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ENVIRONMENTAL VARIATION IN THE MONOTERPENES OF *PINUS MONTICOLA* DOUGL.

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Abstract—The concentrations of five monoterpenes in western white pine cortex oleoresin were measured by gas–liquid chromatography to determine how they vary with respect to certain environmental factors. Position on the tree, relative to the sun, and small differences in tissue age are both shown to have a predictable but small effect on terpene levels relative to genetic effects. Large differences in the age of tissues sampled within a single tree result in strikingly different patterns of terpene composition. The repeatabilities or intraclass correlations of monoterpene concentrations in western white pine are high. Genotypically identical plants (clones) growing in three diverse environments in Idaho show negligible differences in monoterpene levels, indicating that monoterpene concentration is quite stable with respect to many environmental factors. The data show that oleoresin from young, foliage-bearing tissue probably gives the most accurate measure of genotypic values for terpene concentrations.

INTRODUCTION

GENETIC control over the quantitative levels of individual monoterpene compounds in the cortex of western white pine (*Pinus monticola* Dougl.) has been shown to be strong.[1] Although nongenetic variation in monoterpene levels may be comparatively small, the sources and magnitude of such variation should be clearly defined. Otherwise, conclusions cannot be made about either the mode of inheritance or gene control of biosynthetic rates, and the usefulness of terpenes as genetic markers will be impaired.

Environmental variation in the levels of a plant chemical may be attributed to a number of possible sources including climatic factors, nutritional status, time of sampling, tissue sampled, position in the plant, maternal effects, and experimental error. Other investigators have reported that the terpenes in wood oleoresin are little affected by year sampled, season of year, position on the stem, tree age, or elevation.[2–4] Bannister *et al.*[5] suggest that the monoterpene composition of resin collected from resin blisters on a tree might differ from sapwood-derived terpenes. Some information is available about resin composition in relation to its distribution within the tree,[6] but quantitative data on terpene variation due to tissue differences are lacking. Likewise, our knowledge about the effects of climatic or site conditions on terpene levels is meager although Mirov[7] concluded that movement of pines to areas outside their natural range did not alter the chemical composition of their wood turpentine. This conclusion is based mainly on qualitative analyses of wood-derived terpenes. There is

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[1] J. W. HANOVER, *Heredity* **21**, 1 (1966).
[2] R. H. SMITH, *U.S. Forest Serv., Res. Paper* PSW-15 (1964).
[3] M. H. BANNISTER, A. L. WILLIAMS, I. R. C. MCDONALD and M. B. FORDE, *New Zealand J. Sci.* **5**, 486 (1962).
[4] M. M. BLIGHT and I. R. C. MCDONALD, *New Zealand J. Sci.* **7**, 212 (1964).
[5] M. H. BANNISTER, H. V. BREWERTON and I. R. C. MCDONALD, *Svensk Papperstid.* **62**, 567 (1959).
[6] D. B. MUTTON, In *Wood Extractives and their Significance to the Pulp and Paper Industries* (Edited by W. E. HILLIS), p. 331. Academic Press, New York (1962).
[7] N. T. MIROV, *U.S. Dept. Agr. Tech. Bull.* No. 1239 (1961).

good evidence that the monoterpenes of white pine cortex oleoresin exhibit little quantitative variation between vegetative propagules of identical genotypes when grown in a single location.[1]

The present study was undertaken to determine the reliability of a standard individual tree sampling procedure for predicting phenotypic values and also to determine the effect of three different sites or macroenvironments on cortex terpene levels. Emphasis is placed on cortical rather than on wood terpenes. Cortical terpenes are believed to be most reliable for genetic analysis because they are close to the site of terpene synthesis, the epithelial cells, and are also in close association with photosynthetic tissues.

RESULTS

Of the eight terpenes in white pine[1] only five are considered in this study. Two of the eight terpenes are unidentified and another, camphene, is present in such small quantities that precise measurement is difficult. Under the conditions of these analyses, traces of other compounds could be obscured by the predominant terpenes. α-Pinene, β-pinene, myrcene, 3-carene, limonene, and the sum of these five compounds are included in the analyses.

There is only slight variation between growth of different years or between north- and south-facing branches on the three trees sampled (Table 1). However, even this small variation follows a pattern. The south-facing tissues tend to be higher in α-pinene and myrcene. There are also indications that the current-year tissue may have less α-pinene, β-pinene, and limonene and more myrcene and 3-carene than the older tissues. These differences are borne out in much greater degree in the relatively old stem tissue (Table 2). The differences within the younger tissues and between aspects of the tree are small relative to the between-tree, mainly genetic differences. In fact, as is shown, repeatability coefficients[8] (i.e. intraclass correlation coefficients) computed for the monoterpene data of Table 1 are high, which is additional evidence for strong gene control. High repeatability also verifies that a few standard samples taken from specific tissues of the tree are adequate to measure phenotypic values for terpene concentration.

In contrast to the uniformity of monoterpene concentrations in different tissues up to 4 years old, shown in Table 1, samples from the mainstem or from resin blisters are much different (Table 2). α-Pinene and β-pinene concentrations increase, myrcene and 3-carene decrease, and limonene remains unchanged in comparison with samples from the more recently formed tissues. There is also a net decrease in total terpene composition. Some of the tree-to-tree differences are preserved in the older-tissue (mainstem) samples but others are not. Therefore it seems quite necessary, at least in quantitative genetic studies of the monoterpenes, that young, cortical tissue be used to assure precise measurements of monoterpene concentration. Ideally, sampling should be confined to a single year's growth and a single aspect of the tree. Resin blisters should be avoided, although the mainstem cortex and blister samples were very similar in this study. These procedures were followed in the following phase of this study.

Analysis of genotypically identical plants growing at the three Idaho sites and reflecting diverse nutritional conditions revealed practically no differences in monoterpene levels associated with site (Table 3). The slight variation in values between sites is undoubtedly due mostly to experimental error. Considerable differences in monoterpene levels between genotypes are again evident from the data.

[8] D. S. FALCONER, *Introduction to Quantitative Genetics*. Ronald Press, New York (1960).

TABLE 1. VARIATION IN WHITE PINE CORTEX MONOTERPENES WITH AGE OF BRANCH TISSUE AND WITH LOCATION ON THE TREE

Tree no.	Tissue age (yr)	North-facing branches						South-facing branches					
		α-Pinene	β-Pinene	Myrcene	3-Carene	Limonene	Total*	α-Pinene	β-Pinene	Myrcene	3-Carene	Limonene	Total*
		Percent of oleoresin											
19	0	3·0	1·3	3·0	27·1	1·4	35·8	4·6	1·7	4·1	21·5	2·1	34·0
	1	4·3	2·1	3·1	28·5	2·5	40·5	6·1	2·2	3·6	18·6	3·6	34·1
	2	6·4	2·3	2·9	18·8	3·4	33·8	7·1	2·2	3·8	16·3	3·6	33·0
	3	5·6	2·7	3·1	20·2	3·4	35·0	6·9	2·1	3·6	15·8	2·7	36·2
Average		4·8	2·1	3·0	23·7	2·7	36·3	6·2	2·0	3·8	18·1	3·0	34·3
22	0	2·3	3·6	5·8	15·9	2·2	29·8	2·3	3·4	5·7	14·1	0·5	26·0
	1	2·6	4·4	4·3	13·6	1·0	25·9	3·2	5·3	5·5	14·3	0·8	29·1
	2	2·8	4·1	4·2	12·7	1·1	24·9	3·5	4·7	5·4	14·0	1·3	28·9
	3	3·2	4·1	4·1	12·4	1·7	25·5	3·4	4·6	4·7	13·0	1·1	26·9
Average		2·7	4·1	4·6	13·6	1·5	26·5	3·1	4·5	5·3	13·8	0·9	27·7
58	0	4·8	12·9	9·9	7·8	1·8	37·2	5·8	14·9	9·5	7·0	2·0	39·2
	1	5·0	12·7	8·8	8·1	2·6	37·2	5·4	12·9	9·3	7·2	2·3	37·1
	2	5·9	15·8	7·5	6·1	2·1	37·4	5·5	12·4	8·4	5·8	1·5	33·6
	3	5·7	14·2	7·8	5·2	2·7	35·6	6·0	13·7	8·9	6·3	2·0	36·9
Average		5·4	13·9	8·5	6·8	2·3	36·8	5·7	13·5	9·0	6·6	2·0	36·7

* Does not include camphene or unknowns 1 and 2.
Repeatability coefficients (r) are α-pinene 0·76; β-pinene 0·98; myrcene 0·99; 3-carene 0·86; limonene 0·63; total 0·87.

TABLE 2. MONOTERPENE LEVELS IN OLEORESIN COLLECTED FROM MAINSTEM TISSUES

Tree no.	Tissue sampled	α-Pinene	β-Pinene	Myrcene	3-Carene	Limonene	Total*
		Percent of oleoresin					
19	Mainstem blister	14·2	4·1	2·3	1·2	3·8	25·6
22	Mainstem cortex	8·8	18·8	0·0	0·4	0·6	28·6
	Mainstem blister—south	6·7	7·2	2·0	0·9	0·8	17·6
	Mainstem blister—north	7·9	8·0	2·0	1·2	1·3	20·4
58	South fork, stem blister	12·9	17·6	0·0	1·9	3·4	35·8
	North fork, stem blister	11·7	14·9	0·0	2·3	2·3	31·2
	South fork, stem cortex	11·2	15·1	3·8	1·9	2·3	34·3
	North fork, stem cortex	11·5	16·0	2·3	1·4	2·0	33·2

* Does not include camphene or unknowns 1 and 2.

TABLE 3. MONOTERPENE LEVELS IN FOUR CLONES GROWING AT THREE LOCATIONS

Clone no.	Site no.	α-Pinene	β-Pinene	Myrcene	3-Carene	Limonene	Total terpenes*
		Percent of oleoresin†					
I	1	5·0	0·4	5·0	1·2	16·4	28·0
	2	4·6	0·2	4·8	0·8	14·6	25·0
	3	5·4	1·4	4·6	2·2	13·2	26·8
II	1	4·6	13·4	2·9	12·0	2·3	35·2
	2	4·7	14·3	2·2	11·0	1·6	33·8
	3	4·4	13·5	2·6	11·0	2·1	33·6
III	1	5·5	19·2	3·9	0·5	3·3	32·4
	2	7·2	23·5	3·8	0·3	1·7	36.5
	3	7·2	20·8	3·8	1·8	3·6	36·2
IV	1	5·2	5·4	6·6	0·4	13·4	31·0
	2	4·8	5·3	7·6	0·3	10·8	28·0
	3	6·0	4·0	6·0	0·8	10·3	27·1

* Does not include camphene, or unknowns 1 and 2.
† Each value represents the average of two trees.

MATERIALS AND METHODS

The study consists of two phases: (1) variation within individual, mature trees, and (2) variation between grafted, genetically identical trees established at three geographic locations. For the within-tree variation patterns, three trees growing near Fernwood, Idaho, were sampled. On each tree a large limb facing north and one facing south were selected at midcrown position in the tree. Oleoresin samples were taken in July from current-year, 1-, 2-, and 3-year-old tissue at each of four successive internodes. A few samples were also taken at midcrown on the mainstem of each tree from blisters and from smooth bark tissue. One of the trees forked about midway up the stem and samples were taken from each stem of the fork. The tissues represented in this study also differed in the amount of foliage borne. In general, the younger the tissue, the more foliage was associated directly with the tissue. The mainstems from which samples were obtained did not bear any foliage directly.

Plant materials used to study the effects of site variation on terpenes consisted of three

established clonal plantings located at Sandpoint, Moscow, and Clarkia—all in northern Idaho. These locations differed considerably in soil, moisture, and other climatic and site qualities. Two trees from each of four clones were sampled at each location.

The method of sampling oleoresin is the same as described previously.[1] Thirty-μl samples were drawn from the cortex into calibrated 1-mm capillary tubes, placed in a sealed centrifuge tube, and refrigerated until analyses were made.

Each sample was diluted with 50 μl of acetone just before analysis. A 4-μl aliquot from this solution was injected into an F & M Model 500 gas chromatograph with a Model 1609 flame ionization detector and disc integrator. The column was $0{\cdot}6 \times 180$ cm stainless steel packed with 10% polypropylene glycol on 60–80 mesh Diatoport W-AW. Column temperature was 95°, injection port and detector temperatures were both 195°, and helium flow rate was 160 ml/min.

Quantitative measurements of the monoterpenes based on peak areas were obtained from standard curves prepared from known terpenes run on the same column and under the same conditions as the unknown oleoresin samples. Monoterpene composition is expressed as a percentage of the oleoresin, and total terpene values do not include the unknowns.

CONCLUSIONS

The results of this study substantiate earlier findings that monoterpene concentrations in *Pinus monticola* Dougl. are under fairly rigid genetic control and are quite independent of climate or site characteristics. There is some evidence that the monoterpene composition of oleoresin varies only slightly from north to south aspects of the tree but there are substantial differences between recently formed foliage-bearing tissue and relatively old nonfoliage-bearing portions of the tree.

On the basis of these and previous results with western white pine it is concluded that a consistent method of sampling should be followed for terpene analyses with respect to position on the tree, age of tissue, and collection and handling techniques. Relatively young tissue should give more meaningful information about the genetic control of levels of individual terpenes. The nature of the variability in terpenes shown by the data suggests that, for genetic analyses at least, it is desirable to express the concentrations of oleoresin terpenes as a proportion of the oleoresin rather than as a percent of either total terpenes or a reference terpene, as is commonly done. This procedure minimizes the possibility of masking certain genetic differences that may exist in terpene composition.