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WITHIN-TREE VARIATION OF MONOTERPENE HYDROCARBON COMPOSITION OF SLASH PINE OLEORESIN

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Abstract—Within-tree variation of monoterpene hydrocarbons in oleoresin from four 19-year-old slash pine trees (*Pinus elliottii* Engelm. var. *elliottii*) was analyzed by gas–liquid chromatography. Oleoresins from needles, branch cortex, branch xylem, trunk xylem, and root xylem all differed from one another in composition. Needle and branch cortex oleoresins resembled each other more than either resembled the xylem oleoresins. The percentages of certain xylem oleoresin components varied with height of sampling point above the ground. Significant differences in oleoresin composition also occurred among annual rings of the same tree.

INTRODUCTION

EARLY research on monoterpene hydrocarbon composition of oleoresins, such as the studies reported by Mirov,[1] utilized distillation procedures. This method is limited in number of fractions which can be separated and requires large oleoresin samples. Most early work involved composite samples and more attention was given to oleoresin composition for each species than to variation within species.

Since the development of gas chromatography, analyses of oleoresin monoterpenes are much simpler, faster, more accurate and require much smaller samples than do distillation procedures. In recent years, several researchers[2–9] analyzed oleoresin from individual pine trees to determine intraspecific differences in monoterpene hydrocarbon composition and to determine effects of environment on trees from different parts of the species range. There is also much interest in the relationship between the incidence of insect attacks and the monoterpene hydrocarbon composition of oleoresin in individual trees.

Differences in composition of oleoresin from different types of tissue in the same tree have been reported. Squillace and Fisher[8] found differences in oleoresin composition in both branch cortex and trunk xylem among several genetic families of slash pine (*Pinus elliottii* var. *elliottii*). Cortical oleoresins of western white pine (*P. monticola*) from several seed sources

[1] N. T. MIROV, *U.S. Dep. Agr. Tech. Bull.* 1239 (1961).
[2] M. H. BANNISTER, A. L. WILLIAMS, I. R. C. MCDONALD and M. B. FORDE, *New Zealand J. Sci.* **5**, 486 (1962).
[3] RICHARD H. SMITH, *Forest Sci.* **13**, 246 (1967).
[4] RICHARD H. SMITH, Pacific Southwest Forest and Range Exp. Sta., *U.S. Forest Serv. Res. Note PSW*-135 (1967).
[5] JAMES W. HANOVER, *Phytochem.* **5**, 713 (1966).
[6] JAMES W. HANOVER, *Forest Sci.* **12**, 447 (1966).
[7] JAMES W. HANOVER, *Heredity* **21**, 73 (1966).
[8] A. E. SQUILLACE and G. S. FISHER, North Central Forest Exp. Sta., *U.S. Forest Serv. Res. Paper NC*-6, 53 (1965).
[9] M. B. FORDE and M. M. BLIGHT, *New Zealand J. Bot.* **2**, 44 (1964).

planted in three locations were analyzed by Hanover.[5] He found no effect of planting site on monoterpene hydrocarbon composition of oleoresins.

Smith[10] found large differences in monoterpene hydrocarbon composition of oleoresin among ponderosa pine trees (*P. ponderosa*) but only minor variation within individual trees. He also found little difference in oleoresin composition from different annual rings of individual ponderosa pines.[11] Blight and McDonald[12] reported little variation in composition of oleoresin in the trunk xylem from one position to another in individual Monterey (*P. radiata*) and bishop (*P. muricata*) pine trees. Work by Zavarin[13] and Zavarin and Snajberk[14] indicated that the composition of cortical monoterpenes was quite consistent from one part of a fir (*Abies* sp.) trunk to another but minor differences occur in the upper portion.

The purpose of this study was to clarify the within-tree variation in oleoresin composition in equal aged trees of common genetic origin.

RESULTS

Samples came from four 19-year-old slash pine trees (*Pinus elliottii* Engelm. var. *elliottii*) of known parentage growing at Olustee, Florida and previously wounded for naval stores production. Two of these trees were full sibs, known to have high percentages of myrcene and β-phellandrene in their branch cortex oleoresin (high-β-phellandrene trees). The other two trees, also full sibs but with different parents, had extremely small amounts of myrcene and β-phellandrene in the branch cortex oleoresin (low-β-phellandrene trees). Samples from needles, branch cortex, branch xylem, trunk xylem, and root xylem were analyzed by gas–liquid chromatography (GLC). The trees of each pair did not differ significantly in any chemical component at the 0·05 probability level. Therefore data for each pair were averaged for comparison.

Variation by Tissue Types

The monoterpene hydrocarbon composition of oleoresins in needles and branch cortex in high-β-phellandrene trees were similar to each other and quite different from xylem oleoresin (Table 1). Lawrence and Brown[15] reported a similar relationship for resin acids of slash pine oleoresin. They found composition of needle and cortex oleoresins similar to each other and quite different from composition of xylem and phloem oleoresins.

Although needle and branch cortex oleoresins had similar α-pinene and myrcene percentages, they differed in β-pinene and β-phellandrene content. Branch, trunk and root xylem oleoresins were dissimilar and some chemical components were several times greater in one tissue than in another. Myrcene content was too small for accurate measurement in trunk xylem. Neither β-phellandrene nor myrcene was present in measurable amounts in root xylem.

For the low-β-phellandrene trees, no tissues contained sufficient amounts of myrcene and β-phellandrene for accurate measurements (Table 1). Since α-pinene and β-pinene were essentially the only components, their percentages are inversely correlated. In these trees also, needle and branch cortex oleoresins were more nearly alike than either was like the xylem oleoresins. In all xylem oleoresins, α-pinene percentage was greater than β-pinene percentage,

[10] R. H. SMITH, *Science* **143**, 1337 (1964).
[11] R. H. SMITH, *Nature* **202**, 107 (1964).
[12] M. M. BLIGHT and I. R. C. MCDONALD, *New Zealand J. Sci.* **7**, 212 (1964).
[13] E. ZAVARIN, *Phytochem.* **7**, 92 (1968).
[14] E. ZAVARIN and K. SNAJBERK, *Phytochem.* **4**, 141 (1965).
[15] R. H. LAWRENCE, Jr., and C. L. BROWN, *Plant Physiol.* **43**, S-25 (1968).

but the reverse was true for needle and branch cortex oleoresins. Xylem oleoresins were more similar to each other in the low- than in the high-β-phellandrene trees.

TABLE 1. MONOTERPENE HYDROCARBON CONTENT OF DIFFERENT TISSUES OF HIGH- AND LOW-β-PHELLANDRENE TREES

Tissue†	Percentages of major components*			
	α-Pinene	β-Pinene	β-Phellandrene	Myrcene
High-β-phellandrene trees				
Needles	21b‡	46a	14a	17a
Branch cortex	20b	38b	23c	18a
Branch xylem	13a	31c	52d	2b
Trunk xylem	43c	34c	20b	T
Root xylem	82d	17d	T§	T
Low-β-phellandrene trees				
Needles	24a	73a	T	T
Branch cortex	36b	62b	T	T
Branch xylem	58d	40d	T	T
Trunk xylem	59d	37d	T	T
Root xylem	53c	46c	T	T

* Each value is the mean of several (16 to 224) samples.

† Samples came from each of two trees at three crown levels and at three trunk levels; root xylem samples came from four lateral roots per tree.

‡ Figures in each column followed by the same letter are not significantly different at the 0·05 probability level.

§ T indicates trace amounts.

Variation by Height Above Ground

The monoterpenes of oleoresin in the trunk xylem changed with height above the ground in all four trees (Table 2). These changes were greater in high- than in low-β-phellandrene trees.

TABLE 2. MONOTERPENE HYDROCARBON COMPOSITION OF XYLEM OLEORESIN FROM THREE TRUNK LEVELS

Height above ground (ft)	Percentages of major components*		
	α-Pinene	β-Pinene	β-Phellandrene
High-β-phellandrene trees			
1	71	21	6
16	31	40	26
31†	26	40	27
Low-β-phellandrene trees			
1	54	41	T‡
16	59	36	T
31	64	33	T

* Each value is an average for two trees.

† Some cores from the 31 ft level contained enough camphene to affect the total percentage.

‡ T indicates trace amounts.

In high-β-phellandrene trees, differences were greatest at the base. Alpha-pinene percentage decreased and the other two components increased with height above the ground. There was a reverse trend in low-β-phellandrene trees—the α-pinene percentage of trunk xylem oleoresin increased and β-pinene decreased slightly with height above the ground. In the trunk xylem, as well as in other tissues, the range of variability was greater in high- than in low-β-phellandrene trees.

The percentages of trunk xylem oleoresin from near the base of the tree were more similar to the averages for root xylem oleoresin than to the average trunk values in Table 1. However, root xylem oleoresin was significantly different, at the 0·01 probability level, from trunk xylem 1 ft above the ground in three of the four trees. In one low-β-phellandrene tree, the oleoresins from these two tissues were similar.

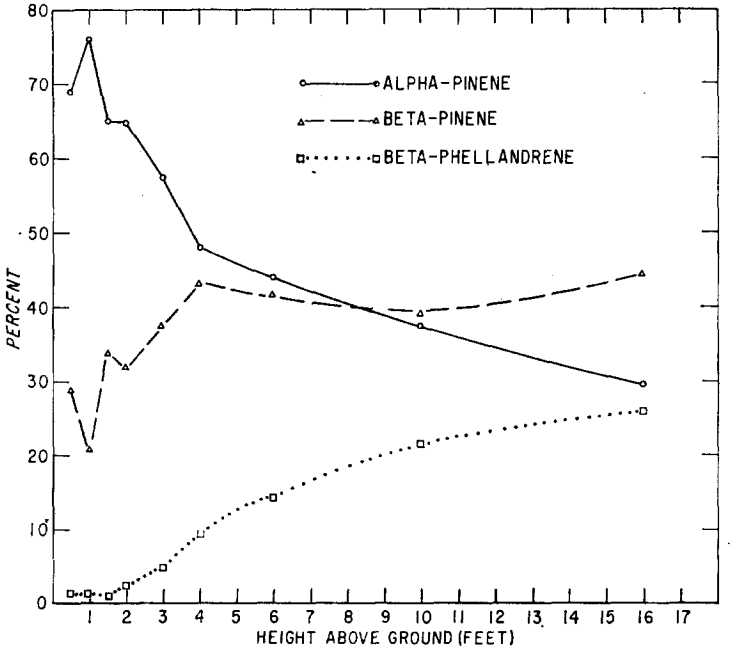

FIG. 1. CHANGES IN MONOTERPENE HYDROCARBON COMPOSITION OF TRUNK XYLEM OLEORESIN (IN ONE HIGH-β-PHELLANDRENE TREE).

Figure 1 shows a plot of an intensive sampling of trunk xylem in one β-phellandrene tree. Alpha-pinene percentage decreases and β-phellandrene increases with height from 2 to 16 ft above ground. Beta-pinene shows some variation but remains at approximately the 40 per cent level.

Some of the variation in trunk xylem oleoresin with height may result from naval stores wounding. However, evidence from some preliminary research[16] indicates that oleoresin composition is changed on the wounded side but not on the unwounded backside. Additional work is needed to establish whether wounding affects monoterpene composition on the unwounded side.

[16] DONALD R. ROBERTS, unpublished data.

Variation by Age—Annual Rings

Results from sampling the five youngest (nearest the bark) and the oldest annual rings of each increment core indicated differences in oleoresin composition among annual rings within some cores. In no case did the annual ring samples show a consistent trend (from high-to-low or low-to-high percentage) for a component the entire length of a particular core.

A study was made of the composition of paired cores selected as examples of the amount of variation found in high- and low-β-phellandrene trees. Gross statistical analyses indicated differences among rings, at the 0·05 probability level, for all chemical components except β-pinene in the high-β-phellandrene tree. Core values differed significantly, at the 0·05 probability level, only for β-pinene and β-phellandrene in the high-β-phellandrene tree. Although sampling intensity was too low to pinpoint which rings differed significantly from others, the oldest ring appears to differ from most of the younger ones. Differences in oleoresin composition between the two cores occurred in high-β-phellandrene trees only. These results contrast sharply with the lack of variation among annual rings of *P. ponderosa* reported by Smith.[11]

Variation by Age—Branch Cortex and Needles

Relatively large between-sample differences were present in the needle and branch data, especially in high-β-phellandrene trees. This variation tended to mask difference in monoterpene composition among crown levels and between branches at each level. No differences were detected in needle oleoresin by internode, branch or crown level. A definite change in oleoresin appeared as branch cortex tissue aged in high-β-phellandrene trees. Alpha-pinene percentages increased and myrcene percentages decreased with age (Table 3). Beta-pinene

TABLE 3. MONOTERPENE HYDROCARBON COMPOSITION OF OLEORESIN IN BRANCH CORTEX OF DIFFERENT AGES IN HIGH-β-PHELLANDRENE TREES

	Percentages of major components*			
Age of tissue	α-Pinene	β-Pinene	β-Phellandrene	Myrcene
Current	15	40	20	22
1 year	20	38	24	17
2 years	24	36	26	14
4 years	35	38	18	8

* Each value is the mean of 24 samples (2 samples/branch; 2 branches/level; 3 crown levels/trees; 2 trees).

percentage changed nonsignificantly; β-phellandrene was variable with no definite trend. Changes with cortex age were not apparent in low-β-phellandrene trees. Since the cortical tissue is rapidly moved outward by activity of the vascular and cork cambiums, it apparently remains metabolically active only a short time. The tissue is rapidly pulled apart, disconnected from the vascular system, and exposed to air, allowing drying and oxidation of the oleoresin. These processes are probably responsible for some of the changes of monoterpene hydrocarbon composition of oleoresin associated with age of the branch cortex.

Additional Components

Camphene and limonene were also present but usually in amounts too small for accurate measurement. In some cores of one high-β-phellandrene tree, 5 per cent or more camphene

was present. Even though camphene and limonene were seldom present in large amounts, they were detected each time the GLC was adjusted to check for their presence. They are probably present in small amounts in all slash pine oleoresin. A peak with the same retention time as α-phellandrene on Carbowax 20M was also present in minute amounts in many samples.

DISCUSSION

The monoterpene hydrocarbon composition of oleoresins in these selected slash pine trees varied greatly from one part of the tree to another. The variability was not limited to differences among tissue types; there was also significant variation within the xylem of individual trees. Among the selected trees studied the degree of variability was quite different in low- and high-β-phellandrene trees.

The variability in trunk xylem terpene composition, coupled with a previous report of changes of monoterpene hydrocarbon composition after wounding,[17] invalidate the term "trunk oleoresin composition" for slash pine. Work reporting "trunk oleoresin composition" of slash pine should specify at least the height at which the sample was taken and whether the value represents a number of replicates or pooled oleoresin. Age or total height of the tree, and evidence of previous wounding, will also be helpful. Comparisons of "trunk oleoresin" from different slash pine trees should be made with samples from comparable trunk locations with respect to height from the ground and proximity to wounds.

Since this work involved only a few selected trees, it may not represent the full range of variability of slash pine trees, but indicates that considerable tree-to-tree, and within-tree variation exists.

EXPERIMENTAL

Sample Collection

The samples for this study were collected from four 19-year-old slash pine trees of known parentage which had been wounded regularly for 2 years for naval stores production. Two of the trees, full sibs, had relatively high percentages of myrcene and β-phellandrene in the cortical oleoresin. The other two trees, also full sibs but from different parents, had only trace amounts of myrcene and β-phellandrene in the cortical oleoresin. At the time of sampling the trees were 50 to 60 ft tall and 11 to 13 in. d.b.h.

Two branches were removed from the lower, two from the middle, and two from the upper crown of each tree. Samples of needles were taken from each internode from each branch. 1- to 2-in. branch segments were cut from current, 1-year-old, 2-year-old, and 4-year-old elongation increments. From each branch segment, tissue samples of cortex, current growth xylem, and oldest xylem were analyzed. Paired 5-mm increment cores, spaced 90° from each other, were removed from the unwounded backside of the trunk of each tree at each of three heights (1, 16, and 31 ft) above the ground. Samples were analyzed from the 1-, 2-, 3-, 4-, and 5-year-old annual rings and the oldest annual ring in each core. At the time of collection, sample material was placed in plastic bags and stored at −20° until removed for analysis.

In another phase of the study, trunk xylem oleoresin of one tree was sampled intensively with regard to distance from the ground. With a ½-in. cork borer, punch wounds were made through the bark to the wood surface at ½, 1, 1½, 2, 3, 4, 5, 10, and 16 ft above the ground. Oleoresin samples were collected in glass vials inserted into these wounds. The vials were sealed and refrigerated to await analysis.

To obtain root oleoresin samples from each tree, four roots (one in each cardinal direction from the trunk) were severed near the soil surface. 10 min later exuded oleoresin on the cut surfaces was collected into separate glass vials, sealed, and refrigerated.

Analysis

All samples were analyzed in duplicate by GLC, using a machine with a flame ionization detector and a recorder with a mechanical integrator. A 20-ft, ⅛-in. copper column packed with 20% Carbowax 20M on

[17] D. R. ROBERTS, *Assoc. Southeast. Biol. Bull.* 15, 53 (1968).

70–80 mesh untreated Chromasorb W was used. The injection port temperature was 250°, oven temperature was 110°, and nitrogen carrier gas flow was approximately 60 ml/min.

All tissue samples were analyzed by a solid sampling method.[18] In this procedure, resin duct segments enclosed in small amounts of tissue were inserted into the injection port of the gas chromatograph for volatilization of the monoterpene hydrocarbons. Whole exuded oleoresin from the intensive trunk sampling and several roots were diluted with pentane and injected with a 1 μl syringe.

Percentages of each constituent were calculated from integrator traces for area under the peaks, compared to the total integrator traces for all monoterpene hydrocarbon peaks. Duplicate analyses of the same sample agreed well. This resulted in a good estimate of the proportion of the turpentine which the particular monoterpene hydrocarbon represents. It gave no indication of the relationship of monoterpene hydrocarbon to the whole oleoresin—when the percentage of one chemical increased, one or more others decreased, since all percentages must total 100.

After arc-sine transformation, the data for each chemical were subjected to analysis of variance to determine significant differences.

[18] D. R. Roberts, *J. Gas Chromatog.* **6**, 126 (1968).