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CHEMOTAXONOMY OF THE GENUS *ABIES*—II.

WITHIN TREE VARIATION OF THE TERPENES IN CORTICAL OLEORESIN*

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Abstract—The variation of the cortical terpene composition along the stem of eleven trees belonging to four *Abies* species was examined by GLC methods. The changes appeared to be the greatest in the youngest part of the stem, before the resin-containing blisters are externally visible. In practically all cases, the systematic variations in the blistered part of the stem were rather small, and in older trees approached the order of magnitude of experimental error. In composition the leaf terpenes were fairly similar to the terpenes from cortex, although in some cases definite differences were apparent. Practically no seasonal change in the terpene composition was observed.

INTRODUCTION

IN OUR first publication of this series we demonstrated that terpenes from the cortical blister oleoresin show strong interspecific and intervarietal differences within the genus *Abies*, and we decided to expand this study into investigations of the geographic variability of these compounds within each of the American species.[1] This was to be followed by investigations of the interspecific introgradation so common to this genus, with the support of the concurrent morphological studies.

In problems of this nature it is of utmost importance for the proper evaluation of the results to obtain a good idea about the magnitudes of the genetic and nongenetic influence on the variables in question—in this case, the terpene content and composition. The whole subject of nongenetic influences affecting the compositions of the various chemical fractions used for chemotaxonomic deductions has been systematically discussed by Schrath.[2]

Practically no information seems to be available in literature on the subject of nongenetic control of the composition of terpenes from cortical oleoresin in *Abies*. A number of studies have been made on the influence of environment on the composition of wood turpentine of *Pinus*; these, up to and including 1960, have been reviewed by Mirov.[3] He concluded that such influences must be relatively small. More recently, using modern GLC methods, Blight and McDonald[4] found that with few exceptions very little difference existed in the composition of wood terpenes between members of the same clone of *P. attenuata* Lemm.

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[1] E. ZAVARIN and K. SNAJBERK, *Phytochem.* **4**, 141 (1965).

[2] E. SCHRATH, *Planta Med.* **11**, 278 (1963).

[3] N. T. MIROV, *U.S. Dep. Agr. Tech. Bull.* No. 1239 (1961).

[4] M. M. BLIGHT and I. R. C. MCDONALD, *New Zealand J. Sci.* **7**, 212 (1964).

XP. radiata D. Don. hybrids, growing at the Intitute for Forest Genetics, Placerville, California. Studies, somewhat more relevant to our investigation, were made in 1966 by Hanover.[5] He worked with clonaly propagated individuals of *P. monticola* Dougl., and using terpenes from cortical tissue found no differences among trees growing in three nutritionally different sites. Based on this work it seemed safe to expect that environmental factors would influence the composition of the turpentine of *Abies* cortical oleoresin very little.

Unfortunately, less could be gleaned from the literature insofar as influence on the composition of cortical terpenes of tree age, season, or within-tree position of the tissue is concerned, even if the entire family Pinaceae is taken in consideration. This is partly due to the fact that a good deal of work has been done on different resin systems (such as wood resin in *Pinus* by

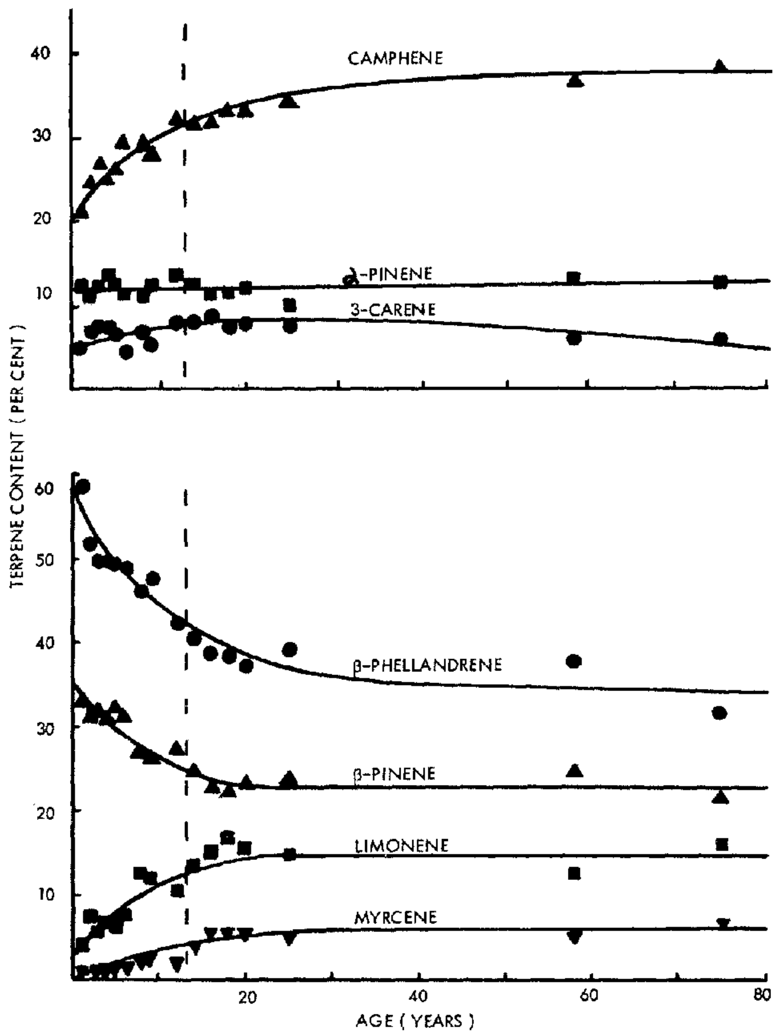

FIG. 1. CHANGE IN PERCENTAGE (TOTAL TERPENE BASIS) OF INDIVIDUAL TERPENES PRESENT IN THE CORTICAL OLEORESIN OF ONE *A. magnifica* TREE AS FUNCTION OF AGE.

[5] J. W. HANOVER, *Phytochem.* 5, 713 (1966).

Bannister *et al.*,[6] Blight and McDonald,[4] and Smith[7]). In other cases insufficient care has been taken to consider various resin systems separately.* The previously mentioned paper by Hanover[5] on cortical terpenes from *P. monticola* seems to be the only one relevant to our work. While he found little year-to-year difference in composition of terpenes from the 0–4 years' growth, the terpenes from young cortex differed considerably from the terpenes of the old, mainstem cortex.

On the basis of this literature survey, we felt that the question of amount of nongenetic contribution to terpene variability due to age and position of a blister on a stem had not been clearly answered and decided to investigate this problem further.

RESULTS AND DISCUSSION

The variation of the cortical terpene composition along the stem of eleven trees—one *Abies concolor* var. *lowiana* (Gord.) Lemm., two *A. balsamea* L. (Mill.), seven *A. lasiocarpa* (Hook.) Nutt., and one *A. magnifica* Murr.—was examined by GLC analysis. Four of the

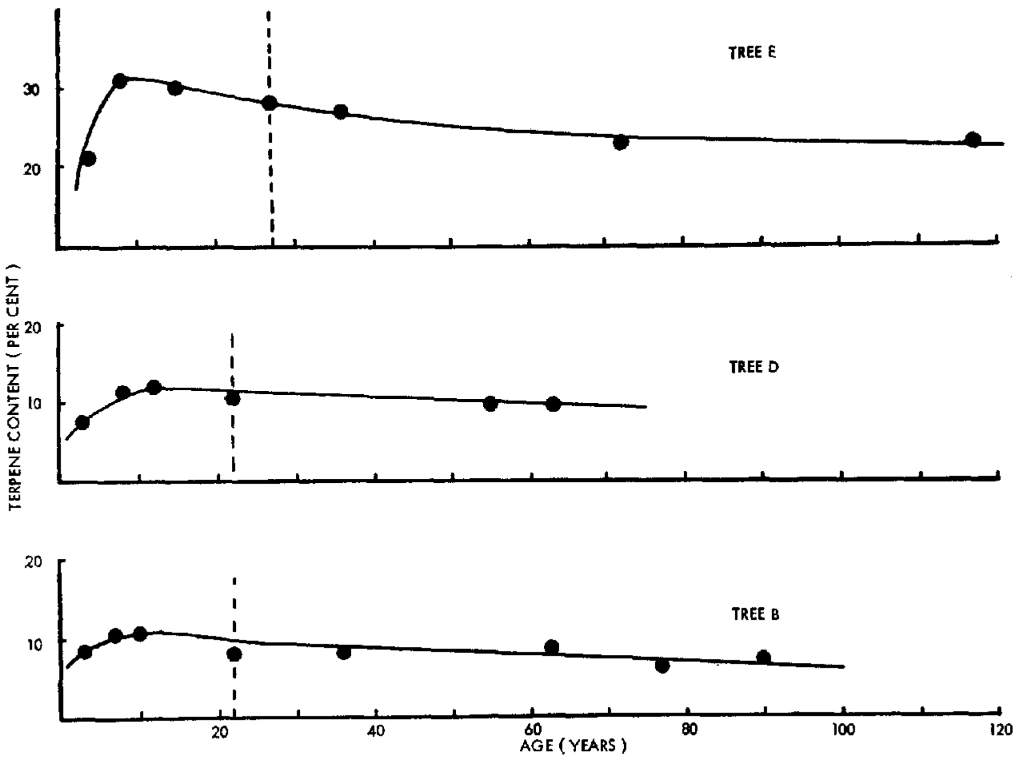

FIG. 2. CHANGE IN PERCENTAGE (TOTAL TERPENE BASIS) OF α-PINENE PRESENT IN THE CORTICAL OLEORESIN OF THREE *A. lasiocarpa* TREES AS FUNCTION OF AGE.

* A tendency seems to exist generally to investigate 'green twigs and leaves' together, which is tantamount to lumping leaf, cortex and xylem resin systems. In one instance trees were separated into needles, young shoots, branches up to 1 cm, thicker stems, and roots for the comparative analysis of terpenes.

[6] M. H. BANNISTER, A. L. WILLIAMS, I. R. C. MCDONALD and M. B. FORDE, *New Zealand J. Sci.* 5, 486 (1962).
[7] R. H. SMITH, *Nature (London)*, **202**, 107 (1964).

TABLE 1. AVERAGE COMPOSITION AND SYSTEMATIC AND RANDOM CHANGES OF THE CORTICAL OLEORESIN TERPENES IN THE BLISTER (B) AND NONBLISTER (N) ZONES OF VARIOUS *Abies* SPECIES*

Tree species	Age (years)	Blisters start (year)	α-Pinene		Camphene		β-Pinene		3-Carene		Myrcene		Limonene		β-Phellandrene		Total terpene content in blister zone	Mean deviation
Older trees: *Abies magnifica*			N	B	N	B	N	B	N	B	N	B	N	B	N	B		
Mean	75	14	12·4	11·6	7·5	13·8	20·1	13·3	6·8	7·2	1·4	5·4	8·2	15·6	43·4	32·8	18·5	1·1
Slope			0·0	0·0	+0·8	+0·1	−0·7	0·0	+0·17	−0·03	+0·25	+0·05	+0·9	+0·1	−1·75	−0·03		
Mean dev.			1·0	0·5	1·5	0·5	0·9	0·6	1·0	0·6	0·6	0·65	1·7	0·8	2·0	1·7		
A. lasiocarpa (A) *A. lasiocarpa*																		
Mean	120	60	27·7	14·4	—	—	33·4	26·9	1·7	3·6	1·0	0·8	1·7	2·3	34·1	51·6	27·1	0·9
Slope			+1·1	−0·15	—	—	+0·05	−0·08	+0·09	+0·025	0·0	0·0	0·0	0·0	1·3	+0·2		
Mean dev.			1·0	0·2	—	—	0·25	0·2	0·3	0·2	0·25	0·13	0·4	0·13	0·3	0·7		
(B) *A. lasiocarpa*																		
Mean	90	21	9·8	7·5	—	—	25·8	21·4	3·1	9·7	0·6	1·2	7·9	14·7	52·4	45·0	30·9	0·7
Slope			ND	0·0	—	—	ND	−0·035	+0·24	0·0	0·0	0·0	+0·75	+0·08	−1·1	−0·05		
Mean dev.			0·4	0·7	—	—	0·7	0·7	0·5	0·1	0·25	0·25	0·2	0·6	0·2	0·9		
(C) *A. lasiocarpa*																		
Mean	112	33	11·8	10·6	—	—	28·4	26·8	6·6	10·3	0·9	1·6	1·9	1·9	49·3	48·0	31·8	ND
Slope			−0·5	−0·02	—	—	ND	−0·05	ND	−0·05	ND	+0·003	ND	−0·013	−0·45	+0·06		
Mean dev.			0·8	0·6	—	—	0·7	0·4	0·8	1·0	0·1	0·2	0·4	0·3	0·5	1·0		
(D) *A. lasiocarpa*																		
Mean	63	22	10·3	9·8	—	—	26·4	24·8	7·4	8·9	0·9	1·3	2·9	1·8	51·1	52·1	26·9	ND
Slope			+0·92	−0·05	—	—	ND	−0·03	+1·6	0·0	0·0	+0·22	−1·0	0·0	−1·4	+0·025		
Mean dev.			0·2	0·4	—	—	0·6	0·3	0·3	1·0	0·0	0·3	0·0	0·1	1·5	1·3		

(E) *A. lasiocarpa*			N	B	N	B	N	B	N	B	N	B	N	B	N	B		
Mean	117	27	17·3	15·2	—	—	35·6	34·7	1·3	2·0	0·9	0·7	2·8	2·8	41·8	45·2	32·7	ND
Slope			+2·3	−0·05	—	—	+1·6	−0·1	0·0	0·0	0·0	0·0	−0·3	0·0	−4·4	+0·1		
Mean dev.			1·3	1·0	—	—	1·0	0·1	0·25	0·5	0·0	0·25	0·2	0·0	1·0	1·5		
(F) *A. lasiocarpa*																		
Mean	95	27	7·4	4·5	—	—	3·4	1·5	13·3	14·8	0·8	0·3	71·9	78·8	2·4	0·0	28·4	1·6
Slope			−1·0	0·0	—	—	−0·5	0·0	ND	−0·05	ND	0·0	+1·1	+0·025	−0·4	—		
Mean dev.			0·0	0·25	—	—	0·5	0·0	1·6	0·4	0·2	0·4	2·0	0·65	0·7	—		
Younger trees:																		
(G) *A. lasiocarpa*																		
Mean	36	13	9·3	8·2	—	—	9·4	12·4	3·2	10·6	1·6	1·0	54·1	38·5	21·8	29·3	24·5	0·9
Slope			+0·57	+0·02	—	—	+0·55	+0·28	+1·07	0·0	+0·19	−0·04	−2·5	−1·0	+1·6	+0·65		
Mean dev.			1·2	0·3	—	—	0·75	0·5	1·2	1·3	0·35	0·2	2·5	1·0	1·5	1·3		
(A) *A. balsamea*																		
Mean	34	11	7·5	7·0	—	—	31·3	42·0	5·0	4·0	3·0	1·0	48·9	41·0	4·0	5·0	33·3	2·0
Slope			−1·0	0·0	—	—	−3·25	+0·25	ND	0·0	ND	0·0	ND	−0·35	0·0	0·0		
Mean dev.			0·4	0·25	—	—	1·2	1·2	1·2	0·65	0·7	0·7	1·3	1·45	0·3	0·45		
(B) *A. balsamea*																		
Mean	21	6	13·0	8·0	—	—	43·8	39·6	—	—	1·5	1·5	37·5	46·8	4·3	4·0	32·4	0·6
Slope			ND	−0·31	—	—	−3·3	−0·1	—	—	0·0	+0·1	+2·1	+0·24	ND	0·0		
Mean dev.			ND	0·3	—	—	0·0	0·6	—	—	0·0	0·2	ND	1·0	ND	0·43		
A. concolor var. *lowiana*																		
Mean	24	7	8·6	10·1	—	—	51·6	54·9	1·6	—	1·2	0·8	3·6	1·2	32·9	32·5	35·9	1·8
Slope			−0·8	0·0	—	—	+3·5	+0·4	ND	—	−0·4	0·0	−3·0	−0·05	ND	−0·3		
Mean dev.			0·8	0·6	—	—	1·0	1·6	ND	—	0·1	0·25	0·25	0·15	1·0	1·6		

* N = non-blister zone; B = blister zone. Increase from tree top to bottom was taken as positive and expressed as change in a terpene percentage per year. Mean deviation for individual terpenes was computed from deviation of experimental values from drawn curves, as explained in the text. Terpene percentages computed on the total terpene basis and total terpene content on oleoresin basis. Total terpene content for nonblister zone has not been determined; expressing terpene percentages on this basis can only decrease slopes of some curves at the expense of increasing slopes of others of the same tree.

trees (the first three and one *A. lasiocarpa*) had an average age of about 30 years, the rest were roughly 90 years old. The variations observed were basically of two kinds—systematic changes along the stem, and random fluctuations. Fig. 1 shows the results obtained with *A. magnifica*. It is apparent that the largest changes occur in the upper part of the tree, decrease downward on the stem, and become zero (or close to it) just before the appearance of the first blisters. In many cases slight changes still take place in the blister zone, although in few instances the curves become horizontal. Generally similar patterns were obtained with most of the other trees examined. In some, however, the direction of the initial change reversed itself toward the blister zone. For instance, with *A. lasiocarpa* B, D, and E trees (Fig. 2), an initial increase in α-pinene changed to a decrease shortly above the blistered part of the stem. Occasionally even slightly more complicated curves were obtained (Fig. 3). In all, the greatest systematic changes in terpene composition occurred in the younger part of the trees, above the blister zone.

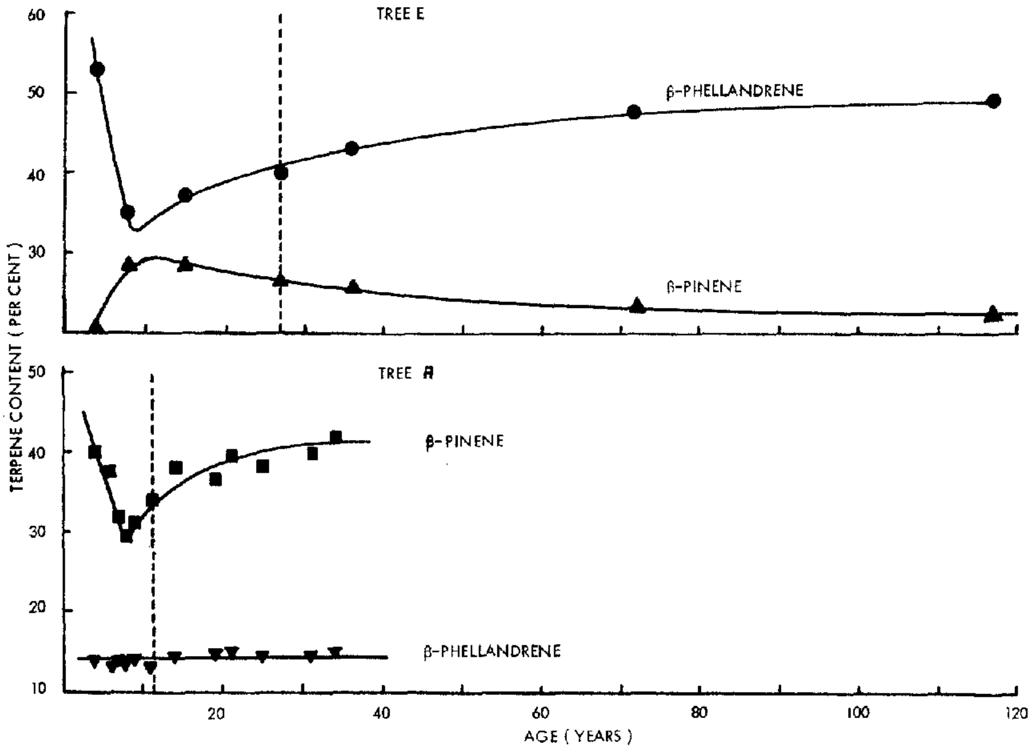

FIG. 3. CHANGE IN PERCENTAGE (TOTAL TERPENE BASIS) OF β-PINENE AND β-PHELLANDRENE PRESENT IN THE CORTICAL OLEORESIN OF ONE *A. lasiocarpa* TREE (TREE E) AND ONE *A. balsamea* TREE (TREE A) AS FUNCTION OF AGE.

The percentage of terpenes in oleoresins was determined for all blister resins available in sufficient amount. The corresponding average values and mean deviations are reported in Table 1. In only one was a systematic increase −0·08 per cent per year—noted. Some researchers[5] prefer to report the percentage of individual terpenes on the basis of total oleoresin rather than on the total terpene. Replotting of the individual curves on the basis of oleoresin values did not change either systematic or random variations in our investigations.

In order to assess quantitatively the changes involved, the slopes for each of the curves were determined separately for the blister zone and the upper part of the nonblister zone. The values obtained are summarized in Table 1, together with the mean values for the terpene compositions in each of the two zones and in cortex of first-year shoots. The random fluctuations of the experimental data from the values given by curves were co-determined, and are presented as mean deviations. In practically all cases the values for the slopes obtained in the nonblister zones were drastically higher (about 18 fold on the average) than those for the slopes in the blister zone. The few exceptions were invariably connected with terpenes present in very small amounts, and the values themselves were very small; thus these exceptions could be the result of experimental errors. It is also noticeable that in all instances, the higher values (0·5 per cent or more per year for the blister zones) were invariably connected with the younger trees. Thus, with the older trees the average slope values were 0·78 and 0·032 per cent per year for the nonblister and blister zones, respectively, whereas the same values for the younger trees were 1·40 and 0·19. It is apparent that the ratio of the two values was also less for the younger trees (24 vs. 7·5), indicating greater similarity between the two zones.

In contrast to the systematic variations, very little difference could be found between the random fluctuations of the terpene compositions in the blister and nonblister zones examined. Average mean deviations for those zones in the older trees were 0·65 and 0·53, respectively, and for the same zones in the younger trees, 0·83 and 0·73. Similarly, no correlation could be established between species or terpenes involved and the direction of the systematic changes.

To find out how the composition of leaf terpenes differs from that of the cortex, leaf terpenes from *A. magnifica*, *A. concolor* var. *lowiana*, and *A. lasiocarpa* (tree G) were separated by steam distillation, and analyzed in the usual way. Separate results for the first- and third-year needles are given in Table 2. Values for the terpenes from the first-year cortex, and the

TABLE 2. COMPARISON OF CORTEX AND LEAF TERPENES FROM THREE SPECIES OF *Abies**

Tree species		Santene	α-Pinene	Camphene	β-Pinene	3-Carene	α-Phellandrene	Myrcene	Limonene	β-Phellandrene	Terpinolene
Abies magnifica	leaf; 1 yr	—	6·5	2·0	19·5	1·5	0·5	2·0	5·0	61·0	2·0
	leaf; 3 yr	—	7·5	2·0	22·0	0·5	—	2·0	5·0	59·0	2·0
	cortex; 1st yr	—	12·5	1·0	23·0	5·0	—	1·0	4·0	53·5	—
	cortex; no blisters	—	12·4	7·5	20·1	6·8	—	1·4	8·2	43·4	—
	cortex; blisters	—	11·6	13·8	13·3	7·2	—	5·4	15·6	32·8	—
A. concolor	leaf; 1 yr	—	7·0	1·5	56·0	—	—	3·0	2·5	30·0	tr
	leaf; 3 yr	—	5·0	1·5	43·0	—	—	3·5	3·5	42·0	1·0
	cortex; 1st yr	—	10·5	—	46·5	—	—	2·0	8·5	32·5	—
	cortex; no blisters	—	8·6	0·4	51·6	1·6	—	1·2	3·6	32·9	—
	cortex, blisters	—	10·1	0·4	54·9	—	—	0·8	1·2	32·5	—
A. lasiocarpa	leaf; 1 yr	1·5	6·5	—	10·5	7·0	—	2·0	56·5	16·0	—
	leaf; 3 yr	2·0	5·0	tr	9·0	8·0	—	1·5	57·0	17·5	—
	cortex; 1st yr	—	7·5	—	5·5	0·5	—	1·0	72·0	13·0	—
	cortex; no blisters	—	9·3	—	9·4	3·2	—	1·6	54·1	21·8	—
	cortex; blisters	—	8·2	—	12·4	10·6	—	1·0	38·5	29·3	—

* In per cent total terpene basis.

average values for the terpenes from the cortex of the blister and nonblister zones are also given for comparison. In most cases there was apparently little difference between leaves sampled in the various years. With few exceptions the general character of the cortex oils seemed to be retained in leaf oils.

Since the blisters* remain on trees for many years with the terpenes probably not subject to any metabolic processes, the seasonal variation in terpene composition is not expected to be high. Seasonal differences in any additional portion of terpenes produced during the current season would be largely suppressed by the bulk of the terpenes already present. We checked this on one tree of *A. concolor* var. *lowiana* growing on the University of California Berkeley campus. The results are presented in Table 3. The differences seem to be rather small, in practically all cases assuming the proportion of an experimental error.

TABLE 3. VARIATION OF TERPENE COMPOSITION WITH SEASON IN ONE TREE OF *Abies concolor* VAR. *Lowiana**

Month	α-Pinene	Camphene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene	Terpinilene	Total Terpenes
October, 1963	8·0	tr	58·0	—	0·5	6·5	26·5	0·5	33·9
January, 1964	8·5	tr	59·0	0·5	1·0	7·0	23·5	0·5	33·2
April, 1964	8·0	0·5	57·0	1·0	0·5	5·5	27·5	—	34·0
July, 1964	8·5	0·5	59·0	0·5	tr	6·0	25·5	—	32·1

* In per cent total terpene basis; total terpenes expressed in per cent total oleoresin basis.

CONCLUSIONS

On the basis of the evidence presented the largest systematic changes in cortical terpene composition along the stem apparently take place in the youngest part of the cortex. In the blister zone, the changes were rather small in most samples, particularly in those from older trees. Although occasionally random fluctuations go as high as 1·7 per cent, they are commonly much less, averaging 0·6 per cent for the blister zones.

No drastic differences seem to exist between the *Abies* leaf oils and the terpenes isolated from cortex, nor between leaf oils of the first- and third-year growths, although more or less minor, quantitative differences are encountered occasionally. The seasonal variations in cortical terpene composition appear negligible.

The best procedure for filtering out any nongenetic contributions to terpene variations is apparently to sample cortex oleoresin from the largest blisters in the butt of the older trees, covering a substantial number of yearly growth sections (to average out any random fluctuation from year to year).

EXPERIMENTAL

The *Abies lasiocarpa* trees A to E came from southern Oregon, HW 230 about 14 miles north of Union Creek, Rogue River National Forest; *A. lasiocarpa* G tree was collected close to Galena Summit, 26·5 miles north of Ketchum, southern Idaho, near HW 93; *A. lasiocarpa* F tree was secured at Lost Trail Pass, western

* They probably develop from very small resin pockets in the cortex of a new shoot.

Montana at the Idaho border, HW 93; both *A. concolor* var. *lowiana* and *A. magnifica* trees were obtained from the Lake Tahoe area, near Echo Summit, California, HW 50; and both *A. balsamea* trees from near Touchwood Lake, eastern Alberta, Canada.

The sections of each tree corresponding to single-year growths were cut out and wrapped in aluminium foil; the balsam was usually drained from the sections containing blisters at the collection spot, and preserved by the method previously described. The sections were stored most of the time in a refrigerator, brought to the laboratory within a few days, and kept in a deep freeze until analyzed.

The age of each section was determined by year-ring count. The composition of the terpenes of the samples secured from blisters was determined by GLC, using the methods previously described,[8,9] and expressed in per cent, total terpene basis. When blisters were not available, i.e. with younger sections, portions of the cortex (about 1·0 g), were cut out and extracted with about 4 ml of CS_2. The resulting solutions were then analyzed as usual by GLC.

For leaf-oil analysis a mixture of about 25 g of leaves and 250 ml of water was macerated at 0° and steam-distilled under nitrogen for several hours. The oils were separated, preserved over pyrogallol, in nitrogen atmosphere at $-5°$ and analyzed by GLC, after dilution with CS_2.

The results were evaluated by plotting percentage values obtained for each terpene against the age of tree sections analyzed, and the curves were drawn as well as possible through the points obtained. The slopes in the blister zone were expressed as a change in terpene percentage values per one year. Because of the continuous slope changes in the non-blister zones the slopes had to be evaluated here at a specific point, namely, between years six and seven, which should give about the average slope for this area. In only a few cases, a point at a slightly earlier age was used. The mean deviation was computed on the basis of deviations of experimental data from the drawn curves. In a few instances, when the random fluctuations were too large or the number of points too small to indicate a definite systematic change, a horizontal line was laid, and the mean deviation was computed on this basis.

Acknowledgements—We extend our sincere thanks to Dr. William B. Critchfield, Pacific Southwest Forest and Range Experiment Station, Berkeley, California for helpful discussions of this problem, and to Mr. Karel Snajberk, Elaine Tsien, and Joanne Chin for their part of the experimental work.

[8] E. Zavarin, W. Hathaway, Th. Reichert and Y. B. Linhart, *Phytochem.* **6**, 1019 (1967).
[9] N. T. Mirov, E. Zavarin and K. Snajberk, *Phytochem.* **5**, 97 (1966).