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COMPOSITION OF THE CORTICAL AND PHLOEM MONOTERPENES OF *ABIES LASIOCARPA*

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Abstract—Monoterpene hydrocarbons from phloem and cortex of sixty-eight trees of *Abies lasiocarpa* var. *lasiocarpa* and var. *arizonica* were analysed by GLC. While the same terpenes were present in either bark tissue, cortical terpenoids were found to be higher in limonene, but lower in α-pinene, β-pinene, 3-carene and myrcene. Statistical calculations indicated that although control of terpene levels by the plant was generally similar in cortex and phloem, small but definite differences were present in some instances. It is concluded that the two oleoresins should be analysed separately in chemosystematic work.

INTRODUCTION

Abies lasiocarpa (Hook.) Nutt. (subalpine fir), a medium-sized tree with a spire-shaped crown, is the most widely distributed fir in western North America. Throughout the central and northern parts of its range all but the oldest trees have smooth bark. This feature, which is common to most firs, is due to the delayed development of the rhytidome (the outer bark, made up of alternate layers of periderm and dead cortical or phloem tissues, and usually rough and furrowed in appearance). The cortex of the primary body together with its thin covering of first-formed periderm, remains intact for many years—sometimes more than a century in subalpine fir. These tissues are eventually sloughed off after rhytidome formation begins, and old trees of subalpine fir have rough bark. From an early stage of development the cortex contains resin canals which are schizogenous in origin. The multicellular epithelium of the canals produces an oleoresin that is chemically similar to that of *Pinus* xylem. As the tree ages these resin-producing ducts increase greatly in number and complexity, and many later-formed ducts lack the elongate, canal-like character of the first-formed ducts. In localized areas of the cortex some of the ducts develop large resin-filled cavities which are externally visible as 'blisters'. Also present in the cortex are many enlarged resin-containing cells which Chang[1,2] has called 'resin passages'.

At the southern end of its range, typical smooth-barked subalpine fir (*A. lasiocarpa* var. *lasiocarpa*) is replaced by the corkbark fir, *A. lasiocarpa* var. *arizonica* (Merriam) Lemm. This distinct geographic race is native to the high mountains of Arizona and New Mexico; it intergrades with the typical form in southern and central Colorado. The unique feature of the corkbark fir is the continuous layer of solid cork (phellem) covering the stem. This layer of cork is produced by the activity of the first-formed cork cambium (phellogen), which originates beneath the epidermis of the first-year stems.[3] It soon develops shallow

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[1] CHANG YING-PE, Anatomy of common North American pulpwood barks. TAPPI Monogr. 14, 249 pp. Tech. Assoc. Pulp and Paper Indus. (1954).
[2] CHANG YING-PE, Bark structure of North American conifers. *U.S.D.A. Tech. Bull.* 1095, 86 pp. (1954),
[3] ELBERT L. LITTLE JR., Some preliminary notes on Southwestern corky barks. Res. Rep. 3, 11 pp. (mimeo.), Southwest. For. and Range Exp. Sta.

furrows due to continuing stem growth, and gives young trees of corkbark fir an appearance quite unlike the smooth-barked appearance of young trees of all other firs. The corky layer, usually less than 1 cm thick even on older trees, envelops the cortex and inhibits production of externally visible resin blisters.

In addition to the cortical resin system, the bark of *A. lasiocarpa* and its close relatives, *A. balsamea* and *A. fraseri*, also has a resin-producing system in the secondary phloem. 'Resin passages' similar to those of the cortex are abundant throughout this tissue. According to Chang[1] they originate from single cells which are usually associated with the phloem rays, but their subsequent development is obscure. Also present in the oldest part of the secondary phloem of *A. lasiocarpa* are much larger resin ducts having a multicellular epithelium. These spherical or tangentially flattened ducts average 1–1·5 mm in dia. They do not seem to have been described previously, and their mode of origin is unknown. Except for their relative uniformity in size and shape, they do not appear to differ from the smallest of the later-formed resin ducts in the cortex.

Our continuing efforts to clarify some of the systematic problems in the genus *Abies*[4–6] have been based on the chemical analysis of cortical oleoresin, which is easily obtained from the resin blisters of most firs. Corkbark fir lacks blisters, however, and obtaining adequate amounts of oleoresin from its cortical resin ducts is a laborious process. The simplest solution would be to extract the oleoresin from all of the living tissues of the bark (cortex and phloem) and analyse the extract by GLC. The validity of this procedure, which would lump the oleroresins produced by the contiguous but independent resin systems of phloem and cortex, rests on the assumption that the two systems produce oleroresin of the same composition. No comparisons of phloem and cortex oleoresins are available in the literature, but the cortex and wood oleoresins of *Pinus elliottii* have been shown to differ in monoterpene composition.[7] This paper compares monoterpene constituents of phloem and cortex resin of subalpine and corkbark firs.

RESULTS AND DISCUSSION

Appreciable quantitative differences between the monoterpenes of phloem and cortex were found throughout the region sampled in this investigation. Our samples were collected from nine stands in Arizona, New Mexico, Utah, and Colorado, and included 68 trees in all. Two stands were typical smooth-barked subalpine fir; the rest were entirely or predominantly corkbark fir (Table 3).

Monoterpene analyses are reported on the individual tree level for Sandia Crest, New Mexico (var. *arizonica*) and Fremont Pass, Colorado (var. *lasiocarpa*) populations, in Tables 1 and 2. Table 3 lists median values for all nine populations examined. The most striking feature is an increase in α-pinene and decrease in limonene in phloem vs. cortical oleoresin; β-pinene, 3-carene and myrcene are apparently also higher in phloem. This tendency is present in both var. *lasiocarpa* and var. *arizonica* populations and is thus characteristic for the whole species.

The variability data were further analysed by plotting the percentages of a terpene from cortex against the percentages of the same terpene from phloem, as exemplified in Figs. 1 and 2, with the results given in Table 4. The scatter is rather strong, and the points as well

[4] E. ZAVARIN and K. SNAJBERK, *Phytochem.* **4**, 141 (1965).
[5] E. ZAVARIN, K. SNAJBERK, Th. REICHERT and E. TSIEN, *Phytochem.* **9**, 377 (1970).
[6] E. ZAVARIN, *Phytochem.* **7**, 92 (1968).
[7] A. E. SQUILLACE and GORDON S. FISHER, Evidences of the inheritance of turpentine composition in slash pine *U S.F.S. Res. Paper* NC-6; 53–60. North Cent. For Exp. Sta. (1966).

TABLE 1. MONOTERPENE COMPOSITION OF CORTEX AND PHLOEM OLEORESIN OF *Abies lasiocarpa* VAR *arizonica*, COLLECTED AT SANDIA CREST, NEW MEXICO

Tissue	% of total								
	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene
Cortex	30·3	0·2	30·2	0·0	0·0	8·0	0·5	30·4	0·0
Phloem	28·5	0·2	31·4	2·0	0 0	10·3	1·0	26·3	0·3
Cortex	5·7	0·0	14·9	0·0	0·3	8·5	36·8	33·6	0·0
Phloem	7·5	0·1	20·9	1·4	0·0	8·7	23·6	37·5	0·3
Cortex	5·7	0·0	14·7	0·0	0·3	8·7	36·7	33·7	0·0
Phloem	65·6	0·4	12·0	tr	0·1	2·4	2·2	17·3	0·0
Cortex	11·6	0·1	14·7	0·0	0·3	2·0	59·0	12·1	0 0
Phloem	19·4	0·1	27·5	1·4	0·0	2·5	35·4	13·7	tr
Cortex	23·1	0·0	15·2	0·0	2·5	0·5	26·3	32·2	0·0
Phloem	44·6	0·2	14·6	5·8	0·0	1·2	7·9	25·6	0·1
Cortex	13·7	0 0	17·4	0·0	0·0	0·8	28·7	39·1	0·0
Phloem	22·2	0·0	22·0	0·2	0·0	1·2	15·6	38·8	0·0
Cortex	23·9	0·0	27·2	0·0	0·7	0·9	19·4	27·6	0·0
Phloem	16·2	0·0	28·2	2·3	0·0	1·2	15·2	36·6	0·0
Cortex	27·8	0·0	17·7	0·0	0·8	0·8	30·0	22·6	0·0
Phloem	30·4	0·2	23·0	3·7	0·0	1·2	20·0	21·0	0·5
Mean values									
Cortex	17·7	0·0	19·0	0·0	0·6	3·8	29·7	28·9	0·0
Phloem	29·3	0·2	22·5	2·1	0·0	3·6	15·1	27·1	0·2

TABLE 2. MONOTERPENE COMPOSITION OF CORTEX AND PHLOEM OLEORESIN OF *Abies lasiocarpa* VAR *lasiocarpa*, COLLECTED AT FREMONT PASS, COLORADO

Tissue	% of total								
	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene
Cortex	11·1	0·5	2 0	6·8	0·0	1·4	74 7	3 2	0·0
Phloem	47·8	1·1	4·5	20·1	0·0	1 6	18 6	6·1	0 0
Cortex	7·6	0·2	3·3	18 2	0·0	1·9	59·8	8·1	0·6
Phloem	6·8	0·2	8·3	26·7	0 0	6·3	37 0	14 4	0 0
Cortex	21·3	0 4	4·5	5·4	0 0	0·9	59·1	8 1	0 0
Phloem	29·5	0·4	6 3	11·4	0·0	1 3	38·5	12 3	0 0
Cortex	5·8	0·6	7·3	3·2	0·0	3·9	63·2	15 7	0 0
Phloem	7·6	0·1	11·7	9·1	0·0	7·5	36·9	26 6	0 0
Cortex	9·6	0·5	1 3	11·7	0·0	5 0	70 6	0·7	0 1
Phloem	18·5	0·5	7·2	18·4	0·0	16·2	26·3	12·4	0 0
Cortex	10 6	0·1	9·1	3 9	0 0	1 1	56 8	18 1	0·0
Phloem	19·0	0·4	13·0	6·2	0·0	2 0	25·6	32·8	0·6
Cortex	7·2	0·1	4·6	0 0	0·0	4·3	73·2	10·3	0 0
Phloem	14·8	0 3	9 0	2·5	0 0	6 7	44 6	21 7	0 0
Cortex	31·9	0 9	17 0	5·7	0 0	1·8	2 2	39·4	0·2
Phloem	27·7	0·6	10·8	17 5	0·0	17·5	3·1	21·2	1 2
Mean values									
Cortex	13·1	0·4	6·1	6 8	0 0	2 5	57·5	13 0	0·1
Phloem	21 5	0 4	8·9	14 0	0 0	7 4	28·8	18·4	0·2

TABLE 3. MEDIAN VALUES FOR THE INDIVIDUAL POPULATIONS TESTED

Population	Tissue	% of total α-Pinene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene
Big Flat, Beaver Co.* Utah	Cortex	6·8	3·8	8·6	1·4	74·5	5·0
	Phloem	9·4	6·6	13·6	3·2	57·4	10·2
Fremont Pass, Summit Co.* Colorado	Cortex	10·1	4·6	5·6	1·8	61·5	9·2
	Phloem	18·8	8·6	14·5	6 5	31·6	17·8
Pass Creek, Mineral Co.† Colorado	Cortex	8·7	8·2	1·5	2·0	46·0	21·6
	Phloem	17·7	12·2	2 6	4·0	23·1	23·2
Cucharas Pass, Huerfano Co.† Colorado	Cortex	12·0	13·8	6·2	1·7	34·6	28·9
	Phloem	26·8	17·8	7·6	4·2	10·8	30·4
Aspen Basin, Santa Fe Co.† New Mexico	Cortex	6·8	14·6	0 0	4·0	37·4	32·8
	Phloem	8·6	18·3	0·8	8·8	18·9	38·8
San Francisco Mt., Coconino Co.‡ Arizona	Cortex	11·6	26·0	0·0	0·9	18·9	47·6
	Phloem	16·2	29·8	0·7	1·4	1·8	39·4
Apache Railroad, Apache Co.‡ Arizona	Cortex	10·4	21·0	0·0	1·5	20·0	42·0
	Phloem	12·5	26·2	1·0	2·1	11·7	41·2
Mt. Graham, Graham Co.‡ Arizona	Cortex	12·3	25·0	0·0	1·1	17·3	39·7
	Phloem	19·9	28·3	0·6	1·9	16·5	31·1
Sandia Crest, Bernalillo Co.‡ New Mexico	Cortex	18·4	16·3	0·0	1·5	29·4	31·3
	Phloem	25·4	22·5	1·7	1·8	15·4	26·0
Mean	Cortex	10·8	14·8	2·4	1·8	37·7	28·7
	Phloem	17·3	18·9	4·8	3·8	20 8	28·7

* Southern stands of var. *lasiocarpa.*
† Stands of varying degrees of intermediacy, but with corky bark predominating.
‡ Stands of var. *arizonica.*

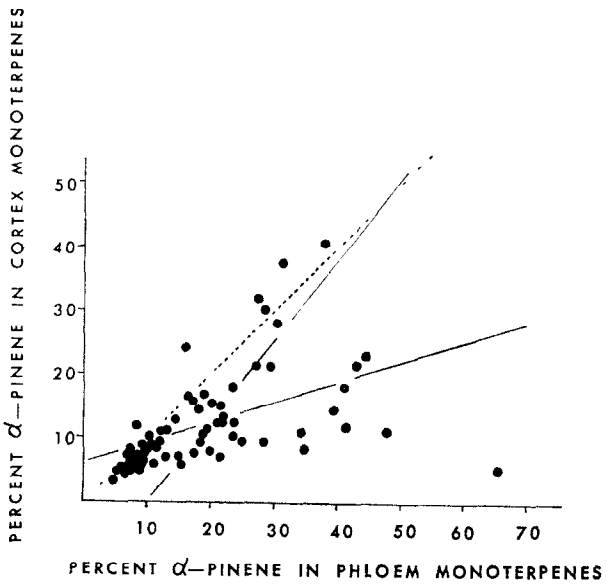

FIG. 1. PLOT OF PHLOEMAL VS. CORTICAL DATA FOR α-PINENE, INCLUDING CALCULATED REGRESSION LINES (———) AND THEORETICAL REGRESSION LINE (— — —).

as the two calculated linear regression lines are positioned (with the probable exception of β-phellandrene) either below or above the theoretical line which assumes identity of the two resin systems (i.e. A = 0, B = +1·0). Still, in all cases an increase in per cent of a terpene in one tissue tends to be accompanied by an increase of the same terpene in the other. This suggests that the same factors control a large part of the terpene variability in both systems. To obtain an idea of the magnitude of this linkage, squared correlations coefficients were computed separately for each terpene (Table 4). The results indicate that with most terpenes, the variability in phloem (or cortex) can explain 61–82 per cent of the variability in the other tissue; α-pinene is a notable exception, however, with only a 24·5 per cent linkage.

Additional comparison between the two resin systems was made by calculation of the χ^2 values for the goodness of fit into a Gaussian curve (Table 4) and by construction of the distribution diagrams (Fig. 3). The largest differences in χ^2 values were met in the case of β-pinene and β-phellandrene data, which fitted satisfactorily the Gaussian with phloem, while exhibiting a markedly non-parametric behavior with cortex. Considering that the samples were collected in different localities, the distribution diagrams for cortex were reasonably close to what would be expected on the basis of our previously published information.[5] The diagrams for α-pinene, 3-carene and myrcene from phloem were not out of line with their cortical equivalents. Stronger discrepancies were observed, however, in the case of β-pinene, limonene and β-phellandrene. This is suggestive of a slightly different control of the terpene levels in the two tissues.

In our previous publication[5] we reported a number of correlations between percentages of different *A. lasiocarpa* cortical monoterpenoids—correlations presumably arising from linkages of biosynthetic pathways—and we gave a biosynthetic interpretation of the regularities observed.[6] In the present investigation, calculation of relevant correlation

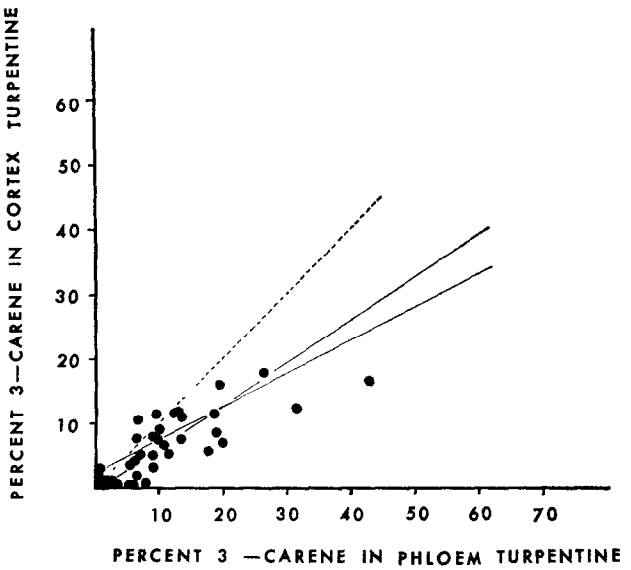

FIG. 2. PLOT OF PHLOEMAL VS. CORTICAL DATA FOR 3-CARENE, INCLUDING CALCULATED REGRESSION LINES (———) AND THEORETICAL LINE (— — —).

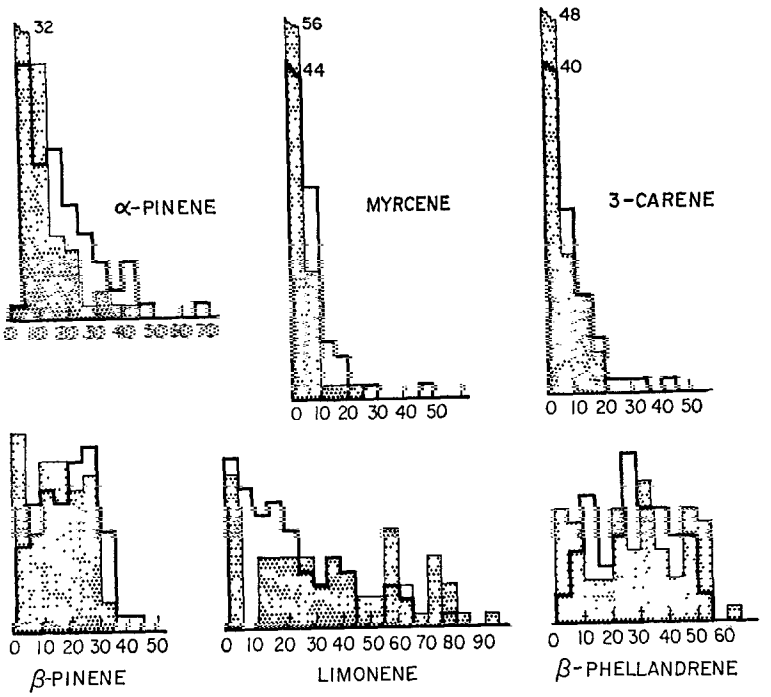

FIG. 3. DISTRIBUTION DIAGRAMS FOR THE SIX MAJOR TERPENOIDS ENCOUNTERED. Heavy lines—phloem; dotted area—cortex. Abscissa in per cent. Ordinate gives number of samples (dimension: 1 sample = 5 per cent).

TABLE 4. CORRELATION STATISTICS FOR CORTEX/PHLOEM TERPENE PAIRS

Terpene	Regression coefficients* Intercept A	Slope B	Confidence interval for slope	Correlation coefficient r	Correlation coefficient square (%)
α-Pinene	6 3	0·31	±0·13	0·495	24·5
β-Pinene	−0·4	0 83	±0·10	0 904	81·8
3-Carene	0 2	0 52	±0·07	0·880	77·5
Myrcene	0·8	0·39	±0 08	0·780	60·8
Limonene	10·5	1·23	±0·18	0·860	74·0
β-Phellandrene	−1·1	1 07	±0·17	0 843	71·0

Chi-square statistics for goodness of fit into Gaussian of terpenes from cortex and phloem

Terpene	Cortex		Phloem	
	Chi-Square	*df*	Chi-Square	*df*
α-Pinene	36·9†	4	28·1†	6
β-Pinene	22·0†	6	8 6	6
3-Carene	140·4†	6	176 7†	7
Myrcene	80·4†	3	79·9†	3
Limonene	47·6†	9	31·1†	9
β-Phellandrene	27·8†	9	10 9	9

* Phloem terpenes as independent variable. Confidence interval at 0·05 level.

† Indicates deviation from Gaussian significant on < 0·01 %; unmarked figures indicate normal distribution significant at > 0 5% level.

statistics for phloem and cortex (Table 5) indicated that qualitatively the nature of the correlations existing was the same as before. Quantitatively, however, the values for the majority of correlation coefficients decreased in phloem. Using sums of r^2 values obtained from all possible terpene pair combinations, the ratio $\Sigma r^2_{\text{phloem}} : \Sigma r^2_{\text{cortex}}$ was calculated as 0·69. Thus, the fraction of the total variance associated with the biosynthetic linkage of reaction paths was less by 31 per cent in phloem, suggesting a more independent control of individual terpene levels in this tissue.

TABLE 5. CORRELATION COEFFICIENT MATRIX FOR VARIABILITY OF TERPENES WITHIN EACH RESIN SYSTEM*

	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene
α-Pinene	0·465	0·116	−0·264	−0 498	0·131
	−0 014	0·023	−0 234	−0·401	−0·284
β-Pinene	—	−0·453	−0 193	−0·926	0 847
		−0·568	−0·354	−0·634	0·697
3-Carene	—	—	0 019	0·249	−0·396
			0·176	0·148	−0·551
Myrcene	—	—	—	−0 086	−0·118
				−0 008	−0·161
Limonene	—	—	—	—	−0 894
					−0·594

* Upper figure refers to cortex, lower to phloem; $r_{0\cdot95} = 0\ 239$, $r_{0\ 99} = 0{\cdot}311$ for $df = 66$.

Thus, in spite of the proximity of phloem and cortical resin systems in bark of *A. lasiocarpa*, the volatile oils obtained from the two tissues differ in their terpene levels, and to some extent in the manner by which these levels are controlled by the plant, so that it seems preferable to analyse the oleoresin constituents from these tissues separately when conducting chemotaxonomic studies.

EXPERIMENTAL

All samples were collected by one of us (W.B.C.) within a 10-day period, and subsequently stored and processed together. The trees sampled were 30–130-yr-old, and ranged from 2·3 and 14·0 in. dia. at 4·5 ft above the ground. Samples of cortical resin were collected from bark blisters of all typical subalpine firs and a few trees in the region of intergradation, using procedures described earlier.[3] All other resin samples were obtained from pieces of bark removed from the tree at 4–5 ft above ground, stored in polyethylene bags, transported in an ice-chest, and frozen until use. Cortical resin was collected from bark samples of corkbark fir by peeling off the layer of cork, removing the outermost layers of underlying tissue with a razor blade if necessary, and tapping the exposed resin ducts. Phloem resin was extracted from slabs of secondary phloem removed from bark samples by tangential cuts through the outer phloem. The boundary between the outer phloem and cortex was determined under a dissecting microscope. Phloem tissues were macerated immediately before analysis into small (about 2 × 2 mm) pieces with a razor blade and covered with about 10 ml of CS_2. After allowing the resulting slurries to stand for 15 min and stirring, the supernatant liquids were used for analysis. That no monoterpenes were lost during maceration was checked by analysing the same macerated tissue immediately after maceration and after allowing the tissue to remain uncovered at room temp. for 1 hr, after maceration no difference in analytical data was noticed. Analysis of monoterpenes was performed by GLC as previously described.[5]

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