

Crown size, which is strongly affected by competitive relations among neighboring trees, has been shown to influence constitutive resin flow and has been recognized as an important factor in stand management for resin production (Schopmeyer and Larson 1955; Clements 1974). Correlative evidence and stand risk-rating studies suggest that trees in thinned stands are less likely to sustain bark beetle infestations (Bennett 1965; Lorio 1978; Lorio et al. 1982; Mason et al. 1985). We hypothesized that the relatively large crowns that result from thinning tend to increase the total carbon budget of trees and therefore increase secondary metabolism, oleoresin synthesis, and resin flow. Mason (1971) and Matson et al. (1987) reported data consistent with this hypothesis. However, in 4 years of study in three different stands ofjuvenile loblolly pine, we have failed to find any effects of stand thinning on constitutive resin flow (Wilkens et al. 1998; M.P. Ayres and P.L. Lorio, Jr., unpublished data). Thus, the question remains: Why are loblolly pines in thinned stands apparently less vulnerable to beetle infestations than pines in unthinned stands?

Preformed resin may be quickly and significantly depleted during the initial stages of bark beetle attack. However, we have sometimes observed elevated resin flow following the onset of southern pine beetle attack, especially when the attacks proceeded slowly (Dunn and Lorio 1993; Lorio et al. 1995). We hypothesized that the capacity of trees for rapid synthesis of resin and refilling of the ducts might be of considerable significance to interactions between loblolly pine trees and southern pine beetles considering that thousands of beetles attack a tree over a time period of ≈7–14 days (Payne 1980). We hypothesized that the strength of this aspect of defense, i.e., the capacity for refilling resin ducts, is positively correlated with tree crown size.

We conducted three experiments to test the hypothesis that loblolly pine subjected to wounding would show initial decreases in resin flow (as the resin ducts are drained) followed by a return to previous levels (indicating refilling of the resin ducts). In two experiments, we compared trees representing different crown size classes to test whether crown size influences the rate at which resin flow returns to constitutive levels. In the third experiment, we compared trees that had and had not been fertilized to test whether soil nutrients could influence resin flow following wounding.

## Methods

### Overview

In the fall of 1996, we conducted three experiments using mechanical wounding to deplete local resin reserves in loblolly pines. We measured resin flow on all trees before and after the wounding treatments. Experiments compared trees subjected to different environmental conditions during the previous 2-5 years (more or less competition for crown space and fertilized or not).

All experiments were conducted in a pure loblolly pine plantation Palustris Experimental Forest in the Kisatchie National Forest of central Louisiana, U.S.A. (31°00′N, 92°37′W). The trees were 15 years old at the time of the study (height 14.7 ± 1.0 m; mean ± SD). They were originally planted with 1.8 x 1.8 m spacing. We measured the diameter at breast height (DBH) of all study trees. Daily mean air temperatures were obtained from the nearby U.S. Forest Service climate station for the Evangeline District of the Kisatchie National Forest.

### Resin sampling

Our protocol for measuring resin flow was the same in each experiment and followed established methods (Lorio et al. 1990) except that the collection time was limited to 3 h. A metal punch was used to remove a 125 $mm^2$ disk of outer bark and phloem tissue at a height of 1-2 m. A plastic vial was placed below the newly exposed xylem surface and a metal tray was fitted to direct resin into the vial. Vials remained on the trees for 3 h after wounding. The first wounds were usually made around 09:00, and the last vials were picked up by 14:30. Trees were sampled in an order that eliminated potential effects of sampling one treatment group earlier in the day than other treatment groups. Vials were weighed to quantify the amount of resin.

Each day, we measured resin flow on two sides of each study tree. One side of each tree was subjected to a wounding treatment and the other side served as a control. We expected that opposite faces would provide good controls, because limited lateral integration of resin ducts would prevent wounding from affecting the control sides of trees. We included a test of this assumption in experiment 2.

### Experiment 1

We subjected 10 trees in each of three crown size classes (unthinned plots, thinned plots, and edge of stand) to repeated wounding (on days 0-4). Experimental trees representing the thinned crown size class and the unthinned crown size class were scattered within 0.25 ha of the plantation. Thinning was conducted in November 1993 by removing a sufficient number of neighboring trees to provide open space for crown growth on all sides of randomly selected study trees. Trees representing the stand edge crown size class were scattered along the southeastern and southwestern edges of the stand facing cleared grassy areas and were within 10-50 m of trees in the unthinned and thinned treatments. On the morning of September 17, we measured pre-treatment resin flow on two opposing sides of each tree.

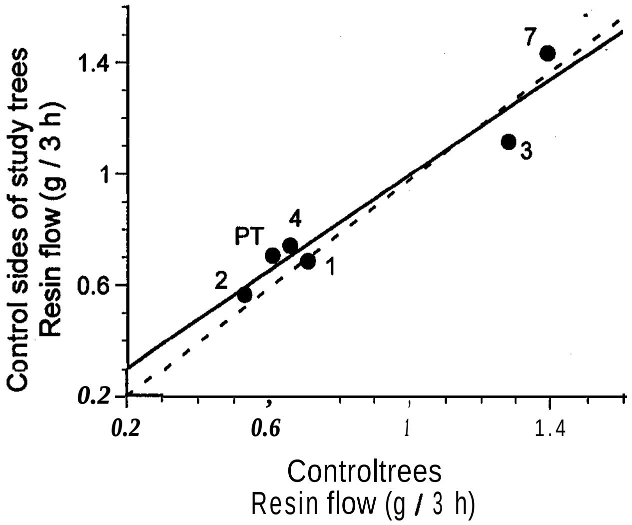
In the afternoon on September 17 (day 0), we began the wounding treatment on one side of each tree. We used the metal punch to add a row of three 125-$mm^2$ wounds 1 cm above the sampling wound and later that day added another row of three wounds 1 cm above the previous row. This wounding protocol was repeated on days 1-4 except that on day 3 we only added one row. On the first day after wounding began (day 1), we measured resin flow on both sides of all trees. Measurement wounds were placed at least 5 cm above and to the side of the previous wound on control sides and 3 cm directly above the last row of wounds on the wounded sides. We measured resin flow again, using the same protocol, on days 2, 4, 7, and 9.

### Experiment 2

This experiment involved severe wounding on 30 trees (10 each in unthinned, thinned and edge-of-stand crown size classes). As an additional control, we included a group of 10 unthinned control trees that were not subject to the wounding treatment. Thinned trees were located in an area of the stand that was thinned in 1993. Edge trees were along the southeastern edge of the stand. Pre-treatment resin flow was measured on two sides of all 40 trees on 10 October.

We began the wounding treatment on 14 October (day 0). We used a bark chipper to remove two 1.5 cm wide strips of bark and phloem on one side of each study tree (one strip at 18 cm above the initial measuring wound and one strip at 18 cm below the initial wound). The length of each horizontal strip was approximately equal to the DBH of the tree. Over an 8-h period, we stripped each tree three times,

**Fig. 1.** Daily mean resin flow on control sides of study trees as a function of daily mean resin flow on separate control trees. PT, pre-treatment; numbers indicate days since initiation of wounding treatment. Broken line is line of equality and solid line represents linear regression ($y = 0.87x + 0.13$, $r^2 = 0.94$).



adding successive strips below the top strip and above the bottom strip. The next day, we stripped each tree once more. Resin flow was measured on both sides of all trees (including unstripped control trees) on the first day after wounding began (day 1) as well as on days 2, 3, 4, and 7. Measurement wounds were placed horizontally adjacent to initial measurement wounds on wounded sides and 5 cm diagonally up and to the side of initial measurement wounds control sides and control trees.

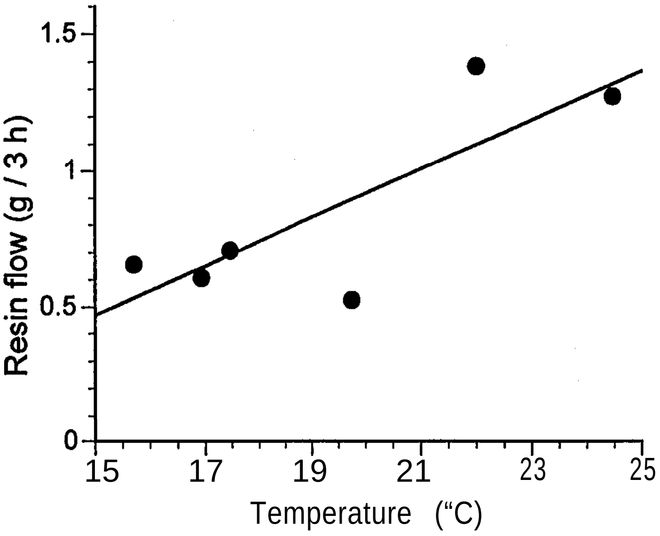
On day 14, we used the metal punch to remove 125-mm$^2$ phloem disks from both sides of all trees. The disks were freeze-dried and weighed to estimate area-specific mass of the phloem. We also measured tree height and height to live crown and used those data to calculate percent live crown. Because of the asymmetry of the edge tree crowns, we estimated their percent live crown by averaging estimates for two sides (facing the stand and facing the clearing). On these edge trees, we also mapped the location of the wounded and control sides relative to the clearing. We used eq. 1 (where $d$ is the short distance around the bole to the middle of the sunny aspect and $C_t$ is the circumference of the tree) to calculate a metric for each side of each tree that ranged from 0.0 (sunny side) to 1.0 (shady side):

$$[1] \quad \mathrm{EdgeOrient} = \frac{d}{0.5C_t}$$

### Experiment 3

On October 30, a severe wounding treatment, identical to the wounding treatment on day 0 of Experiment 2, was imposed on unfertilized and fertilized trees ($n = 12$ in each group). The trees had been fertilized with diammonium phosphate applied at a rate of 746 kg/ha (supplying 150 kg/ha of phosphorus and 134 kg/ha of nitrogen) in the winter of 1993-1994. Pre-treatment resin flow was measured on 7 October (collection time for this date only was extended to 24 h). On the 5th day after wounding, resin flow was measured on both sides of all trees.

**Fig.** 2 Daily mean resin flow on control trees as a function of daily mean temperature ($y = 0.09x - 0.88$, $r^2 = 0.66$).



### Statistics

The day-to-day variation in resin flow on control sides of trees in experiment 2 was almost fully explained by the daily means of resin flow on separate control trees (Fig. 1; $r^2 = 0.94$). This indicated that the control sides of study trees made adequate controls (i.e., they were virtually unaffected by the wounding). The day-to-day variation in control tree resin flow appeared to be due primarily to variance in temperature (Fig. 2; $r^2 = 0.66$). which is known to be a major factor affecting resin flow (Harper and Wyman 1936; Clements 1974). Our resin flow data also included variance attributable to genetic and phenotypic variation among trees within crown size classes ($F_{27,134} = 7.03$, $p$ c 0.0001, and $F_{27,135} = 8.52$, $p < 0.0001$, for effects of tree within crown size class on resin flow from the unwounded sides of trees in experiments 1 and 2). Because our hypotheses were chiefly concerned with the effects of wounding and tree crown size class, we used resin flow data from the control (unwounded) sides of trees to adjust resin flow measurements for random variation among days and among trees within crown size classes. Equation 2 describes adjusted resin flow ($R_{\mathrm{adj}}$):

$$[2] \quad R_{\mathrm{adj}} = R_{\mathrm{ctd}} + (\mathrm{RC}_{\ldots} - \mathrm{RC}_{..d}) + (\mathrm{RC}_{c..} - \mathrm{RC}_{ct.})$$

where $R_{\mathrm{ctd}}$ is the observed resin flow from the wounded side of tree $t$ within canopy class c on day $d$, $\mathrm{RC}_{\ldots}$ is the the grand mean of control-side resin flow, $\mathrm{RC}_{..d}$ is the mean control-side resin flow for each day (averaged across crown size classes and trees within crown size class), $\mathrm{RC}_{c..}$ is the mean control-side resin flow for each crown size class (averaged across days and trees within crown size class), and $\mathrm{RC}_{ct.}$ is the the mean control-side resin flow of tree $t$ within crown size class c (averaged across days). This adjustment was the equivalent of employing day and tree within crown size class as covariates; it imparted no bias and reduced the experimental error (MSE) by 28–40%. Adjusted resin flow was analyzed with an ANOVA model that included date, crown size class, and date x crown size class; trees were treated as a random blocking variable nested within crown size class.

Tree diameter (experiments 1 and 2) and percent live crown (experiment 2) were all analyzed with a one-way ANOVA that tested for an effect of crown size class. Phloem area-specific mass (experiment 2) was analyzed with an ANOVA that included crown size class, trees nested in crown size class and side (wounded or control) as main effects as well as the crown size class x side interaction.

Resin flow data from experiment 3 were analyzed with an ANOVA that included fertilization and side as main effects, baseline resin as a covariate and fertilization x baseline, fertilization x side, baseline x side and fertilization x baseline x side interactions.

## Results

### Experiment 1

Edge trees were of larger diameter than trees from thinned or unthinned crown size classes (means ± SE: 19.6 ± 0.8, 16.3 ± 0.8 and 17.0 ± 0.5 cm, respectively; $F_{2,27} = 8.30$, $p < 0.01$).

Fig. 3. Mean (±SE) resin flow following wounding in loblolly pine representing three crown size classes (unthinned, thinned, and edge of stand) in experiment 1. PT, pre-treatment resin flow. Moderate wounding was performed on days O-4.

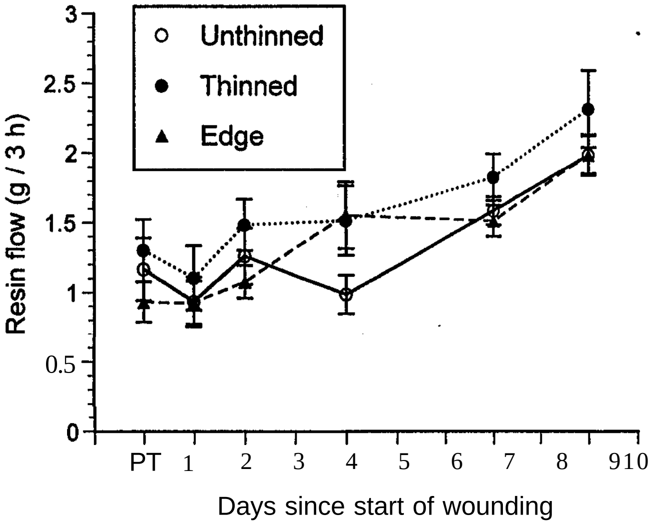
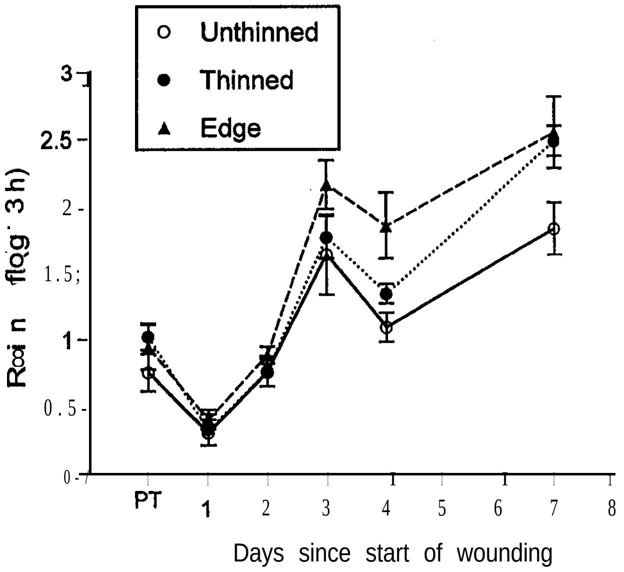
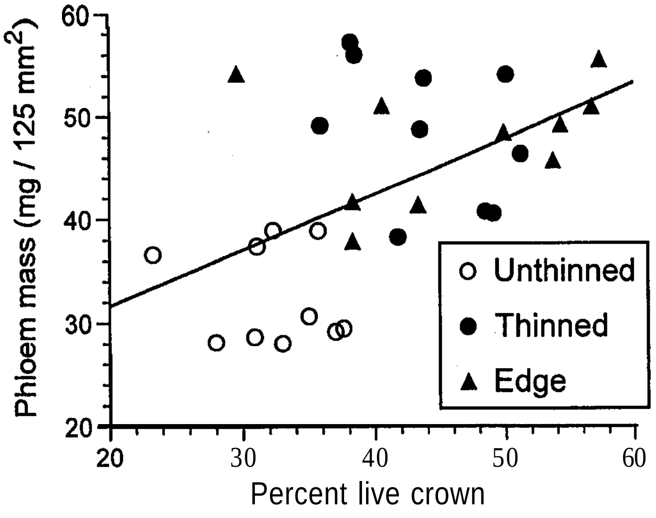


Fig. 4. Mean (±SE) resin flow following wounding in loblolly pine representing three crown size classes (unthinned, thinned, and edge of stand) in experiment 2. PT, pre-treatment resin flow. Severe wounding was performed on days 0 and 1.



Resin flow on wounded sides of trees was strongly affected by date ($F_{5,134}$ = 16.32, $p$ c 0.0001; Fig. 3). Because these resin flow values had been adjusted for day-to-day variation in the controls, the effect of date represents the response to the wounding treatment that began on the afternoon of day 0. There was no change in resin flow on days 1 or 2, but resin flow began to increase on day 4, was much greater on day 7 and was nearly double pre-treatment values on day 9 (least square means ± SE: 1.13 ± 0.10, 1.35 ± 0.10, 1.65 ± 0.10 and 2.10 ± 0.10 g/3 h for pre-treatment and days 4, 7, and 9, respectively). Resin flow was also affected by crown size class ($F_{2,27}$ = 3.20, $p$ = 0.05) with trees in the thinned class having greater flow than trees in both the unthinned and edge classes (least square means ± SE: 1.59 ± 0.07 vs. 1.32 ± 0.07 and 1.33 ± 0.07 g/3 h, respectively). There was no date x crown size class interaction ($F_{10,134}$ = 0.73).

Fig. 5. Phloem thickness (area-specific mass) as a function of percent live crown in loblolly pine ($y = 0.5x + 19$, $r^2 = 0.27$).



## Experiment 2

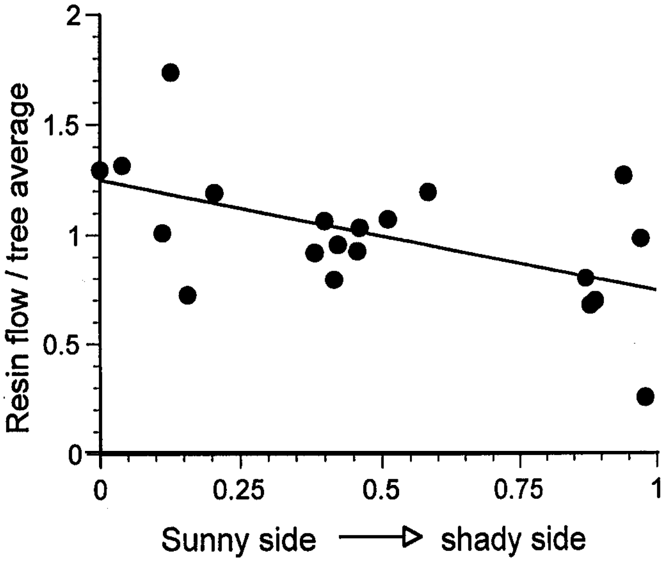
Trees in the edge crown size class had the largest diameter followed by the thinned class and the unthinned class ($F_{2,27}$ = 18.86, $p < 0.001$; mean ± SE: 20.0 ± 0.8, 16.8 ± 0.6 and 14.7 ± 0.5 cm, respectively).

As in the first experiment, resin flow in the second experiment was strongly affected by the wounding treatment ($F_{5,134}$ = 74.99, $p < 0.0001$, effect of date; Fig. 4). Resin flow dropped to less than half of pre-treatment values by day 1 (least square means ± SE: 0.90 ± 0.09 vs. 0.35 ± 0.09 g/3 h), returned to pre-treatment values by day 2 (0.80 ± 0.09 g/3 h) and more than doubled by day 3 (1.85 ± 0.09 g/3 h). Resin flow remained high on days 4 and 7 (1.42 ± 0.09 and 2.28 ± 0.09 g/3 h). This positive response to wounding was very consistent among trees. Unadjusted flow was higher on wounded sides than on control sides on 29, 27, and 27 trees of 30 study trees on days 3, 4, and 7 respectively. Crown size classes differed in resin flow with edge trees having the greatest flow followed by trees in the thinned class, followed by trees in the unthinned class (least square means ± SE: 1.46 ± 0.07, 1.28 ± 0.07 and 1.06 ± 0.07 g/3 h, respectively; $F_{2,27}$ = 4.84, $p$ = 0.016). There was no date x crown size class interaction ($F_{10,134}$ = 1.60).

Trees in edge and thinned classes had similar percent live crown, and both had more crown than trees in the unthinned group (means ± SE: 0.48 ± 0.02, 0.49 ± 0.02 and 0.33 ± 0.015%, respectively; $F_{2,27}$ = 23.48, $p < 0.001$). Trees in edge and thinned classes also had higher area-specific phloem mass than trees in the unthinned class ($F_{2,27}$ = 11.80, $p < 0.001$; means ± SE: 46 ± 3, 44 ± 2 and 32 ± 1 mg/125 mm², respectively). Across crown size classes, there was a positive relationship between percent live crown and phloem thickness ($r^2$ = 0.27; Fig. 5).

On edge trees, initial measuring wounds facing more towards

Fig. 6. Relationship between pre-treatment resin flow and location of wounding relative to stand edge ($y = -0.51x + 1.25$, $r^2 = 0.29$). Resin flow was divided by the mean flow of the two wounds on each tree to remove variation among trees. Negative slope indicates that, within trees, pre-treatment resin flow tended to be higher on the sunny side of the tree (facing the clearing).



the interior of the stand tended to have lower pre-treatment resin flow ($r^2$ = 0.29; Fig. 6).

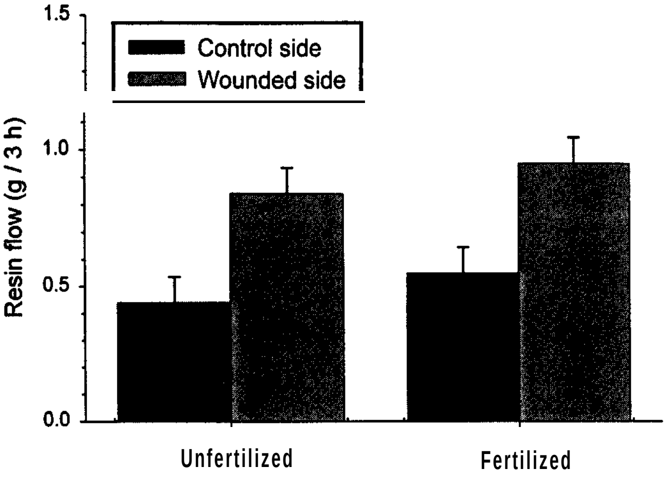
### Experiment 3

Again, trees responded to wounding with increased flow (least square means ± SE: 0.90 ± 0.07 vs. 0.47 ± 0.07 g/3 h, wounded vs. control sides on day 5, $F_{1,40} = 5.35, p = 0.026$; Fig. 7). Resin flow was higher on the wounded sides on 22 of 24 of study trees. Fertilization did not have any effect on resin flow on control or wounded sides ($F_{1,40} = 0.12$, $p > 0.50$). Baseline resin flow had a strong positive relationship with post-wounding resin flow (F, $_{40}$ = 37.37, $p$ c 0.0001). There were no significant interactions ($F_{1,40} < 1.80$).

## Discussion

Our original research objective was to use mechanical wounding to deplete resin reserves in loblolly pine trees and measure the return of resin flow to baseline values. We were surprised to find that, in all three experiments, practically every tree eventually responded to the wounding with an increase in resin flow relative to pre-treatment values. In fact, in experiment 1 we did not detect any decline in resin flow in response to the wounding treatment (Fig. 3). Instead, resin flow appeared unaffected by wounding until it began to increase some time between 4 and 7 days after wounding. Experiment 2 was designed to include a more severe wounding treatment. By the first day after wounding began, resin flow had declined considerably as anticipated (Fig. 4). However, by day 2, resin flow returned to pre-treatment values and then kept increasing, reaching two times pre-treatment values on day 3. Results from experiment 3 also indicated a significant increase in resin flow in response to wounding. This observed increase in resin flow is consistent with naval stores methods developed during the 1950s and 1960s indicating that appropriate repeated wounding yielded the greatest amount of resin (Clements 1974). In a study of resin flow as a function of height in loblolly pine, Tisdale and Nebeker (1992) incidentally found some evidence of a similar response to wounding. With their heaviest sampling regime (eight sampling wounds per height), resin flow was significantly higher when they sampled the same trees again after 48 h. Damage to the root crown or bole during thinning operations has also been shown to result in increased resin flow (Nebeker and Hodges 1983).

Fig. 7. Mean (±SE) resin flow on the 5th day after wounding in loblolly pine that were and were not fertilized.



Because resin flow after wounding reached double pre-treatment values in our study, the refilling of resin ducts does not seem like an adequate explanation. Perhaps the increased resin flow reflects translocation of resin from elsewhere in the tree. It is also possible that our results reflect an induced response in which gene activation results in increased resin synthesis.

It has been suggested that *Pinus* species, which generally have high constitutive levels of monoterpene synthesis, do not respond to mechanical wounding with increased synthesis but instead depend on the translocation of preformed resin (Lewinsohn et al. 1991*a*, 1991b).

The fact that phloem area-specific mass was not lower adjacent to wounds is consistent with this translocation hypothesis, because increased resin synthesis locally might be expected to reduce phloem mass because of carbohydrate depletion. However, anatomical studies have indicate that the resin ducts in pines do not constitute a true network and that large-scale translocation of resin is unlikely (Esau 1965; Fahn 1970). If increased resin flow is due to translocation, there would necessarily be decreased resin flow on parts of the bole distal to the wounds.

Is it possible that our results reflect an induced response? If the increase in resin flow following mechanical wounding is the result of changes in the genetic regulation of oleoresin synthesis in the resin ducts, then it represents an inducible response in the strict sense (Karban and Myers 1989; Tallamy and Raupp 1991). Genetic controls of oleoresin synthesis might involve the regulation of monoterpene cyclase activity (Lewinsohn et al. 1991b). While there have been many studies on trees' reactions to inoculations of fungi associated with bark beetles, there have been conspicuously few studies that address induced responses to the mechanical components of bark beetle attack. However, Scots pine (*Pinus sylvestris* L.) seedlings responded to bark stripping with increased concentrations of resin acids (Gref and Ericsson 1985) and grand fir (*Abies grandis* (Dougl.) Lindl.) saplings increased terpene synthesis in response to wounding (Steele et al. 1995). The duration of our experiments was long enough to allow for increased synthesis. because labeled $^{14}C$ (introduced as $CO_2$) can be incorporated into resin within 24 h (Popp et al. 1991). It seems possible that our results reflect an induced response to mechanical wounding, but confirmation would require direct evidence of altered gene expression and increased resin synthesis.

In bark beetle – conifer systems, induced defense has generally been thought to require the presence of certain chemical elicitors, usually of fungal origin (Paine et al. 1997). The resin found in lesions at the site of fungal inoculation differs from the constitutive resin in the ducts in the concentrations of various monoterpenes (Paine et al. 1987). The resin synthesis and lesion formation are restricted to nonspecialized cells in dying phloem tissue adjacent to the wound (Schwarz 1983; Christiansen and Ericsson 1986; Paine et al. 1997; Dunn and Lorio 1992). In contrast, our reported increase in resin flow following mechanical wounding is a property of the resin duct system in the xylem. The secondary metabolites are the product of living epithelial cells that line the resin ducts and are differentiated for this purpose prior to any wounding (Lorio 1986). Regardless of whether increased resin flow results from translocation or de novo synthesis, the response is physiologically distinct from the induced reaction involving lesion formation.

If the trees respond to the initial depletion of resin during beetle attack with an increase in resin flow, this could have an important impact on the oviposition activity of initial colonizers and on the attack success of beetles that arrive on subsequent days. The outcome of beetle attacks might be influenced by beetle population size and spacing of host trees (both of which would likely impact attack rate on any given tree). If the increase in resin flow is due solely to translocation of preformed resin, beetles that attack less infested areas of the bole may encounter lower resin flow.

The outcome of beetle attacks may also be strongly affected by environmental factors that affect the trees' potential for increased resin flow. Based on the growth-differentiation balance model, we had predicted that fertilization would have a negative effect on resin flow after wounding. We did not find such an effect (Fig. 7), but this result is inconclusive because most effects of the fertilization treatment (including increased tree growth) had disappeared by the time these experiments were conducted. In earlier measurements, these and other trees showed reduced constitutive resin flow following fertilization (Wilkens et al. 1998).

Trees with larger crowns had higher resin flow after wounding (experiment 1: thinned > edge of stand = unthinned, Fig. 3; experiment 2: edge of stand > thinned > unthinned, Fig. 4). Edge trees had similar calculated percent live crown as thinned trees but had much longer lower branches and appeared to have substantially more foliage (experiment 2). Edge and thinned trees also had thicker phloem than unthinned trees (experiment 2). The effect of crown size class on resin flow after wounding is presumably the result of greater overall carbon budgets that allow for higher rates of resin synthesis and (or) translocation.

Carbon budgets are known to have an effect on various aspects of plant defense in conifers. Paine and Stephen (1987) found that suppressed loblolly pines produced smaller lesions. Removal of photosynthetic tissue, by pruning, increases susceptibility to blue-stain fungus in Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (Christiansen and Fjone 1993; Solheim et al. 1993). Pruning also increases susceptibility to the common pine shoot beetle (*Tomicus piniperda* L.) in Scots pine (Långström et al. 1992). Other studies indicate that trees in thinned stands have higher rates of constitutive resin flow (Mason 1971; Matson et al. 1987) and that trees with larger crowns produce more gum (Schopmeyer and Larson 1955; Clements 1974). In our study, pre-treatment resin flow was higher on the sides of edge trees with abundant foliage again suggesting a relationship between carbon resources and constitutive resin flow (Fig. 6). However, pre-treatment resin flow did not differ among crown size classes in the present study. In addition, our other studies with loblolly pine in central Louisiana, including 4 years of measurements at the same site as the present study have found no difference in constitutive resin flow of juvenile pines in thinned and unthinned plots (Wilkens et al. 1998; unpublished data). Evidently, tree carbon budgets affect the potential for responses to wounding more than they affect constitutive resin flow. Differences in the response to mechanical wounding may help explain why trees in thinned stands are more resistant to southern pine beetle infestation (see Bennett 1965; Lorio 1978; Lorio et al. 1982; Mason et al. 1985).

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