pine

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REPORT

Environmental effects on constitutive and inducible resin defences of *Pinus taeda*

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Abstract

The ecological literature abounds with studies of environmental effects on plant antiherbivore'defences. While various models have been proposed (e.g. plant stress, optimal allocation, growth-differentiation balance), each has met with mixed support. One possible explanation for the mixed results is that constitutive and induced defences are differentialiy affected by environmental conditions. In this study, constitutive oleoresin flow from *Pinus tadea* was least during periods of rapid tree growth and most when drought conditions limited growth; this is as expected if constitutive secondary metabolism is a function of the carbohydrate pool size after growth has been maximised. Induced increases in resin flow, however, were greatest in the fastest growing trees during the season of greatest growth. Apparently, resin production becomes an allocation priority after wounding but not before. Understanding environmental effects on plant antiherbivore defences requires physiological and evolutionary models that account for the differences between constitutive and induced secondary metabolism.

Keywords

Pine, *Pinus taeda,* plant defence, induced defence, bark beetles, Scolytidae, environmental effects, growth-differentiation balance.

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INTRODUCTION

Phenotypic variation in plant secondary metabolism has far-ranging ecological consequences for herbivores, detritivores, plant parasites, the persistence of plant populations, and decomposition processes (Waring & Cobb 1989; Herms & Mattson 1992; Halbrendt 1996; Strand *et al.* 1999). Models explaining environmental patterns in plant secondary metabolism include carbon-nutrient balance (Bryant *et al.* 1983), growth-differentiation balance (Loomis 1932; Lorio 1986; Herms & Mattson 1992), the plant stress hypothesis (White 1984; Mattson & Haack 1987), substrate/enzyme imbalance (Waterman & Mole 1989), and optimal allocation (Tuomi et *al.* 1991). Many empirical patterns can be explained by one or more of these models, but there is no consensus of support for any single model (Koricheva et al. 1998a). One complication is that plant resistance to herbivores is 'a function of both constitutive and inducible secondary metabolism. We now know that the defensive repertoire of many plants includes rapid increases in biologically active secondary metabolites following wounding (short-term inducible responses; Karban & Baldwin 1997). However, most studies of environmental effects on plant resistance have emphasized constitutive defences. Much less is known about the effects of plant physiological status on short-term inducible responses. Most inducible responses are a result of changes in gene expression that influence the biochemical regulation of secondary metabolite synthesis, turnover, or transport (Karban & Baldwin 1997). If internal regulation is very strong, inducible responses should be largely a function of the environmental stimulus (wounding) and the plant genotype. Alternatively, responses could be modulated by plant physiological status, for example if carbohydrate pool size influences product formation (supply side-effects in the terms of Lerdau *et* al. 1994). If inducible responses are influenced by environmental effects on plant physiology, the patterns could match environmental effects on constitutive resistance, implying that a single physiological model might explain supply side-effects on both constitutive and inducible defences, or patterns might differ, which could explain why environmental stress on host plants tends to benefit some herbivores and harm others (Koticheva *et al.* 1998b). It has been hypothesised that inducible defences tend to be more responsive than constitutive defences to current rates of resource acquisition (Tuomi et *al.* 1991; Herms & Raffa 1995).

Loblolly pine (*Pinus taeda* L.) provides a suitable model system to address these questions because the anatomy, biochemistry, and ecology of its secondary metabolism is relatively well understood, because simple measurements of oleoresin flow provide ecologically meaningful assays of herbivore resistance, and because loblolly pine naturally encounters extensive variation in resource availability and has a long evolutionary history with potentially lethal herbivores (especially the Scolytidae, bark beetles). Oleoresin, a mixture of monoterpenes and resin acids, is the most obvious and widespread defence of coniferous trees, including loblolly pine. The amount, viscosity, and chemical composition of oleoresin can influence tree suitability for herbivores such as bark beetles (Mason 1971; Berryman 1972; Hodges *et al.* 1979; Lewinsohn *et al.* 1991; Popp *et al.* 1991; Nebeker et al. 1992; Reeve *et al.* 1995; Steele et *al.* 1995).

It is well known that constitutive resin flow in pine trees can be affected by environmental conditions (Lorio & Hodges 1968; Mason 1971; Paine & Stephen 1987; Lorio *et al.* 1995; Wilkens *et al.* 1997; Kytö et *al.* 1998). Furthermore, conifer defences also involve facultative changes in the resin system that are induced by mechanical wounding (Lewinsohn et *al.* 1991; Tisdale & Nebeker 1992; Ruel et *al.* 1998), fungal inoculation (Popp *et al.* 1991) or bark beetle attack (Dunn & Lorio 1993; Lorio *et al.* 1995). Inducible increases in oleoresin may enhance tree defences against bark beetles by (1) decreasing beetle attack rates; (2) decreasing survival of attacking adults; (3) decreasing oviposition success; (4) decreasing larval survival and (5) preventing the establishment of other insects and fungi that are secondary colonisers of trees that have been attacked by bark beetles (Berryman 1972; Raffa 1991).

In this study, we compared environmental effects on inducible and constitutive resin defences in loblolly pine. We tested for effects of crown size (which influences carbon gain), fertilisation, and seasonal drought on constitutive and inducible resin flow in mature loblolly pine. Results show strong environmental effects on both constitutive and inducible resin flow, but indicate that different physiological and evolutionary models are required to explain the effects.

MATERIALS AND METHODS

Study site

Experimental plots were within a loblolly pine plantation in Camp Beauregard near Pineville, Louisiana (31°22′ N, 92°22′ W). The study included 90 trees within an even-aged stand (approximately 25-years-old): 30 study trees were within a plot that was thinned in 1990 and GO were within an unthinned plot. Study trees from the unthinned plot were stratified into 30 trees of codominant crown class (crowns shared the upper canopy) and 30 trees of intermediate crown class (crowns partly overgrown by neighbouring trees). All trees in the thinned plot had codominant crowns (intermediate trees had been removed by the thinning) which were substantially larger than codominant crowns in the unthinned plots because of crown expansion following the removal of neighbours. Consequently, we treat these experimental groups as three levels of crown class. Baseline measurements for unthinned intermediate vs. unthinned codominant vs. thinned codominant were: mean height ± SD = 18.21 ± 1.20 vs. 20.88 ± 1.09 vs. 19.67 ± 1.20 m, respectively; mean diameter ± SD = 14.6 ± 1.2 vs. 24.1 ± 1.2 vs. 24.2 ± 1.1 cm; and mean basal area ± SD = 40.6 ± 7.5 vs. 36.5 ± 6.8 vs. 18.4 ± 4.4 m^2/ha (as measured with an English BAF 10 prism). In each plot and crown class combination, half the trees (15) were fertilised in the spring of 1997 and 1998 with diammonium phosphate applied at 746 kg/ha (150 kg P and 134 kg N per ha).

Daily maximum and minimum air temperature and daily precipitation *were* used to calculate potential evapotranspiration, soil water depletion, water recharge, water storage, and water deficits with a Thornethwaite model parameterized for the Kisatchie National Forest (Zahner & Stage 1966; Lorio *et al.* 1995).

Tree growth and morphology

Height growth during 1997 and 1998 was measured with a clinometer. Percent live crown was measured as the percentage of total tree height that included live branches. Diameter growth was measured biweekly using dendrometer bands affixed to each tree 1.5 m above ground. We extracted cores from each tree to reconstruct the history of diameter growth in each tree and quantify the number and density of vertical resin ducts in xylem produced during the previous five years (average number of vertical resin ducts per year per 3 mm transverse section of xylem and average number of vertical resin ducts per cm^2 of xylem). Total annual height growth and total annual diameter growth *were* analysed with an ANOVA model that included crown class (thinned–codominant, unthinned-codominant, and unthinned–intermediate), fertilisation, and year as fixed main effects, and tree nested within crown class and fertilisation as a random effect. We used correlation analyses to test for relationships among trees between resin flow and various measures of tree growth, tree morphology, and stand conditions.

Constitutive resin flow

Resin flow in all trees was measured 4 times in 1997 and 5 times in 1998. On each measurement day, we removed two 125 mm^2 disk of bark and phloem from each tree (thus exposing the surface of the xylem) and collected the resin that flowed over the following 3 h from the exposed lateral resin ducts, which conduct preformed resin outward from vertical resin ducts (Dunn & Lorio 1993; Wilkens et *al.* 1997; Ruel et *al.* 1998). Samples were collected from opposite sides of the bole at 1.5 m above ground, Phloem disks were freeze-dried and weighed to give a measure of phloem thickness (mg/125 mm'). Constitutive resin flow was analysed with the same ANOVA model as for tree growth except with the addition of date (May, June, and July) as a fixed effect. Measurements of constitutive resin flow after wounding treatments (see below) were made on the unwounded sides of trees, which were unaffected by the wounding treatment.

inducible resin flow

We used mechanical wounding to deplete the reservoir of oleoresin and measured subsequent changes in resin flow. Half of the trees in each treatment group (15 trees x 3 groups) were measured on G-13 June 1998 during the season of rapid growth in early summer and half were measured on l-8 September 1998 during the season of reduced growth in late summer. Trees were allocated to the two measurement times such that there were no differences between groups in constitutive resin flow. To minimise the error contributions of variance among trees, we used a design with paired measurements of control and wounding responses for each tree (Ruel et *al. 1998).* On day 0 of the experiments, resin flow was measured as before on both sides of each tree. Then we wounded one side of each tree by using a bark chipper to remove two horizontal strips of bark and phloem (1.5 cm wide) 22 cm above and below the site of resin measurements. Wounds did not penetrate the xylem, but did expose the lateral resin ducts, which permitted oleoresin to flow from the resin duct system within the xylem. The orientation of the wounding was random with respect to the direction of the closest codominant tree. After one hour, we expanded the upper and lower wounds by removing one additional strip of bark and phloem. The next day (day 1), we measured resin flow on the wounded and control side of each tree (= W_1 and C_1) and then expanded the wound by removing a 4th strip of bark and phloem. On day 7, we sampled resin flow with two measurements on both sides of each tree (W_7 and C_7 = resin flow on wounded and control sides, respectively). Trees wounded in June were measured again on days 35 and 63 (20 July and 18 August), This method matches that of Ruel *et al.* (1998), which was modelled after techniques employed by the naval stores industry to maximise resin yield from longleaf pine (Clements 1974). The technique was not intended to mimic a beetle attack, which, in addition to depleting preformed oleoresin, also wounds the phloem and introduces fungi. Our tests isolated the capacity of the resin duct system in the xylem to replace oleoresin. This physiological challenge is relevant to tree-beetle interactions because the reproductive success of attacking beetles depends upon the dynamics of oleoresin depletion and synthesis during mass attack (Raffa 1991), which typically lasts 5-10 d for *Dendroctonus frontalis* (Payne 1980).

Analyses indicated that the unwounded side of trees was unaffected by the treatment (no difference in resin flow between the unwounded side of experimental trees and unwounded trees: $F_{1,88} = 0.87$, $P = 0.35$), presumably because of limited radial connections between resin ducts (Lapasha & Wheeler 1990). Ruel *et al.* (1998) reported the same observation. Consequently, analyses of inducible changes in resin flow were based on the difference between wounded and control sides of each tree ($W-C$). We analysed this difference on Day 1 and Day 7 with an ANOVA model that included crown class (thinned-codominant, unthinned-codominant, and unthinned-intermediate), fertilisation, and season (early summer vs. late summer) as fixed main effects.

To further test for spatial and temporal variation in tree responses to a depletion of preformed resin, we conducted identical induced response experiments during August 1997 in the Catahoula district of the Kisatchie National Forest of Louisiana (31'25' N, 92°17' W) and during April 1999 in the Talladega National Forest of Alabama (32°45' *N,* 86°50' W).

RESULTS

Climatic conditions

Soil water regimes differed dramatically between years (Fig. 1). Spring and early summer in 1997 were characterised by well-watered soil conditions. Moisture deficits did not appear until mid-July of 1997. In 1998, precipitation was lower and temperatures were higher than in 1997. Precipitation from 1 May to 9 September totalled only 13 cm in 1998 compared to 36 cm in 1997. As a consequence, soil moisture in 1998 was depleted to half of field capacity by early June and to 15% of field capacity by late July. Cumulative moisture deficits reached 400 mm by September of 1998 compared to a maximum of < 200 mm in 1997. Based on the Palmer drought severity index 1998 was the 7th most severe drought in central Louisiana during the last 75 years (NOAA 1999).

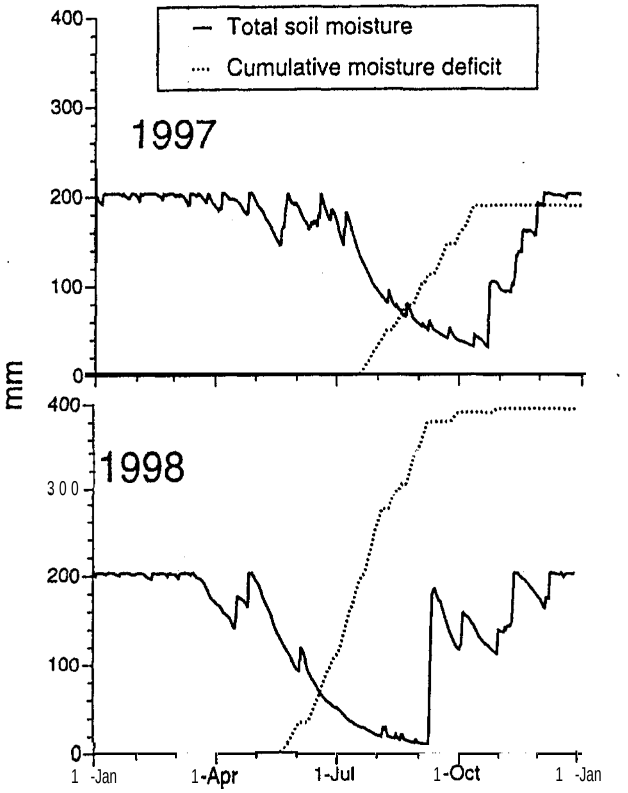


Figure 1 Calculated soil moisture and cumulative moisture deficits indicate that the dry summer period came earlier and was more severe in 1998 than in 1997.

Tree growth

In both years, height growth tended to be least in intermediate trees within unthinned stands and greatest in codominant trees within unthinned stands, but there were no differences in height growth between years, in spite of the climatic differences: mean ± SE for unthinned-intermediate trees, unthinned-codominant trees, and thinned-codominant trees, respectively, = 0.60 ± 0.09, 0.85 ± 0.09 and 0.55 ± 0.06 m in 1997 and 0.40 ± 0.06, 0.75 ± 0.07 and 0.63 ± 0.06 m in 1998; $F_{2,84} = 9.73$, $P = 0.0002$). There was no effect of fertilisation on height growth (least square means ± SE = GO.7 ± 4.0 vs. 65.1 ± 4.0 in unfertilised vs. fertilised, respectively; $F_{1,84} = 0.61$, $P = 0.43$), no effect of year, and no significant interactions.

Diameter growth differed dramatically among crown classes in both years (Fig. 2; mean ± SE for unthinned intermediate trees, unthinned codominant trees and thinned codominant trees, respectively, = 0.71 ± 0.14, 4.85 ± 0.20, and 6.32 ± 0.29 mm in 1997 and 1.45 ± 0.26, 7.44 ± 0.30 and 8.88 ± 0.39 mm in 1998; $F_{2,84} = 202.07$, $P < 0.0001$). Total diameter growth was greater during the drought year of 1998 than during 1997 ($F_{1,84} = 337.91$, $P < 0.0001$). Growth ceased for about a month during late June and July of 1998, but this was more than compensated by rapid diameter growth during spring and autumn (Fig. 2). Fertilisation produced modest increases in diameter growth, especially in trees with the largest crowns (thinned codominants) during autumn of 1998 ($F_{1,84} = 4.10$, $P = 0.02$ for main effect of fertiliser; Fig. 2).

Constitutive resin

Constitutive resin flow was sensitive to environmental conditions (Fig. 3, Table 1). Average resin flow was higher in 1997 than in 1998 (least square mean ± SE = 1.99 ± 0.05 vs. 1.75 vs. 0.05, respectively; main effect of year in Table 1). Years differed even more dramatically in the seasonal pattern of resin flow. In 1998, resin flow was relatively low during May when tree growth was rapid, and then increased by about 2-fold following the mid-summer drought (Fig. 3). In contrast to 1998, resin flow in 1997 was relatively high in early summer and relatively low in late summer (Fig. 3, date–year interaction in Table 1). Trees of intermediate crown class tended to vary less across dates than trees of codominant crown class, especially in 1997 (Fig. 3, crown class-date interaction in Table 1). There was no effect of fertilisation on resin flow. Individual trees within a treatment tended to retain their relative rank in resin flow (tree effect in Table 1).

Inducible changes in *resin* flow

Resin flow was strongly affected by previous wounding. In all experiments, resin flow on the wounded side of the tree declined immediately following wounding (day 1) and then increased to levels higher than the original flow by day 7 (Fig. 4). The wounded side of trees produced significantly less resin than the control side on day 1 (mean difference ± SE = -0.75 ± 0.07, respectively; $t_{89} = 10.80$, $P < 0.0001$ from paired t-test) and significantly more resin on day 7 (mean difference ± SE = 1.06 ± 0.09, respectively; $t_{89} = 12.11$, $P < 0.0001$). Inducible changes in resin flow were sensitive to season and crown class. During early summer, trees retained higher resin flow on the first day after wounding than during late summer (least square means ± SE = 0.62 ± 0.07 vs. 0.16 ± 0.07 g/wound, respectively; $F_{1,78} = 18.97$, $P < 0.0001$). Resin flow on day 1 tended to decrease with decreasing crown size (least square means ± SE = 0.55 ± 0.09 vs. 0.35 ± 0.09 vs. 0.26 ± 0.09 g/wound; $F_{1,78} = 5.16$, $P = 0.035$ for linear contrast), especially in early summer. Following wounding, resin flow on day 7 increased to the highest levels in

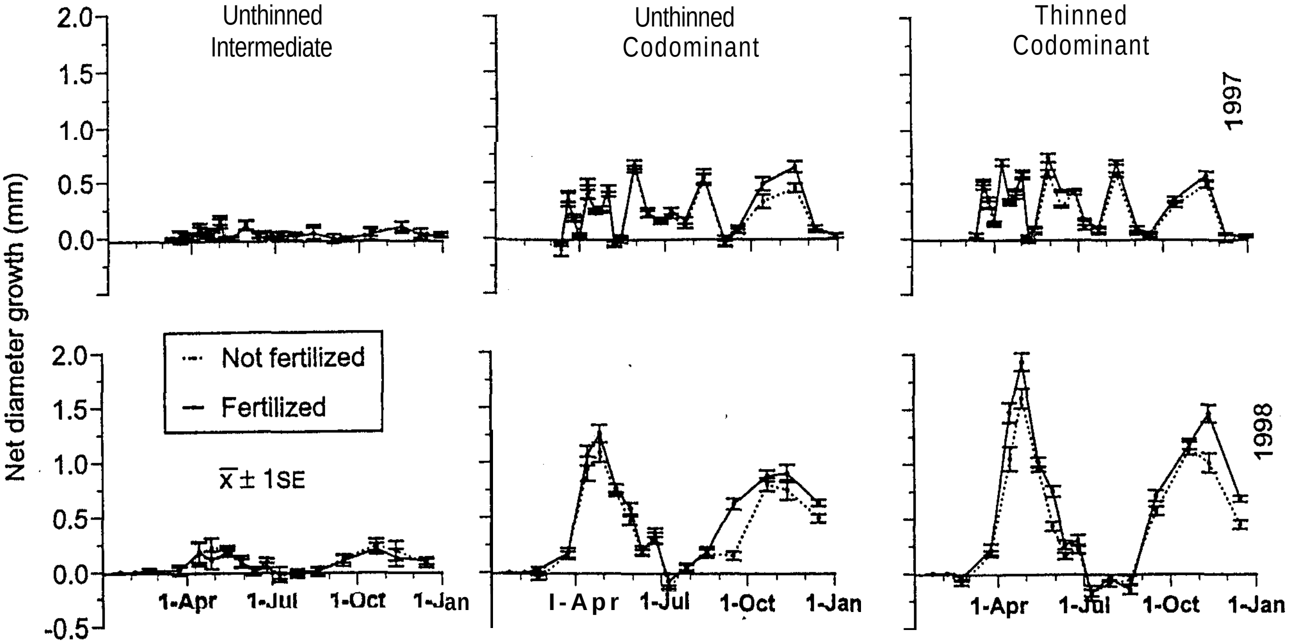


Figure 2 Diameter growth during 1997 and 1998 indicates that trees with smaller crowns grew less (unthinned-intermediate < unthinned-codominant < thinned-codominant) and that growth largely ceased for G-8 weeks during the drought of 1998, even though total annual growth was greater during the drought year. Fertilisation had only a modest effect on diameter growth.

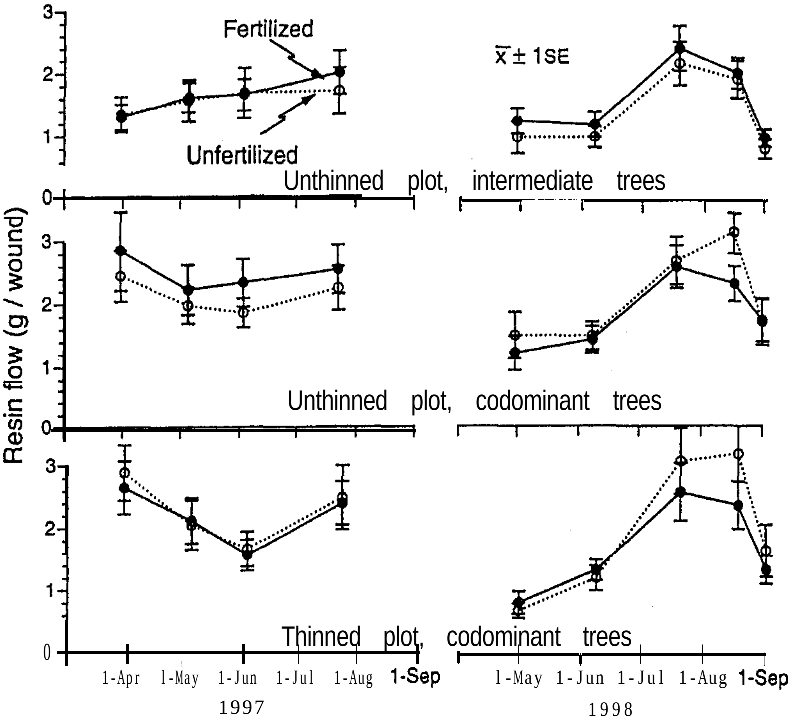


Figure 3 Seasonal changes in constitutive resin flow were more pronounced in 1998 than in 1997, with the highest resin flow corresponding to the period of drought when diameter growth had ceased.

trees with the largest crowns (Fig. 4; crown effects on W_7 and W_7–C_7: $F_{2,78} = 9.23$, $P = 0.0003$ and $F_{2,78} = 12.06$, $P < 0.0001$, respectively), The effect of crown size on responses to wounding was especially dramatic in late summer, when trees with the largest crowns (thinned-codominant) increased their resin flow by 172% compared to only 21% in trees with the smallest crowns (unthinned-intermediate) (Fig. 4; crown-season interaction for W_7

and W_7-C_7: $F_{2,78} = 3.94$, $P = 0.023$ and $F_{2,78} = 4.79$, $P = 0.011$). There were no effects of fertilisation on inducible changes in resin flow ($F_{1,78} < 0.98$, $P > 0.32$).

Table 1 ANOVA testing for effects of crown class, fertilisation, date and year on constitutive resin flow in loblolly pine

Source	d.f	MS	F^a	P
Crown class	2	4.74	0.69	0.50
Fertilisation	1	0.10	0.01	0.90
Date	2	49.45	78.75	< 0.0001
Year	1	7.57	12.06	0.0006
Crown class x Fertilisation	2	1.11	0.16	0.85
Crown class x Date	4	2.45	3.90	0.004
Crown class X Year	2	1.63	2.60	0.07
Date x Year	2	20.18	32.14	< 0.0001
Fertilisation x Year	1	1.63	2.60	0.11
Crown class x Fertilisation x Date	4	0.82	1.30	0.27
Crown class x Fertilisation X Year	2	0.29	0.46	0.63
Crown class x Date x Year	4	1.25	1.99	0.09
Fertilisation x Date x Year	2	0.88	1.40	0.25
Crown class x Fertilisation x Date x Year	4	0.25	0.40	0.80
Tree[Crown class, Fertilisation]	84	6.86	10.92	< 0.0001

[a] F-test denominator = MS_{Trees} for Crown class, Fertilisation and Crown class x Fertilisation and MSE for others.

Resin flow remained elevated through at least the remainder of the summer following wounding in June: W_d-C_d ± SE (% of control) = 2.08 ± 0.28 (165%) and 0.96 ± 0.18 (131%) for days 35 and 63 following wounding.

Replicate experiments in the Kisatchie National Forest during August 1997 and in the Talladega National Forest in April 1999 further indicated that loblolly pines respond to a depletion of oleoresin by rapidly refilling the resin reservoir to about twice its previous level. In both experiments, mean resin flow declined on day 1 following wounding to 12–29% of controls and increased by day 7 to 197–221% of controls ($P < 0.0001$ for paired t-tests of W_1-C_1 and W_7-C_7 in both experiments).

Correlations among trees

Constitutive resin flow was uncorrelated with any measures of tree-specific growth, morphology, or stand conditions (Table 2). Neither were inducible increases in resin flow during early summer correlated with any other measured tree attributes. However, inducible increases in resin flow during late summer were correlated with most measures of tree growth, morphology, and stand conditions (Table 2). Resin flow increased the most in trees with large diameters, rapid diameter growth, large

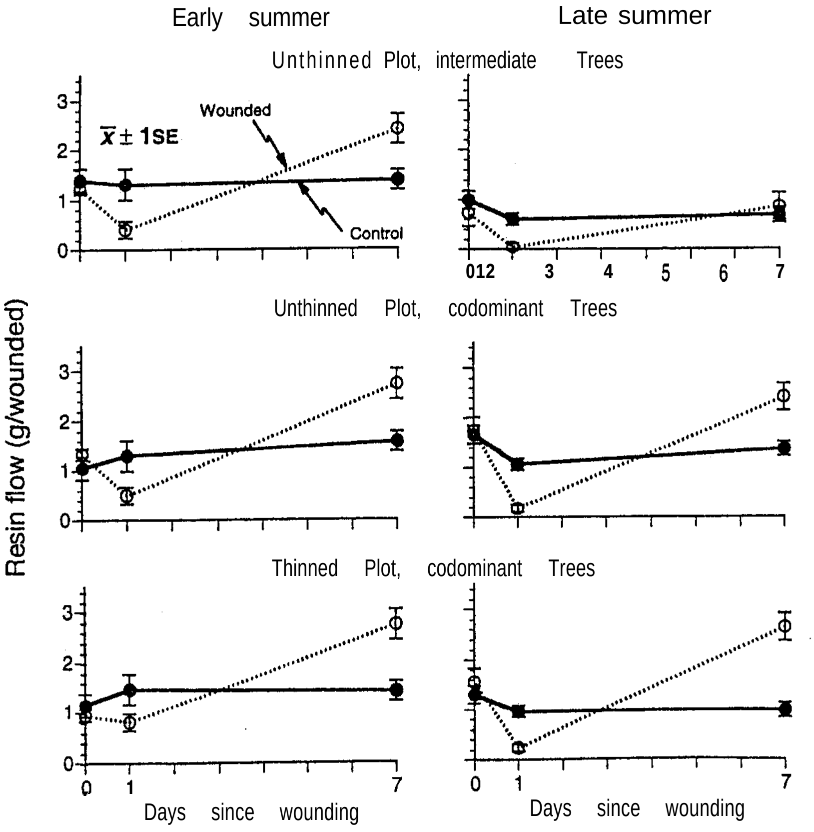


Figure 4 In the early summer of 1998, trees of all crown classes responded to wounding with initial decreases in resin flow (from depletion) following by a near doubling of resin flow within 7 days. In late summer, the initial decrease **was** greater in all crown classes and the subsequent increase was strongly related to crown size (unthinned-intermediate < unthinned–codominant < thinned-codominant).

Table 2 Correlations (r) between constitutive and inducible resin flow in early summer and late summer and various measures of tree-specific growth, morphology, and stand characteristics. (Each correlation based on 45 trees)

	Early summer		Late summer	
	Constitucive resin”	Inducible resin”	Constitutive resin^a	Inducible resin^b
Diamerer at breast height	0.16	0.07	0.00	0.65***
Basal area	-0.10	-0.15	-0.01	−0.61***
Height in 1997	0.04	0.14	0.19	0.11
Height growth in 1997	0.16	0.12	-0.04	0.00
Height growth in 1998	0.19	−0.10	0.03	0.24
Radial growth 1993 – 97	0.09	0.16	0.05	0.63***
Radial growth 1998	0.15	0.11	0.02	0.62***
Percent live crown	0.07	-0.03	0.00	0.48***
Phloem thickness	0.29	0.13	-0.15	0.40**
Resin duct density/cm”	0.01	0.03	-0.15	−0.41**
Resin ducts/year	0.07	0.25	-0.15	0.31*
Conseitutive resin		0.34*		0.02
Inducible depletion		0.11		0.22

“Based on average of 5 dates for early summer and 4 dates for late summer. ^{b}Difference between resin flow on wounded and control sides of free on day 7 following wounding (W_7-C_7).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

crowns, thick phloem, and large numbers of vertical resin ducts per year of xylem growth (but low densities of vertical resin ducts/cm^2 of xylem); resin flow increased the least in trees experiencing a high local density of other trees (high basal area). There was a weak positive correlation between constitutive resin flow and inducible increases in ‘resin flow during early summer but no correlation dyring late summer.

DISCUSSION

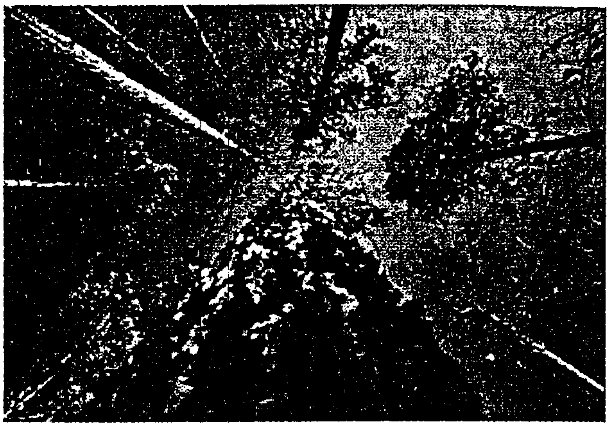
Patterns in constitutive resin flow of loblolly pine are consistent with a simple and longstanding physiological model which postulates that carbohydrates are invested preferentially in growth, and phenotypic variation in carbon-based secondary metabolism is a function of the pool size of remaining carbohydrates that cannot be invested in growth because of growth limitation from other resources. Moderate deficiencies of water or minerals usually limit plant growth more than photosynthesis and should therefore result in a larger pool of carbohydrates that are left after carbohydrate demands for growth have been met. This model is a foundation of the growth:differentiation balance concept as developed by Loomis (1932), applied to pines by Lorio (1986), and extended to plant defences in general by Herms & Mattson (1992). Similarly, source-sink models of whole-tree allocation patterns (e.g. Luxmoore *et* al. 1995) postulate that (I) growth is more sensitive than photosynthesis to water stress and nutrient deficiency; (2) growth is the priority sink for carbohydrates; (3) carbohydrates tend to accumulate in the presence of water or nutrient deficiencies and (4) pine trees tend to increase carbohydrate storage when there is little growth activity. Environmental conditions may have similar effects, for the same reasons, on carbohydrate allocation to storage and constitutive secondary metabolism. This explanation does not require a tradeoff at the level of the whole plant between secondary metabolism and growth (carbohydrates that go into secondary metabolism could not have been used to increase growth because of other resource limitations on growth). The explanation neither invokes nor precludes natural selection operating on secondary metabolism (e.g. secondary metabolites can be viewed as biochemical garbage or adaptive antiherbivore defenses, among other things). In our study, tree diameter growth was moderate but stable through the 1997 growing season and constitutive resin flow also remained stable (Figs 2, 3). In contrast, diameter growth during 1998 was very rapid during the early summer until the onset of a drought, when growth ceased almost completely (Figs 1,2). During 1998, constitutive resin flow was low during the season of rapid growth and then increased by more than two-fold during the drought (Fig. 3). We were unable to measure below-ground growth in this study, but root growth in loblolly pine also tends to be higher in the early summer than in the late summer and positively correlated with soil moisture (Sword et al. 1998). Previous studies have similarly indicated that water deficits that limit growth in loblolly pine can increase constitutive resin flow and decrease host suitability for bark beetles (Dunn & Lorio 1993; Reeve et *al.* 1995). This pattern is exactly opposite that predicted by the plant stress hypothesis and falsifies the model that trees tend to partition carbohydrates equally between growth and secondary metabolism. Results also falsify the hypothesis that internal regulation of secondary metabolism is so strong that environmental effects on whole-plant physiology can be disregarded.

Extreme water deficits must eventually lead to a collapse of the carbon budget and concomitant decreases in secondary metabolism (Lorio 1986; Herms & Mattson 1992) and accordingly, resin flow in our study began to decline by early September of 1998, presumably because of declining photosynthesis associated with the extreme drought and sustained high temperatures (Teskey *et al.* 1995). This is consistent with some previous studies showing that extreme drought stress can lower the resin defenses of loblolly pine (Lorio & Hodges 1968; Lorio *et al.* 1995). However, even in the advanced stages of a severe drought, resin flow remained at least as high as during times of rapid growth (Fig. 3). Apparently, most droughts in this region will tend to increase constitutive defenses rather than decrease them.

As in previous studies (Wilkens *et al.* 1997; Ruel *et al.* 1998), constitutive resin was largely unaffected by crown size (Fig. 3) even though the thinned-codominant trees had relatively massive crowns compared to unthinned–intermediate trees. Apparently, the higher carbohydrate gains of trees with large crowns tend to be invested in growth and storage rather than constitutive defences. Our study revealed no effects of fertilisation on resin flow, which was surprising because previous studies with loblolly pine had all indicated that resin flow decreases with fertilisation (24% decrease in Wilkens et *al.* ***1997; 30***-100% decrease in Warren et *al.* 1999; 39% decrease in Wilkens, Ayres & Lorio, unpublished data). Our experiment was also unique among these studies in that fertilisation produced very little growth increase. Evidently, fertilisation only decreases resin flow when growth is limited by nutrients deficiencies, which is as predicted if secondary metabolism is a function of the pool size of carbohydrates that remain after growth has been maximised. Constitutive resin flow' was uncorrelated with the number or density of vertical resin ducts, which implies that resin ducts are not normally filled to capacity with resin, and indicates that genetic or environmental factors that increase the number of vertical resin ducts (Mergen et *al.* 1955; Lorio 1986; Lapasha & Wheeler 1990) do not necessarily increase constitutive resin flow.

The speed and magnitude of induced increases in resin flow suggest that this response may be important in the defence system of loblolly pine. Resin flow was doubled within 7 days. With the same wounding, but more frequent sampling, Ruel et al. (1998) found that most of the increase was within 3 days. Mass attacks by ***D. frontalis*** are sometimes completed within 3 days but commonly extend beyond 7 days (Payne 1980). In Reeve *et* al. (1995), a doubling of resin flow from 1 to 2 g (compare to Figs 3, 4) halved beetle reproductive success. It makes evolutionary sense that pine trees should have a rapid inducible response to wounding because most bark beetles use aggregation pheromones to coordinate mass attacks on individual trees, so wounding implies a high probability of continued attacks during the days and weeks to come. Inducible increases in resin flow from the xylem resin duct system probably pose a third barrier to colonisation of pine trees by insects-in addition to constitutive resin flow and necrotic lesion formation (Berryman 1972; Cook & Hain 1987; Raffa 1991; Paine *et al.* ***1997;*** Christiansen *et* al. 1999). Increases in resin ***are*** apparently the result of de novo synthesis of resin (Lawrence 1971) by the epithelial cells that line vertical resin ducts and synthesise constitutive resin (Lewinsohn et *al.* 1991). We hypothesise that wounding increases the level to which resin ducts are filled before there is negative feedback to the biosynthetic pathway. Because of induced increases in secondary metabolism within the resin duct system, trees that have previously sustained low levels of beetle attacks may sometimes be better defended against subsequent attacks. Perhaps for this reason, *Picea abies* exposed to wounding and fungal inoculation had enhanced resistance to subsequent fungal inoculations Christiansen et *al.* (1999). Induced increases in defence should select for aggregation behaviour in bark beetles that facilitates rapid mass attack of host trees (Berryman 1972; Raffa 1991).

The inducible response characterised by our measurements differs from the resinosis and necrotic lesion formation that typically occurs within the phloem of conifers exposed to beetles and/or fungi (Berryman 1972; Raffa 1991; Paine ***et*** *al.* 1997). We measured changes in the amount of oloeresin that flowed from the resin duct system within the xylem, which permitted us to compare environmental effects on constitutive vs. induced secondary metabolism of the same tissue system (vertical resin ducts). By design, our wounding produced no visible resinosis or lesion formation in the phloem. There was



Pinus in Louisiana, U.S.A., resists mass attack from bark beetles by exuding oleoresin from a system of resin ducts within the xylem. Photograph provided by Erich Valley, Southern Research Station, USDA Forest Service, Pineville, LA.

little or no contribution to our oleoresin samples from secondary metabolism within phloem tissue. Induced responses of the resin duct system and the phloem are apparently both elements of conifer defence systems. They are related because changes in phloem chemistry during beetle attack are partly a product of oleoresin that soaks into the phloem from the xylem. However, changes in the phloem are also a result of rapidly induced synthesis of terpenes and phenols within the phloem tissue itself (Berryman 1972; Raffa 1991). Some studies indicate that the efficacy of induced defences within the phloem is greatest in the most rapidly growing trees during the season of most rapid growth, as we observed for induced changes in the resin duct system (Christiansen & Fjone 1993; Paine *et al.* 1997).

Inducible increases in resin flow were weakly correlated or uncorrelated with constitutive resin flow (Table 2) implying that the physiological and genetic controls on inducible resin flow differ from the controls on constitutive resin flow. Furthermore, the response of induced increases in resin flow to changes in the environment and tree physiological status contrasted sharply with the response of constitutive resin flow. The lowest levels of constitutive resin defences were measured in the fastest growing trees during the season of most rapid growth (Figs 2, 3), while the lowest inducible responses were in the slowest growing trees during a season of minimal growth (Figs 2, 4). Patterns in constitutive resin flow in loblolly pine across crown classes and season can be explained if secondary metabolism is simply a function of the carbohydrate pool that exceeds growth requirements. In contrast, the patterns for inducible resin flow suggest that secondary metabolism within resin ducts becomes an allocation priority following wounding. Inducible responses were greatest during the early summer when growth was greatest, and among the trees with the largest crowns and greatest photosynthetic potential (Figs 3, 4). After the drought period in 1998, when growth had nearly ceased, inducible resin flow was strongly correlated with growth rates prior to the drought and with phloem thickness, a measure of carbohydrate storage (Table 2). Apparently, wounded trees increased resin flow by drawing on the pools of current photosynthates and/or stored carbohydrates that could otherwise have been allocated to growth, respiration, or storage. This scenario predicts tradeoffs between tree growth and inducible increases in resin flow, which is consistent with many evolutionary models to explain inducible defences (Karban & Baldwin 1997). Costs of secondary metabolism are difficult to estimate. However, the case for oleoresin within the resin ducts of P. taeda is simplified somewhat because all of the main constituents (> 1%) are monoterpenes and diterpenes (Hodges et *al.* 1979) that are synthesized from the same pathway within the same cells, and have a similar content by mass of carbon and energy. Evidence of costs to pine trees from inducible responses comes from the naval stores industry, which learned that repeated wounding of trees maximised resin production, but led to reduced growth and increased mortality (Clements 1974; Harrington 1969).

The apparent role of carbohydrate supply as a determinant of secondary metabolism in pine trees does not preclude the importance of biochemical controls and downregulation. Indeed, there is a heritable basis to the oleoresin yield of wounded *Pinus elliottii* (Mergen et *al.* 1955) which clearly indicates genetic control, and many elements of the biochemical controls on monoterpene synthesis in conifers have already been elucidated (Lewinsohn ***et al.* 1991;** Steele ***et al.* 1995).** In ***Nicotiana sylvestris***, internal regulation of secondary metabolism is apparently so strong that growing conditions of the plant have little effect on inducible defences (Ohnmeiss & Baldwin 1994). However, it seems obvious that inducible defences in pines, like constitutive defences, can only be fully understood in the context of environmental effects on whole-plant physiology. Too few plants have been studied thus far to generalise about the prevalence of environmental effects on inducible responses. The frequency and magnitude of such effects is relevant to bioengineering programs focused on inducible plant defences (Karban & Baldwin 1997; Robison & Raffa 1997).

The striking differences between environmental effects on constitutive and inducible defences of pines suggests an explanation for the highly variable effects of plant stress on herbivores (Koricheva *et al.* 199813). It may be that environmental conditions that limit plant growth (stress) tend to favour herbivores that are most impacted by rapid inducible defences and disfavour herbivores most impacted by constitutive defences (Herms *et al.* 1997). Factors that influence the relative importance of constitutive and inducible defences probably include plant physiology (e.g. development of constitutive vs. inducible systems), herbivore feeding guild (e.g. consumers of mature vs. developing tissue and miners vs. gallers vs. folivores), herbivore mobility, and herbivore detoxification systems. Predicting phenotypic patterns in plant secondary metabolism, and understanding the ecological consequences of environmental variation in resource availability for plants, probably requires physiological and evolutionary models that account for the differences between constitutive and inducible plant defences.

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