Cortical Monoterpene and Fusiform Rust
Resistance Relationships in Slash Pine

D. L. Rockwood

Research Associate, Forest Physiology-Genetics Laboratory, University of Florida, Gainesville 32611.
The author gratefully acknowledges the cooperation of D. R. Roberts, Project Leader, Naval Stores Project;
and that of A. E. Squillace, Chief Plant Geneticist at the Southeastern Forest Experiment Station, Forest
Service, USDA, Oustee, Florida, for providing chromatographic facilities and experimental data from a slash
pine seed source study, respectively. The author also thanks the Container Corporation of America,
Continental Can Company, International Paper Company, and the St. Joe Paper Company for their kind
provision of experimental material.
Journal Series Paper No. 5042 of the Florida Agricultural Experiment Station.
Accepted for publication 8 February 1974.

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 \( \beta \)-phellandrene. High amounts
of \( \beta \)-phellandrene may be genetically, as well as
phenotypically, related to resistance. Suggestions for
potential use of \( \beta \)-phellandrene for increasing rust resistance
of slash pine and examples of these applications are given.
Phytopathology 64:976-979.

Additional key words: Cronartium fusiforme, Pinus elliottii var. elliottii, indirect selection.

Indirect selection for resistance to fusiform rust
( caused by Cronartium fusiforme Hedge & Hunt ex
Cumm.) on the basis of the cortical monoterpene, 
\( \beta \)-phellandrene, has been suggested for lobolly 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 monoterpene 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 3.048 mm by 6.35 mm (10 ft
by 0.25 in) copper columns with 20% Carbowax 20M
on Chromosorb W 80- to 100-mesh; sample size, 0.5
\( \mu \)lter oleoresin diluted with pentane. The content of
each monoterpene was expressed as a percent of total
monoterpenes.

RESULTS.—Four monoterpene were major
constituents in the sample trees: \( \alpha \)-pinene, \( \beta \)-pinene,
myrcene, and \( \beta \)-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 \( \beta \)-phellandrene content was common to the
resistant or uninfected category in each study except
Greenville (Table 1 and 2). High \( \beta \)-phellandrene was
significantly correlated phenotypically with resistance
in three studies, while the difference between
resistant and susceptible groups for \( \beta \)-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 \( \beta \)-phellandrene were less
TABLE 1. Monoterpene characterization relative to fusiform rust resistance for families in four slash pine studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Rust category</th>
<th>Avg monoterpene contenta (% of total monoterpenes)</th>
<th>Correlation between β-phellandrene and rust resistanceb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>α-pinene</td>
<td>β-pinene</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>27.9</td>
<td>50.9</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>30.0</td>
<td>50.7</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>26.4</td>
<td>51.8</td>
</tr>
<tr>
<td>G-49d</td>
<td>Resistant</td>
<td>27.0 s</td>
<td>43.9</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>37.6</td>
<td>46.3</td>
</tr>
<tr>
<td>Ludowicié</td>
<td>Resistant</td>
<td>33.6</td>
<td>46.1</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>36.2</td>
<td>47.9</td>
</tr>
<tr>
<td>Bainbridgef</td>
<td>Susceptible</td>
<td>33.3</td>
<td>58.2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Rust category</th>
<th>No. of trees</th>
<th>Avg monoterpene contenta (% of total monoterpenes)</th>
<th>Correlation between β-phellandrene and rust resistanceb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>α-pinene</td>
<td>β-pinene</td>
<td>myrcene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ludowicié</td>
<td>Uninfected</td>
<td>46</td>
<td>35.0</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>84</td>
<td>35.6</td>
<td>48.3</td>
</tr>
<tr>
<td>Cappsd</td>
<td>Uninfected</td>
<td>9</td>
<td>27.0</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>9</td>
<td>20.7</td>
<td>49.7</td>
</tr>
<tr>
<td>Greenvilled</td>
<td>Uninfected</td>
<td>6</td>
<td>24.7</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>6</td>
<td>27.7</td>
<td>49.4</td>
</tr>
</tbody>
</table>

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 monoterpenone 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 monoterpenones on fungi and insects suggest that β-phellandrene is not the most growth-limiting monoterpenone 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-inhabiting 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 concen of β-phellandrene would be responsible for greater resistance to fusiform rust.

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

One application of monoterpenones 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 the Capps sample was high β-phellandrene common to the uninfected trees.

A more conservative approach, in view of current knowledge, would utilize monoterpenone 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 monoterpenone content could lead to vertical, rather than horizontal, resistance if a monoterpenone were genetically associated with a specific resistance mechanism, or if a monoterpenone 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.

**LITERATURE CITED**


