Cortical Monoterpene and Fusiform Rust Resistance Relationships in Slash Pine

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ABSTRACT

Monoterpene composition of branch cortex oleoresin and fusiform rust resistance of slash pine were compared in six studies. Correlations between monoterpene content and rust resistance were generally variable, but a consistent relationship was observed for β-phellandrene. High amounts of β-phellandrene may be genetically, as well as phenotypically, related to resistance. Suggestions for potential use of β-phellandrene for increasing rust resistance of slash pine and examples of these applications are given.

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Indirect selection for resistance to fusiform rust (caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm.) on the basis of the cortical monoterpene, β-phellandrene, has been suggested for loblolly pine, *Pinus taeda* L. (5). Fusiform rust is also a major disease of slash pine, *P. elliottii* Engelm. var. *elliottii,* and identification of resistance via characterization of a correlated trait would be a valuable tool to aid in selection for increased rust resistance in this species. Reported below are relationships among the branch cortex monoterpenes and fusiform rust resistance of slash pine in Georgia, Florida, and Mississippi and suggestions for application.

PLANT MATERIALS AND METHODS.–Six sets of material were utilized: (i) Fifty-two 11- to 17-yr-old resistant, intermediate, or susceptible clones (UF) were sampled in an orchard at Gainesville, Florida. The rust value given to each clone was a composite over several tests with a clone's score in a test being its deviation from check lots divided by the standard deviation of the test. Oleoresin samples were collected from one ramet of each clone.

(ii) Twenty-five wind-pollinated families (G-49) in a 10-yr-old seed source study established in Lake City, Florida, Gulfport, Mississippi, and Macon, Georgia, were utilized. Each family was either uniformly resistant or susceptible at least at Lake City and Macon, and rust values were calculated as for the UF clones. Oleoresin samples were taken from three trees in each family at Lake City.

(iii) Thirteen full-sib families were analyzed in a 6-yr-old progeny test near Ludowici, Georgia. Each sample tree was assigned a "c-score" value. C-score is the severity index used by Blair (1) for rating a tree by the number of stem and branch galls present and ranges from 1 (no stem or branch galls) to 10 (dead or with multiple stems due to infection). Ten trees per family were sampled for oleoresin.

(iv) Ten trees in a known susceptible wind-pollinated family in a 3-yr-old rust test at Bainbridge, Georgia, were sampled for oleoresin.

(v and vi) Pairs of trees (each pair consisting of an infected and uninfected tree in close proximity) in 12- to 14-yr-old 90+% rust-infected plantations of unknown seed source at Capps and Greenville, Florida, were sampled. Nine pairs were sampled at Capps, six pairs at Greenville. Each tree was given a c-score value.

In all cases, oleoresin samples were taken by excising vigorous branch tips, and the oleoresin exuding from cortical tissues was collected in capillary tubes. The tubes were deposited in glass vials which were subsequently sealed with Teflon Tape and screw caps. Samples were frozen until analysis.

Analyses of monoterpene contents were conducted on a Microtek GC 2000-R chromatograph under the following representative conditions: injection port, 180 C; column, 115 C; detector, 190 C; helium flow, 25 cc/min; hydrogen flow, 60 cc/min; air flow, 283 cc/min; dual 3.048m by 6.35mm (10 ft by 0.25 in) copper columns with 20% Carbowax 20M on Chromosorb W 80- to 100-mesh; sample size, 0.5 μliter oleoresin diluted with pentane. The content of each monoterpene was expressed as a percent of total monoterpenes.

RESULTS.–Four monoterpenes were major constituents in the sample trees: α-pinene, β-pinene, myrcene, and β-phellandrene (Table 1). Such monoterpene contents are as expected for slash pine from the northern part of the range for the species (A. E. Squillace, *unpublished*).

Higher β-phellandrene content was common to the resistant or uninfected category in each study except Greenville (Table 1 and 2). High β-phellandrene was significantly correlated phenotypically with resistance in three studies, while the difference between resistant and susceptible groups for β-phellandrene was significant only in the UF study. Also, for 945 trees from 54 seed sources in the G-49 plantation at Lake City, trees containing high β-phellandrene were less

TABLE 1. Monoterpene characterization relative to fusiform rust resistance for families in four slash pine studies

Study	Rust category	Avg monoterpene content[a] (% of total monoterpenes)				Correlation between β-phellandrene and rust resistance[b]
		α-pinene	β-pinene	myrcene	β-phellandrene	
UF[c]	Resistant	27.9	50.9	1.0 s	19.6 s	
	Intermediate	30.0	50.7	7.9 st	10.6	0.31*
	Susceptible	26.4	51.8	9.6 t	12.2	
G-49[d]	Resistant	27.0 s	43.9	12.1	14.0	
	Susceptible	37.6	46.3	4.8	10.6	0.43*
Ludowici[e]	Resistant	33.6	46.1	3.5	15.9	
	Susceptible	36.2	47.9	1.7	13.4	0.22
Bainbridge[f]	Susceptible	33.3	58.2	1.8	1.8	—

[a]For each monoterpene, any means in a study not sharing the same letter are significantly different ($P = 0.05$) based on unpaired *t*-tests.
[b]Phenotypic correlation between resistance evaluation and β-phellandrene content for all families in a study.
[c]Eleven- to 17-year-old clones with 20, 12, and 20 clones in the resistant, intermediate, and susceptible categories, respectively.
[d]Ten-year-old wind-pollinated families, three trees per family, with 11 and 14 families in the resistant and susceptible categories, respectively.
[e]Six-year-old full-sib families, 10 trees per family, with four and nine families in the resistant and susceptible categories, respectively.
[f]10 trees in a 3-year-old wind-pollinated family.
* = Significant difference, $P = 0.05$.

TABLE 2. Monoterpene characterization relative to fusiform rust resistance for individuals in three slash pine studies

Study	Rust category	No. of trees	Avg monoterpene content[a] (% of total monoterpenes)				Correlation between β-phellandrene and rust resistance[b]
			α-pinene	β-pinene	myrcene	β-phellandrene	
Ludowici[c]	Uninfected	46	35.0	45.7	1.4	15.3	.19*
	Infected	84	35.6	48.3	1.8	13.6	
Capps[d]	Uninfected	9	27.0	48.1	1.7 s	22.6	.36
	Infected	9	20.7	49.7	14.3	14.6	
Greenville[d]	Uninfected	6	24.7	50.3	11.5	12.8	−.23
	Infected	6	27.7	49.4	1.3	18.4	

[a]For each monoterpene, any means in a study not sharing the same letter are significantly different ($P = 0.05$) based on unpaired *t*-tests for Ludowici and paired *t*-tests for Capps and Greenville.
[b]Phenotypic correlation between c-score and β-phellandrene content for all trees in a study.
[c]Six-years-old.
[d]Twelve- to 14-years-old.
* = Significant, $P = 0.05$.

infected, although not significantly, than other trees.

Other monoterpenes were occasionally significantly associated with resistance. Alpha-pinene was lower for the resistant families in the G-49 study (Table 1), and the phenotypic correlation was −0.54. Myrcene content was lower in the resistant clones in the UF study (Table 1) and in the uninfected trees at Capps (Table 2), and myrcene was correlated with resistance in those two studies, −0.36 and −0.55, respectively. The correlation between β-pinene and resistance for individual trees at Ludowici was −0.21.

DISCUSSION.—The overall consistency of the association between high β-phellandrene and fusiform rust resistance was notable. Especially relevant is the significantly higher β-phellandrene level in the resistant families in the UF study since the families in the study have been repeatedly tested for resistance. Also, the significant correlation in the G-49 data is relatively important because the families have been tested at least twice. The phenotypic correlations from the UF, G-49, and Ludowici studies were based on family means and consequently are the best available indication of the genetic correlation which may exist between β-phellandrene and resistance.

Assignment of the level of β-phellandrene that may indicate resistance is difficult. Oleoresin samples for the six studies were collected at different dates, and time of sampling can influence β-phellandrene content although no clear seasonal pattern has been established (10). At any given data of sampling, however, differences among trees remain relatively constant. The UF samples were collected in March, and 15 of the 20 resistant clones had β-phellandrene contents of 14% or more.

Rationalization for the high cortical β-phellandrene and resistance relationship in slash pine is not evident. In loblolly pine, β-phellandrene, the one monoterpene which had potential as a marker for fusiform rust resistance, commonly occurred in quantities of less than 8% in resistant families (5). Dissimilar patterns for β-phellandrene and relative resistance exist in the four major southern pines: shortleaf pine, *P. echinata* Mill. - high β-phellandrene (52% – A. E. Squillace, *personal communication;* 43% – D. L. Rockwood, *unpublished*), highly resistant; longleaf pine, *P. palustris* Mill. – virtually no β-phellandrene (4), resistant; loblolly pine – low β-phellandrene (8%) (6), susceptible; and slash pine – intermediate β-phellandrene (13%) (9), highly susceptible. However, established geographic trends indicate that slash pines in the northern part of the species' range have lower amounts of β-phellandrene (A. E. Squillace, *unpublished*) and incidence of fusiform rust is greater in this area. As observed here, high β-phellandrene slash pine from the northern part of the range tend to be more resistant than low β-phellandrene trees.

Specific tests of the effects of monoterpenes on fungi and insects suggest that β-phellandrene is not the most growth-limiting monoterpene of slash pine. Myrcene and limonene were more inhibitory than β-phellandrene on the growth of *Fomes annosus* and four species of *Ceratocystis* (2). Limonene was slightly more inhibitory than myrcene to the growth of some wood-inhibiting fungi, while myrcene was more inhibitory than either α-pinene or β-pinene (3). Limonene was more toxic to bark beetles than myrcene, which was more toxic than α-pinene or β-pinene (8). Thus, it appears unlikely that slightly higher concns of β-phellandrene would be responsible for greater resistance to fusiform rust.

Concentrations of monoterpenes may be associated with other characteristics which determine resistance to fusiform rust. If monoterpenes are genetically linked with controlling traits, rather than being causal components, they may still be useful for increasing resistance. Thus, the importance of well-substantiated genetic correlations among the monoterpenes, particularly β-phellandrene, and resistance is increased.

One application of monoterpenes to improvement in rust resistance is indirect selection of resistant trees. On the basis of having a high β-phellandrene level, for example, a presumably resistant slash pine may be selected. Only select trees would subsequently be further tested with successfully tested trees then being utilized in breeding programs.

The Greenville and Capps data were examples of indirect selection of individual trees. Fusiform rust incidence was 90+% in the Greenville and Capps plantations, and the uninfected trees were presumably resistant. However, only in the Capps sample was high β-phellandrene common to the uninfected trees.

A more conservative approach, in view of current knowledge, would utilize monoterpene contents of families rather than individual trees. Direct selection for resistance using progeny tests of select trees or families for selection can be moderately productive (7). High β-phellandrene in a family could be an indication of resistance and could serve as a supplement to actual performance of the family in rust tests to further verify resistance.

The Bainbridge study was a successful application of indirect selection of families (Table 1). The family sampled was highly susceptible in previous rust tests and at Bainbridge. As expected from the UF, G-49, and Ludowici observations, it had low β-phellandrene content. The family would thus be eliminated as a source of rust resistance.

Selection on the basis of monoterpene content could lead to vertical, rather than horizontal, resistance if a monoterpene were genetically associated with a specific resistance mechanism, or if a monoterpene were a causal agent for resistance. The experimental material utilized here was chosen, when possible, for uniform resistance or susceptibility in a number of tests, often established over a large geographic area. Resistance to fusiform rust on many widely distributed sites, instead of resistance in a restricted region, is a major objective of resistance breeding in slash pine.

Effective indirect selection requires a relatively low heritability for the desired trait, a high heritability for the correlated trait, and a strong genetic correlation between the two traits. The heritability of rust resistance in slash pine is very low for individuals and intermediate for families (7). The heritability of β-phellandrene composition is high (9). Based on present data, the genetic correlation between the two traits may be strong enough to justify efforts at indirect selection. Indirect selection potentially has a time advantage over direct selection in that it may identify resistance almost immediately while direct selection, based on evaluation of rust symptoms, commonly requires several months to several years.

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