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Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle

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Abstract

Oleoresin characteristics of first-generation (F_1) progeny of loblolly pines (*Pinus taeda* L.) that escaped mortality from the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), despite heavy mortality of neighbors, were evaluated and compared to trees from a general (i.e., trees produced from bulk seed sources) population over the course of two and a half years in southcentral Mississippi (USA). Trees were 21–25 years old and growing in a common-garden type planting when sampled. The relative concentrations of five monoterpenes, five resin acids, and one phenylpropanoid were determined from oleoresin collected on five dates over 18 months. Multivariate analysis of variance showed that the concentration of 11 oleoresin chemical components did not differ between trees from escape and general populations ($P > 0.619$), providing evidence against the importance of this potential resistance factor. Univariate analyses on three individual resin constituents that were deemed important prior to the study—α-pinene, 4-allylanisole, and limonene-showed that only 4-allylanisole ($P < 0.0339$) varied significantly between populations; however, its concentration was higher in trees from the general population ($\bar{x} = 1.4$ vs. 0.9% of oleoresin weight). which does not support the hypothesis that higher concentrations of 4-allylanisole in oleoresin facilitated escape from *D. frontalis* attack. Oleoresin flow, on the other hand, was significantly higher in escape trees-averaging 1.65 times higher than general population trees over the course of 28 months (eight sampling times). This strongly supports the hypothesis that oleoresin flow can impact the host selection process of *D. frontalis*, and suggests that increased flow can improve survival under heavy pressure from *D. frontalis*. These results also may provide an indirect estimate of the magnitude of increase in flow necessary for producing a 'real world' impact on the outcome of the interaction between *D. frontalis* and a preferred host. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Pinus taeda*; *Dendroctonus frontalis*; Host resistance; Oleoresin flow; Oleoresin chemistry

1. Introduction

The southern pine beetle, *Dendroctonus frontalis* Zimm. (Coleoptera: Scolytidae), is native to forests of the southeastern United States. Despite the native status of *frontalis*, its populations outbreak

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periodically and impact mature pine forests more dramatically than any other insect in this region of North America (Drooz. 1985). The frequency and severity of outbreaks have increased in recent decades (Price et al.. 1998), a result likely due to changes in the quality of habitat since European settlement. For the past 300 years, pine forests of the southeastern US have been subjected to rapid (on an evolutionary scale) changes from cutting, altered fire regimes, modified species composition and spacing, and nonnative introductions (Martin and Boyce, 1993). Often these alterations leave resources with increased susceptibility to *D. frontalis* (Cameron and Billings, 1988; Clarke et al.. 2001). For example, the area of longleaf pine (*Pinus palustris* Mill.) savanna, a community resistant to disturbance by *D. frontalis*, has decreased by an estimated 97% since settlement by Europeans (Frost, 199 1). Mixed pine-hardwood forests have also lost tremendous area, with about 3% remaining from that which was present prior to settlement (Ware et al.. 1993). Meanwhile the standing volume of loblolly pine, *Pinus taeda* L., a preferred host of *D. frontalis*, has continued to increase, nearly doubling since about 1950, and now accounting for more than half the standing volume of southern yellow pine (Schultz, 1997).

The southern pine beetle is probably the most aggressive scolytid in North America, and is distinctive in the spatial pattern of mortality it causes. Like most aggressive bark beetles, *D. frontalis* must kill its host to reproduce. This process is facilitated by aggregation pheromones which promote mass attack and provide a mechanism, i.e., depletion of host defenses, by which apparently healthy trees can be successfully attacked (Raffa et al., 1993). Because it is not limited to decrepit trees, *D. frontalis* has a more extensive host population from which to choose (compared to less aggressive species) and can produce widespread mortality during outbreaks. During periods of locally high population densities, *D. frontalis* kills trees contiguously in space as groups or ‘spots’, missing few trees as mortality spreads through the forest. Individual trees are occasionally ‘missed’ during infestation expansion, resulting in trees that are attacked later than expected, or in trees that completely escape attack from D. *frontalis*. Reasons for tree ‘escape’ are unknown, but probably include such factors as random chance, wind shifts, and perhaps tree resistance. For example, shifts in winds and pheromone plumes likely cause infestations to change direction through time, perhaps leaving some trees alive. Alternatively, tree factors that increase resistance may promote escape. To our knowledge the potential resistance of *D. frontalis* escapes has not been evaluated. but the general resistance characters of trees resistant to *D. frontalis* have been outlined previously (Hodges et al., 1977, 1979; Cook and Hain, 1987; Nebeker et al., 1988; Lorio et al., 1995). Currently, there is no single reliable measure of resistance, but escape trees provide a potentially untapped resource for evaluating both the existence and level of traits suspected in resistance.

The susceptibility of pines to *D. frontalis* is affected by many variables. It is widely believed that no tree of suitable species and size is immune to D. *frontalis*. However, some individual trees are considered more resistant than others; a trait probably resulting from a combination of factors, the most important of which appears to bc constitutive oleoresin flow (Hodges et al., 1977, 1979; Lorio et al., 1995; Reeve et al., 1995; but see Popp et al., 199 1; Ruel et al., 1998). In addition to flow, chemical constituents of oleoresin have been proposed as important for pine resistance to bark beetles (Coyne and Lott, 1976; Cook and Hain, 1987; Raffa et al., 1993). Monoterpenes, which can be both semiochemicals and poisons, have generally received the most attention with two in particular, α-pinene and limonene, prompting wide interest due to their effects on behavior and physiology. Typically, the most prevalent volatile component of loblolly oleoresin is α-pinene (Mirov, 196 1; Hodges et al., 1979), which is a powerful synergist of the aggregation pheromones of *D. frontalis* (Renwick and Vité, 1969, 1970). Limonene is the most toxic (to *D. frontalis*) of the monoterpenes that commonly occur in the southern pines and has been implicated in the resistance of trees to both insects and disease agents (Coyne and Lott, 1976; Bridges, 1987; Michelozzi et al., 1995). Field studies have not provided support for the importance of monoterpenes in impacting tree-D. *frontalis* interactions (Hodges et al., 1979; Cook and Hain, 1987). However, since resin acids have been little studied with regard to *D. frontalis*, and a recent finding suggests that 4-allylanisole may be an active semiochemical deterrent for *D. frontalis* (Hayes et al., 1994b), it is possible that other components of oleoresin chemistry provide important contributions to resistance.

The phenylpropanoid 4-allylanisole occurs at relatively low concentrations in the oleoresin and other tissues of pines including loblolly (Mirov, 1961; Hayes et al., 1994a). It has been found to have antiaggregant or deterrent effects toward *D. frontalis* when eluted at relatively high rates from traps (Hayes et al.. 1994b; Strom et al., 1999), and in laboratory assays (Hayes et al., 1994a,b). Effects at low concentrations have not been evaluated, but one study found that the infusion of loblolly pines with a mixture of sodium *N*-methyldithiocarbamate MS + dimethylsulfoxide (DMSO)-which causes a well-documented *increase* in pine susceptibility to bark beetles (Roton, 1987; Hayes et al.. 1994a; Miller et al., 1995)—was followed by a reduced concentration of 4-allylanisole in the oleoresin that was coincident with *D. frontalis* attack (Hayes et al., 1994a). This suggests that *D. frontalis* may preferentially select trees with lower concentrations of repellent compounds such as 4-allylanisole; this hypothesis, however, has not been experimentally evaluated.

Escape trees may be useful to both managers and researchers for a variety of reasons. They may provide clues about *D. frontalis* behavior, a much-needed quantitative (or at least relative) measure of resistant traits, and be a source of genetic material for incorporating *D. frontalis* resistance into existing breeding programs. Tree breeders have made tremendous progress toward improving growth and disease resistance in loblolly pine, but have largely ignored resistance to *D. frontalis* (Nebeker et al., 1988). Preventive measures aimed at reducing future *D. frontalis* damage will likely need to rely on a variety of silviculturally-based resistance factors in order to be both effective and ecologically legitimate. Resistant trees could be one important component of these strategies provided that they can be identified; escapes, if found to possess resistant traits, may aid in this function.

Our objectives in this study were to evaluate whether trees that escaped *D. frontalis* attack differed in oleoresin composition or total flow from trees in a general population (i.e., one unselected with respect to traits known or hypothesized to be important in resistance to *D. frontalis*). To do this, first-generation (F_1) loblolly pine progeny of parents that escaped II. *frontalis*-caused mortality during local outbreaks (i.e., those living pines that were standing alone after *D. frontalis* destroyed all or nearly all adjacent trees) in the early 1960s were sampled using traditional methods: analysis of constitutive oleoresin flow and composition. In addition to the more typical chemical analysis of monoterpenes, the concentration of 4-allylanisole and resin acids were quantified. By evaluating both flow and composition concurrently, it was hoped that a determination could be made of which, if either, of these two characters was associated with the observed escape from *D. frontalis*.

2. Materials and methods

Trees from both escape and general (i.e., trees produced from bulk seed sources) populations were sampled simultaneously for comparison. Sampling of oleoresin took place over 3 years and during three seasons-spring, summer, and fall-so that measurements were made during periods of the growing season that are thought to differentially affect oleoresin characteristics and hence resistance to *D. frontalis* (Lorio et al., 1990). Ail samplings were done at the Harrison Experimental Forest. Saucier, MS (Harrison County, latitude: 30 37′30″, longitude: 89°3′, elevation: -70 m). Trees were sampled on the following dates: 3 June 1995, 13 October 199.5, 29 March 1996, 5 June 1996, 8 October 1996, 22 March 1997, 2 July 1997, 10 October 1997. Hereafter, March samples are referred to as spring; June/July as summer; and September/October as fall. Two sites, approximately 2.7 km apart, planted in 1973 and 1974 were sampled for oleoresin flow and chemical composition. Site 1 consisted of trees from both escape ($n = 18$) and general ($n = 26$) populations, while site 2 consisted only of escape trees ($n = \mathbf{44}$).

2.1. Site, stand and tree measurements

The soil at both sites is classified as McLaurin fine sandy loam (Smith, 1975). Diameter at breast height (dbh), total height, and height to the lowest green branch were measured on each tree in April 1998. Live crown ratio (LCR) ([total height − height of lowest green branch]/total height) also was determined for each tree so that its effect on response variables could be assessed.

2.2. Selection of escaped trees

Original selection of escaped trees began in 1963 (Coyne, 1974). To be selected as escapes, trees had to meet the following criteria: (1) susceptible species; (2) surrounded on all sides for 100 ft by at least 90% mortality caused by *D. frontalis*; (3) may or may not have pitchouts present; (4) dominant or co-dominant, good form and free of other pests (Coyne, 1974). Scions were removed from selected escape trees and established in a clone bank at the Harrison Experimental Forest so that seed for progeny tests could be produced as desired.

The escape population in this study was made up of first-generation (F_1) progeny from seed of the originally selected escape trees. Our population of escape trees consisted of 62 individuals from 21 half-sibling families (i.e., at least the maternal parent was known to be an escape), with original maternal parents being from Louisiana (two families, six individuals), Mississippi (six families, 18 individuals) and Texas (13 families, 38 individuals). The trees from our general population consisted of 26 individuals from five seed sources (one Louisiana source, two Mississippi, two Texas). Hereafter we use 'seedlot' to identify families in the escape and general populations. Trees were selected using seedlot survival information followed by field corroboration of survival plus visual inspection to ensure each tree's status as dominant or co-dominant in the stand.

2.3. Oleoresin sampling

Total oleoresin flow was determined following the methods of Lorio and Sommers (1986) with the minor modification of using a specially designed tin funnel to direct resin into vials (Ostrom and True, 1946; Lorio et al., 1990). Briefly, trees were wounded by punching a 1.27 cm hole through the outer bark to the face of the sapwood. Oleoresin was allowed to flow into the sampling vial for 24 h (± 1.5 h), at which time vials were collected, sealed. and stored on ice until they were weighed (usually within 24 h). Because 16 samples were taken from each tree over the course of the study (eight each for flow and chemistry), we wounded trees using a spiral pattern (Lorio et al., 1990) to avoid lesions formed from our earlier samples. To minimize injuries, one fow sample was taken from each tree during each sampling period.

Oleoresin was sampled for chemical analysis during each of the sampling periods listed above; however, its chemical composition was determined for only five sampling periods (June 1995–October 1996). Samples for chemical analysis were collected differently to avoid the loss of volatile components during the sampling period. Holes (1.27 cm× ∼ 2 cm) were drilled at an upward angle so that resin would Bow directly into vials, which were immediately twisted into the holes to create a sealed vessel. The exposed portion of each vial then was covered with kraft paper to reduce exposure to sunlight during the sampling period. Therefore, oleoresin sampled for chemical analysis was protected from both air and light. Drill bits were rinsed with acetone between each sample. Vials (7.4 ml) were collected after about 4 h, immediately sealed and placed on ice until returning to the lab, where they remained frozen until analysis. Chemical analysis was conducted at the Mississippi Forest Products Laboratory (Starkville, MS) using a Varian Star 3600 gas chromatograph (GC) equipped with a J & W DB-625 capillary column. A subset of samples was further evaluated by gas chromatography/mass spectrometry (GUMS) (Carla Erba/Kratos MS-SO GUMS system) to ensure that identification of compounds by GC was correct. The concentration of each chemical constituent was calculated using percent weight of the oleoresin (Birks and Kanowski, 1988).

2.4. Statistical analyses

Seasonal differences in oleoresin composition were not anticipated; therefore, we determined mean values for each chemical constituent in each tree over the five sampling periods. We used these tree means in all statistical analyses. Multivariate analysis of variance (MANOVA) was used to evaluate the effect of site on chemical composition of oleoresin from escapes only (because only escape trees were present at both sites). Site had a significant effect on chemical composition of oleoresin ($F_{10,10} = 5.7$: $P = 0.0055$) (Proc GLM, SAS, 1988, v. 6.12). All further analyses of chemical composition used only data from site I. where both escape and general trees were present. Site also had a significant effect on oleoresin flow of escapes (site 1: $\bar{x} = 5.57$, site 2: $\bar{x} = 3.50$; $F_{1,19} = 17.86$, $P = 0.0005$)

(Proc Mixed, SAS Institute, v. 6.12), so further analyses of flow were also done using only data from site 1.

Averages for each of the 11 chemical constituents obtained from each of the 44 trees at site 1 were subjected to MANOVA using methods for analysis of compositional data (Aitchison, 1984). The independent variables used were population and seedlot nested within population. Response variables were average concentrations of chemicals for each tree. Because this study was not designed to investigate the magnitude of variation within populations, evaluation of scedlots was not pursued. However, all tests of population effects used seedlot(population) as the denominator of F-tests, so this factor was included in statistical models. A nonparametric univariate approach (Wilcoxon rank-sum test) also was used to evaluate the effect of population on each of the three oleoresin chemicals determined *a priori* to be of interest: α-pinene, limonene, and 4-allylanisole. The mean values from each seed source also were used in the rank-sum tests.

LCR has been shown to significantly affect oleoresin flow of pines (e.g., Schopmeyer and Larson, 1954; Ruel et al., 199X). We included LCR as a covariate after determining that it was not significantly different between our two populations ($F_{1,40} = 0.22$; $= 0.64$). Therefore, we used repeated measures analysis ofcovariance (ANCOVA) to test for effects of population on oleoresin flow (Proc Mixed, SAS Institute, v. 6.12). Values of oleoresin flow were square-root transformed prior to analysis to better meet the assumptions of parametric statistics.

Correlation analyses were performed to evaluate the relationship between tree parameters (dbh, height, LCR) and resin variables (total flow, concentration of 4-allylanisole, α-pinene, and limonene). In addition, estimates of correlation coefficients between oleoresin flow and oleoresin chemicals were also obtained. Fifteen correlation coefficients were estimated, so the critical value of ***P*** was adjusted to 0.0034 (from 0.05) using the method of Dunn-Sidak (Sokal and Rohlf, **1995).**

Because flow and chemistry are not independent in their effects on semiochemistry, we evaluated two host compounds known to affect the behavior of *D. frontalis*—α-pinene and 4-allylanisole—for their potential effect on the semiochemical bouquet. For a preliminary evaluation, we used the product of the mean concentration of each chemical and the mean flow of oleoresin (using the same five sample periods used for chemistry) from each tree to describe the amount of α-pinene and 4-allylanisole potentially released into the air from the half-inch wounds. We use this only as a relative measure because other factors (e.g., vapor pressure of individual components) are important for determining the actual release of volatile components from oleoresin. The mean of this product, which we call yield, for each chemical and seed source was subjected to a Wilcoxon rank-sum test to evaluate the difference between escape and general populations.

3. Results

Tree height, dbh, and LCR were not significantly different between escape and general trees at site 1 (Table 1). The effect of site was significant for both oleoresin chemistry and flow, so only those data from site 1 were subjected to statistical analysis for evaluating differences between tree populations. Results of the MANOVA on the concentration of components in the oleoresin, transformed and analyzed

Table 1

Tree diameter, height, and LCR ($\bar{x}$ ± SEM) from trees at two sites near Saucier, MS, containing escape trees (first-generation progeny from parents that 'escaped' *D. frontalis* attack) and general trees (trees with unknown parents that originated from sources in Louisiana, Mississippi and Texas)

Site	Population	n	$\bar{x}$, dbh (cm)	$\bar{x}$, height (m)	$\bar{x}$, LCR
1[a]	Escape	18	21.8 ± 1.1 a	17.2 ± 0.6 a	0.43 ± 0.02 a
1	General	26	24.3 ± 1.0 a	18.4 ± 0.4 a	0.44 ± 0.01 a
2[b]	Escape	44	23.0 ± 0.6	19.7 ± 0.4	0.36 ± 0.01

[a] Means within a column that are followed by different letters are significantly different (protected LSD, $P < 0.05$).

[b] Data from site 2 were not subjected to statistical analysis because only escape trees were growing there.

using the recommendations of Aitchison (1984), indicated that there was no significant difference in composition between escape and general populations (Wilk's $\lambda = 0.3689$; $F_{10,4} = 0.6844$; $P > 0.715$). Nonparametric univariate analyses of α-pinene and limonene also revealed no significant differences between populations ($P > 0.05$). However, 4-allylanisole was significantly higher in trees from the general population ($\chi_1^2 = 9.66$, $P < 0.0339$). This result does not support the hypothesis that any negative effect of 4-allylanisole toward *D. frontalis* was partially responsible for tree escape (Table 2).

Resin flow on the other hand differed significantly between our tree populations grown on a common site. Over the two and a half years of the study, trees from the escape population produced significantly more

Table **2**

Mean concentration of chemical constituents measured in oleoresin of trees from escape and general populations. Due to the design of the plantings, all statistical analyses used data only from site 1. Data from site 2 is only included to increase the scope of the information

Site	Population	n	Chemical name	Chemical concentration	
				$\bar{x} \pm$ SEM	Range
	Escape	IX	α-Pinene	17.4 ± 0.72	12.99–24.53
			β-Pinene	12.4 ± 1.11	3.03–19.42
			Camphene	0.2 ± 0.01	0.16–0.36
			Limonene	1.9 ± 0.29	0.0X-4.35
			Myrcene	0.9 ± 0.10	0.36–2.27
			4-Allylanisole	0.9 ± 0.10	0.22-1.59
			Pimaric acid	4.9 ± 0.26	3.29-7.31
			Iso/Levo/Palm acid[a]	13.5 ± 0.82	8.X9-20.64
			Dehydroabietic	3.4 ± 0.25	2.05-5.66
			Abietic acid	18.6 ± 1.36	10.31–31.35
			Neoabietic acid	9.6 ± 0.45	6.72–13.68
			Total	X3.7	
	General	26	r-Pinene	17.3 ± 0.66	12.17-23.04
			β-Pinene	12.0 ± 0.84	4.21-x.71
			Camphene	0.2 ± 0.01	0.12–0.33
			Limonene	0.9 ± 0.1 x	0.10–2.96
			Myrcene	0.8 ± 0.12	0.20–2.57
			4-Allylanisole	1.4 ± 0.10	0.37–2.45
			Pimaric acid	4.2 ± 0.22	2.90-x.39
			Iso/Levo/Palm acid[a]	13.5 ± 0.85	5.53–28.30
			Dehydroabietic	4.1 ± 0.26	2.24–7.51
			Abietic acid	16.5 ± 0.x')	10.71–29.61
			Neoabietic acid	8.6 ± 0.33	5.8X-11.53
			Total	79.5	
	Escape	44	a-Pinene	17.9 ± 0.41	11.67–22.54
			β-Pinene	9.3 ± 0.34	4.22–14.25
			Camphene	0.2 ± 0.01	0.0x-0.27
			Limonene	0.9 ± 0.12	0.09–2.98
			Myrcene	0.7 ± 0.07	0.13–2.31
			4-Allylanisole	1.1 ± 0.08	0.24–2.51
			Pimaric acid	5.5 ± 0.21	3.3X-X.78
			Iso/Levo/Palm acid[a]	14.4 ± 0.53	7.47-23.4x
			Dehydroabietic	5.3 ± 0.28	2.52–10.44
			Abietic acid	19.7 ± 0.81	10.13–32.37
			Neoabietic acid	9.7 ± 0.30	5.X1-13.89
			Total	x4.7	

[a] Isopalmaric acid, levopalmaric acid and palmaric acid (Iso/Levo/Palm acid) were not separated during chemical analysis

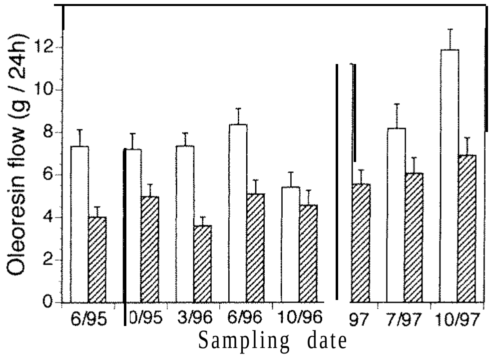

Fig. 1. Average flow of escapes vs. general trees for each sampling period (site 1 only; $n = 18$ for escape and 26 for general trees). Mean values of escape trees are shown by open bars, while mean values of general trees are shown by hatched bars. Error bars show one standard error of the mean.

oleoresin per 24 h ($\bar{x} = 8.34$ g) than did trees from the general population ([$\bar{x} = 5.07$ g], $F_{1,13} = 22.03$; $P = 0.0004$; Fig. 1). On average, escapes produced about 1.65× the resin flow of general population trees. Escapes yielded more resin at every sampling time (Fig. 1), suggesting that differences between populations are not affected in an important way by physiological changes across seasons (see Lorio and Sommers, 1986).

Correlation analysis was used to explore relationships between tree and oleoresin characteristics. Correlations of 4-allylanisole and α-pinene with oleoresin flow produced nonsignificant results ($P > 0.0034$, i.e., $P = 0.05$ adjusted for 15 tests); however, limonene was positively correlated with flow ($r = 0.50$; $P < 0.0001$). As expected, oleoresin flow also was positively correlated with LCR ($r = 0.37$; $P = 0.0004$). Tree dbh ($r = 0.31$: $P = 0.0032$) and height ($r = 0.45$; $P < 0.0001$) were positively correlated with the concentration of 4-allylanisole in the oleoresin. but neither limonene nor r-pinene was significantly correlated with any tree measurement ($P > 0.0034$).

Total chemical yield, i.e., the product of the mean α-pinene or 4-allylanisole concentration and oleoresin flow, was used to investigate the combined effects of chemical concentration and flow on the potential semiochemical bouquet at the wound site of each tree. Yield of x-pinene was significantly higher for escape trees ($\bar{x} = 1.23 \pm 0.10$ g) than general trees ($\bar{x} = 0.76 \pm 0.08$ g; Wilcoxon rank-sum test, $\chi_1^2 = 6.125$, $P = 0.0133$), suggesting that more r-pinene was available for release from escapes. Yield of 4-allylanisole in escape trees ($\bar{x} = 0.067 \pm 0.011$) did not differ significantly from general trees ($\bar{x} = 0.060 \pm 0.009$; $\chi_1^2 = 0.125$. $P = 0.7237$).

4. Discussion

The unique behavior of *D. frontalis* populations leads to large contiguous areas of tree mortality, making missed trees apparent and suggesting that random, extrinsic, or resistance factors may have played a role in their escape. The 'random miss hypothesis' has some merit and plausibility because of the apparent stochasticity in the pattern of tree attack as *D. frontalis* spots expand. However, this study strongly suggests that this is not generally the case. Escapes in this study, which were F_1's of actual survivors, yielded more oleoresin than general trees at all eight of the sampling periods (Fig. 1). Across all time periods, the average increase in flow was 1.65 ×, suggesting that escapes did not avoid *D. frontalis* colonization due to random chance. This supports the conclusions of previous studies that resin flow is an important defense mechanism against *D. frontalis* (Hodges et al., 1979; Cook and Hain, 1987; Lorio et al., 1995) and provides an estimate of the relative increase in flow necessary for trees to survive when surrounded by significant pressure from *D. frontalis*.

Families of loblolly pine have been shown to vary in oleoresin flow (Nebeker et al., 1992). This finding suggests that oleoresin flow in loblolly pine is at least partly influenced by genetic composition, a conclusion supported by the results of this study. To our knowledge, heritability for oleoresin flow has not been estimated for loblolly pine, but in slash pine (*Pinus elliottii* Engelm. var. *elliottii*) heritability of gum yield is estimated to be quite high (about 55%; Squillace and Bengtson, 1961). Accordingly, a tree improvement program designed specifically to increase gum yields of slash pine (for naval stores products) resulted in a gain of about 64% over unselected progeny after one generation of selection (McReynolds and Gansel, 1985); a result nearly identical to ours. Assuming similar values of heritability and variance of' flow between loblolly and slash pines, this suggests that *D. frontalis* escapes were subjected to a selection differential similar in magnitude to that used by tree breeders.

Although it is widely believed that oleoresin flow affects tree resistance, the mechanisms underlying this are complex and poorly understood. Besides the physical resistance offered by oleoresin (Hodges et al., 1979; Cook and Hain, 1987), its chemical constituents may have semiochemical (Renwick and Vité, 1969, 1970; Hayes et al., 1994a,b) and toxic (Coyne and Lott, 1976) effects on *D. frontalis*—neither of which is independent of flow. The differences in flow between escape and general trees, without differences in composition, could still result in differences in the quantity of airborne host compounds released from wounds or during *D. frontalis* attack. Semiochemical effects of oleoresin flow may be particularly important early in the attack process, when the ratio of aggregation pheromones to host-produced volatiles are dominated by the latter. As attacks accumulate and become numerous, insect-produced volatiles would increase as host-produced volatiles decreased (due to a reduced flow of oleoresin as the tree succumbs). The relationship between constitutive host chemistry and the semiochemistry of the attack process of *D. frontalis* has not been quantitatively described. These events are inherently complex, and are further complicated by induced resin flow (Ruel et al., 1998), stereochemistry (Grosman et al., 1997), and other factors. Additional research is necessary before the relationship between oleoresin flow and semiochemical bouquet, and thus semiochemical-based tree resistance, can be adequately described and properly understood.

Previous authors have suggested that susceptibility to bark beetles may be higher in trees with low concentrations of deterrent host chemicals (Heikkenen and Hrutfiord, 1965; Cobb et al., 1968; Bordasch and Berryman, 1977; Hayes et al., 1994a.b). Whether or not pine susceptibility to *D. frontalis* is affected by intrinsic concentrations of 4-allylanisole has been considered (Hayes et al., 1994a,b), but remains unevaluated. In this study we did not test this idea directly; however, we found no evidence to suggest that concentrations of 4-allylanisole in the oleoresin were involved in abetting tree escape from *D. frontalis*. In fact, trees in our general population had a significantly greater concentration of 4-allylanisole in the oleoresin than did our escapes. Because sufficient quantities of airborne 4-allylanisole are known to affect aggregation behavior of *D. frontalis* at traps (Hayes et al., 1994b; Strom et al., 1999), we evaluated further its potential semiochemical effects by estimating the total relative yield of 4-allylanisole potentially released to the air from our half-inch wounds. This estimate, though perhaps not a valid quantitative measure of what was released, does provide a relative measure for comparing individual components between our two tree populations which did not differ in oleoresin composition. Using these estimates, the total potential release of 4-allylanisole from escapes was slightly, but not significantly, higher (about 10%) than general trees. Therefore, the higher flow in escapes made up for the lower concentration of 4-allylanisole in their oleoresin, producing slightly more 4-allylanisole yield. With α-pinene, which was not significantly different in concentration between tree populations, the higher resin flow of escapes produced a significantly greater total yield (about 38%). Higher airborne levels of α-pinene increase attraction of *D. frontalis* to traps baited with synthetic aggregation pheromone (Billings, 1985), and it has been suggested that resistant trees would ideally have low concentrations of α-pinene (Nebeker et al., 1988); therefore, it is unlikely that α-pinene aided tree escape. Although more semiochemical experiments are required before our understanding of attack and attraction processes is complete, our results suggest it is unlikely that oleoresin composition affected survival of the escape trees.

With the exception of 4-allylanisole, none of the chemical constituents of oleoresin we measured differed between tree populations, indicating that oleoresin composition was not responsible for the trees being missed by *D. frontalis*. Previous studies have concluded that monoterpenes are not as important for *D. frontalis* resistance of southern yellow pines as oleoresin flow (Hodges et al., 1979; Cook and Hain, 1987), and our results support this conclusion. Monoterpenes are considered highly heritable in southern pines (Rockwood, 1973; Squillace et al., 1980). In fact, cortical monoterpenes are used as genetic markers in loblolly pine (Squillace et al., 1980). This implies that significant differences in oleoresin composition of parent escape trees likely would have been observed in F_1 progeny. In addition, we found no significant differences in resin acid concentrations between escape and general trees.

Resin acids typically make up >60% of loblolly oleoresin by weight (Hodges and Lorio, 1975; Table 2) and are thought to be the most highly toxic resin component to *D. frontalis* (Nebeker et al., 1992); however, we found no evidence to suggest that they had a significant role in the escape of these trees from *D. frontalis*.

Identifying, producing and planting high resin yielding loblolly pines could be an important part of a multifaceted silvicultural approach for reducing both economic and ecological damage from *D. frontalis*. Forests in the southeastern US are quite different from what they were prior to European settlement, and *D. frontalis* outbreaks have become more extensive and damaging in recent decades. Although native to the southeastern US, *D. frontalis* is not native to the forests that now grow there. We believe that strategies to prevent unacceptable damage from *D. frontalis* over long periods of time and large geographic areas must emphasize silviculture to be reasonable and effective. We, and others (e.g., Nebeker et al., 1988, 1992), envision that effective silviculturally-based approaches will include multiple complementary strategies, each being integrated with primary management objectives. Many forest management decisions that impact *D. frontalis* will necessarily be implemented over long time periods, but in the mean time, important strides may be made by planting more resistant individuals and striving for more resistant communities.

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