Monoterpene-Fusiform Rust Relationships in Loblolly Pine

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ABSTRACT

Correlations among the monoterpenes in the stem xylem and branch cortex of loblolly pine and fusiform rust resistance are reported. Low amounts of branch cortex β-phellandrene were phenotypically and genetically associated with resistance. Indirect selection for rust resistance on the basis of low content of β-phellandrene may be more productive than direct mass selection.

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The use of genetic markers to select for resistance to the fusiform rust fungus, *Cronartium fusiforme* Hedgc. & Hunt ex Cumm., would be profitable since selection on the basis of rust symptoms is uncertain. Fatty acids in the cortex oleoresin of mature loblolly pine, *Pinus taeda* L., have been suggested as possible markers for resistance (10). Monoterpenes were inhibitory to several fungi which attack ponderosa pine, *P. ponderosa* Laws. (2). Hare (5) found that trees in geographic locations where loblolly pine is resistant to fusiform rust differed from trees in locations where it is susceptible in content of two stem xylem monoterpenes. This paper reports the relationships among monoterpenes in stem xylem and branch cortex oleoresins and fusiform rust resistance in loblolly pine in southwestern Georgia.

MATERIALS AND METHODS.–Plant materials were taken from the Loblolly Pine Heritability Study, which is maintained cooperatively by International Paper Company and North Carolina State University on International Paper Company's Southlands Experiment Forest near Bainbridge, Georgia. Stonecypher (9) presented a complete description of the design and establishment methods employed.

Thirty-six full-sib families, obtained by crossing each of 12 males with three different females, were sampled. Stem xylem oleoresin was taken from 346 5-year-old trees in September, 1969 (two to five trees per family in each of two replications). The same trees, plus 11 more, were sampled for branch cortex oleoresin in March, 1971. Two fusiform rust measurements, c-score, and percentage of trees infected, were taken at the end of the sixth growing season. C-score is the severity index used by Blair (1) for rating an individual 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).

Stem xylem oleoresin was captured in a one-dram glass vial secured in a hole drilled into the stem xylem. Branch cortex oleoresin from an excised branch tip was collected in a capillary tube and deposited in a glass vial. Sampling height was approximately 4.5 ft for the stem samples and 5.0 ft for the branch samples. All vials were tightly sealed. Xylem samples were refrigerated until analysis; cortex samples were frozen.

Oleoresin samples were analyzed by gas-liquid chromatography. Representative operating conditions on the F&M Model 700 Chromatograph were: copper column, 8 ft by 0.25 inch; support, Chromosorb P AW/DCMS 80-100 mesh; substrate, 20% Carbowax 20M; injection port, 170 C; column, 125 C; detector, 150 C; helium flow, 25 ml/min; sample size, 1 μliter oleoresin dissolved in an equal volume of ethyl ether. Each monoterpene was expressed as a percentage of total monoterpenes.

Phenotypic correlations were made and tested according to Steel & Torrie (8). Genetic correlations, which indicate genetic associations among the characteristics (free of environmental influences), were based on components derived from analyses of variance and covariance. For example, the genetic correlation ($r_{a_{xy}}$) between monoterpene level x and fusiform rust measurement y was calculated as follows:

$$r_{a_{xy}} = \frac{\sigma_{m_{xy}}}{\sqrt{\sigma^2_{m_x} \cdot \sigma^2_{m_y}}}$$

where $\sigma_{m_{xy}}$ was the covariance component due to males and $\sigma^2_{m_x}$ and $\sigma^2_{m_y}$ were the variance components due to males for monoterpene x and rust measurement y, respectively. Genetic correlations were subsequently used in indirect selection formulae as described by Falconer (3).

RESULTS AND DISCUSSION.–No stem xylem monoterpene was phenotypically correlated with rust. Genetic correlations among the stem xylem monoterpenes and rust were weak to moderate. The standard errors of the genetic correlations were large, indicating a low precision of estimation (Table 1)

TABLE 1. Genetic correlations, r_a, and phenotypic correlations, r_p, among stem xylem and branch cortex monoterpenes and indices of fusiform rust infection in loblolly pine

	C-score[c]		Percent infection[d]	
	r_a	r_p	r_a	r_p
Stem xylem monoterpenes:[a]				
α-pinene	−.29 ± .45[e]	−.03	−.45 ± .44	−.17
β-pinene	.23 ± .42	.04	.43 ± .40	.21
myrcene	−.24 ± .43	−.05	−.36 ± .42	−.19
Branch cortex monoterpenes:[b]				
α-pinene	−.81 ± .75	−.04	−.94 ± .70	−.13
β-pinene	−.20 ± .43	−.05	−.45 ± .39	.01
myrcene	.37 ± .45	−.01	.54 ± .50	−.04
limonene	.08 ± .39	.02	.10 ± .42	.05
β-phellandrene	.54 ± .32	.15**	.78 ± .26	.28*

[a] 346 5-year-old trees.
[b] 357 6-year-old trees.
[c] Rust severity index, expressed as $\sqrt{\text{c-score} + 1}$.
[d] Based on 72 plot means, expressed as arcsin $\sqrt{\text{percent}}$.
[e] Standard error of genetic correlation, based on techniques presented by Mode & Robinson (6) and Grossman (4).
* and ** indicate significance at the 5% and 1% level, respectively.

The tendency for resistant trees to be lower than susceptible trees in stem xylem β-pinene was opposite the observation of Hare (5).

Branch β-phellandrene was phenotypically related in an inverse manner to rust resistance. The more resistant families had β-phellandrene contents of less than 8% (Table 2).

The high genetic correlations of branch cortex α-pinene with c-score and percentage infection, −.81 and −.94, respectively, were questionable since the precision of the estimates was very low. Furthermore, these two values were probably inflated by a small and nonsignificant variance component for α-pinene.

Branch cortex β-phellandrene had genetic correlations that were estimated with more precision. The values suggest that low amounts of β-phellandrene are associated genetically with resistance of loblolly pine to fusiform rust.

Gains in resistance through selecting trees on the basis of their monoterpene content were compared to the gains possible by phenotypically selecting resistant trees. A simple mass selection scheme was assumed, with six of the 12 males selected on the basis of observable rust symptoms. Four of the 12 males were selected on the basis of desirable monoterpene content. Branch cortex β-phellandrene was the only monoterpene for which the indirect selection gain in resistance surpassed the gain from direct selection.

The branch cortex monoterpenes of loblolly pine may be sampled easily and analyzed quickly. All are remarkably consistent regardless of environment and time, and some are highly heritable and may be evaluated in young trees (7). The utility of the monoterpenes as markers depends on the genetic correlations with resistance, and the correlation between β-phellandrene and rust is promising. However, further work is needed to substantiate the genetic correlations reported here.

Branch cortex tissues are closely associated with the initial colonization of *C. fusiforme*. Consequently, the branch cortex monoterpenes are more likely to be involved in resistance than are the stem xylem monoterpenes. The reported correlations further support this hypothesis.

A potential application suggested by these findings is indirect selection for fusiform rust

TABLE 2. Relationship between branch cortex β-phellandrene and fusiform rust resistance in loblolly pine as evidenced by the progenies of 12 males

Male group[a]	Average β-phellandrene content[b] (%)	Average rust infection[c] (%)
2	3.3	36.7
4	6.4	20.0
5	12.4	43.3
6	6.7	33.3
18	10.1	50.0
23	5.0	6.7
24	12.2	53.3
26	7.9	13.3
32	11.5	36.7
41	11.5	43.3
45	2.7	36.7
58	4.3	17.5

[a] Three full sib families derived from crossing the male with three females, 28 to 30 trees per male group.
[b] β-phellandrene content for each tree in a male group was expressed as a percentage of total monoterpenes.
[c] For each family, percentage of trees infected was calculated for each of two replications (four or five trees per replication).

resistance, such as a screening technique for field selections of loblolly pine. Assessment of branch cortex monoterpene composition could identify trees low in β-phellandrene and presumably rust resistant. Field testing, or other methods of rust evaluation, would be necessary to verify resistance in the low β-phellandrene trees.

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