

GEOGRAPHICAL VARIATION IN THE TERPENE COMPOSITION OF THE LEAF OIL OF DOUGLAS FIR†

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ABSTRACT

The two forms of Douglas fir, *Pseudotsuga menziesii* var. *menziesii* and var. *glauca*, differ quite considerably with regard to the monoterpenes of their leaf oils. Several chemical races, differing quantitatively in certain monoterpenes, appear to exist in each variety and the leaf oil composition seems to be particularly useful in classifying intermediate forms.

INTRODUCTION

Chemosystematic studies based on terpene analysis have advanced from the qualitative approach, as used by Erdtman¹ in his pioneer work on the heartwood extractives of conifers, and a semi-quantitative one by Mirov² with the turpentine of most pine species, to a quantitative method with the development of gas-liquid chromatography (GLC)³. Several groups of workers³⁻¹² have shown that quantitative analysis can lead to useful systematic correlations at the species and sub-species levels. Qualitative differences appear to be significant only in higher taxa. Since one is dealing with volatile compounds precautions in sampling, transportation and storage of the plant material as well as in steam-distillation, recovery and GLC analysis must be taken to ensure reproducible results. The relative amounts of individual terpenes in an oil can be determined with a reproducibility of 0.1 per cent, but this increases to 0.5 to 1 per cent when different columns and instruments are used. Losses owing to volatility, autoxidation and polymerization can increase the error further. To this one must add the inherent biological variability within a given plant sample. Hence, an error of 2 to 5 per cent in the individual values must be accepted, but this is usually well within the variation of one plant or population of plants to another and hence is entirely satisfactory for most quantitative chemosystematic studies.

In our chemosystematic studies of North American conifers we have concentrated on the volatile oils of the leaves (or foliage) for several reasons. These oils contain a wide spectrum of mono- and sesqui-terpenes and in some cedar species also diterpenes¹³. In contrast, the volatile oils of the wood, blister resins or bark^{2, 9, 10, 14} are composed mainly of monoterpene

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hydrocarbons. Therefore, the leaf oils meet better the first requirement which Harborne¹⁵ put forward for chemosystematic studies. This states that chemical complexity and structural variability are highly desirable features. The presence in leaf oils of many oxygenated terpenes in addition to related hydrocarbons is a distinct advantage even though this adds to the analytical problems. However, the use of several GLC columns with different liquid phases resolves most separation problems, and in addition, provides replicate quantitative data³.

In conifers which have distinct needles there is a further advantage because a single anatomical organ is at hand. The resin canals of the needles of e.g. pines¹⁶ are not connected with those of the twigs and hence cross-contamination with the oil of the xylem, cortex or phloem can be avoided. As shown in our recent study of seasonal variation in the foliage oils of *Picea glauca*¹⁷ the buds and twigs have a different oil composition from the leaves and these should be removed in critical studies. In contrast with the wood or bark of conifers, the leaves offer young plant material in which a choice of age is readily available. Leaves which are older than four or five years should, however, be avoided since these may be dead or dying and hence no longer provide representative data.

In contrast to Erdtman's work with heartwood extractives¹, we dealt with live plant tissue in the dormant state (autumn or winter collection) which may be subject to physiological changes. Hence, transportation and storage in the dark and cold is usually required. As shown in several of our studies^{3, 4, 8, 15, 17} the leaf oil composition does not change during the autumn and winter. Von Schantz and Juvonen⁶ made a study of the leaf, branch, wood and root oils of many *Picea* species, but unfortunately they collected their plant material in summer when the quantitative variation in the new leaves is very high¹⁷. This limited their systematic correlations and makes comparison with our data difficult. If the foliage is collected in the dormant season, and the precautions mentioned are taken, reproducible results may be obtained year after year¹⁷. This reproducibility suggested to us that the composition of the leaf oils of conifers may be under strict genetic control and hence offers a sound basis for chemosystematic studies.

Our work with the leaves or foliage (leaves, buds and twigs up to four years old) of North American spruce^{7, 8, 18-21} pine²², Douglas fir²³ and juniper^{4, 24-29} and cedar species¹³ has shown that:

- (a) Most species have characteristic quantitative leaf oil compositions. Species of the same genus usually (but not always) produce the same terpenes but in different amounts. Qualitative differences are usually found only amongst different families.
- (b) In many species the within-tree variation is smaller than the experimental error, provided a sufficiently large leaf or foliage sample is taken during the dormant season.
- (c) The tree-to-tree variation within a population is often small. When this is so, possible differences amongst populations of the same species can be studied in a quantitative manner.
- (d) Provenance samples (5 years and older) give the same results as the parent population irrespective of ecological differences.
- (e) Hybrids have terpene compositions that are intermediate to those of

the parents. Introgression may be studied when the leaf oil compositions of the species involved differ sufficiently to permit detection of different hybrids.

This accumulated information indicated that in species which show a relatively low tree-to-tree variability within typical populations, geographical differences may be studied quantitatively by leaf oil analysis. This was confirmed in part by the initial results obtained with Douglas fir²³. We have now extended this study over a wider geographical region and find that inherent geographical differences may be described in a quantitative manner.

TERPENOID VARIATION IN DOUGLAS FIR LEAVES FROM DIFFERENT LOCALITIES

The Douglas fir, *Pseudotsuga menziesii* (Poir.) Franco (syn. *P. taxifolia* (Poir.) Britt.), has a very wide natural range occurring on the western portion of the North American continent from 37° to 53° latitude at the coast and 23° to 55° in the interior with an overall longitudinal range from 100° to 128°³⁰. The elevation may range from sea level to 3500 metres and the climatic extremes from +45 to -40°C and 200 to over 4000 mm precipitation. Such a wide geographical and ecological range would lead to the expectation of wide genetic variations within Douglas fir³⁰⁻³², and many attempts of subdividing this species into different varieties or even species have been made. Notwithstanding considerable morphological variation, authorities agree only on two major subdivisions, the coastal or green variety, *P. menziesii* var. *menziesii* (syn. var. *viridis*), and the Rocky Mountain or blue variety, var. *glauca*. Harlow and Harrar³³ recognized these two varieties as clearly distinguishable taxa, but pointed out that intermediates occur in central British Columbia. These intermediate forms are known to European foresters as var. *caesia*, but only the coastal and Rocky Mountain forms can be satisfactorily distinguished by morphological characters³¹.

The difficulty in classifying various forms of Douglas fir and in distinguishing intermediate forms offered a severe test for our chemosystematic method. Fortunately, the two recognized varieties have leaf oil terpene compositions which differ markedly²³. That of the coastal variety is characterized by relatively large amounts of β -pinene, sabinene, α - and γ -terpinene, terpinolene and 4-terpinenol and smaller amounts of citronellol, citronellyl acetate and geranyl acetate. However, there was considerable tree-to-tree variation in many coastal populations and a north-south cline was suspected to exist²³. The Rocky Mountain variety has a leaf oil with relatively large amounts of camphene, bornyl acetate, limonene, santene and tricyclene, much less β -pinene than the coastal variety, and less than two per cent of the other typical coastal variety terpenes (*Table 1*). The tree-to-tree variability was found to be low when populations in the Canadian Rockies and Alberta foothills were examined. The samples from central British Columbia had various intermediate terpene patterns. In the interior dry-belt regions the Rocky Mountain terpenes predominated, whereas in the Cascades (Fraser Canyon and east of Hope) the coastal terpenes were present in larger amounts.

Previously, we encountered difficulties in determining the amount of sabinene when relatively large amounts of β -pinene were present and reported

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Table 1. Characteristic relative percentages of the significant terpenes of the leaf oil of Douglas fir from British Columbia

Compound	Peak No.†	Coastal	Percentage Range		Rocky Mountain
			Coastal Intermediate	Interior Intermediate	
Santene	3	—	0.1-1	1-4	3-5
Tricyclene	4	—	0.1-1	1-3	2.5-4
α -Pinene	5	7-15	8-15	12-18	15-20
Camphene	6	0-0.2	0.3-8	15-25	20-30
β -Pinene	7a	20-35‡	15-30	5-20	5-10
Sabinene	7b	2-15	2-12	0.5-5	0.1-0.5
α -Terpinene	10a	2-5	1-3	0.1-1.5	0-0.3
Limonene	11b	0.5-1.5	1-3	3-10	5-10
γ -Terpinene	14	3-8	2-8	0.1-4	0.1-1
Terpinolene	15	5-20	5-15	1-5	0.5-3
4-Terpinenol	21	5-15	5-15	1-5	0.5-3
α -Terpineol	22	1-3	1-3	0.5-2	0.2-1
Citronellol	23	1-5	1-3	0.5-2	0.1-1
Bornyl acetate	24	0-0.3	0.5-5	15-25	20-30
Citronellyl acetate	25	2-4	2-6	1-3	0.1-2
Geranyl acetate	26	1-3	2-5	0.5-2	0.1-1

† As designated previously from GLC data^{2,3}

‡ Central coastal population: higher in northern, lower in southern populations (see Figure 1)

these two components as a combined percentage. Improvements in the gas chromatographic technique have overcome this problem and re-analysis of the leaf oil samples examined previously shows that the relative amounts of β -pinene and sabinene, as well as those of α - and γ -terpinene, terpinolene and 4-terpinenol can serve as an excellent measure of variation in coastal populations when 10 trees per population are sampled. Using the data from previous and new collections it could be confirmed that a north-south cline, or possible clustering of different ecotypes, exists. This is born out by the histograms shown in Figure 1, which compares the mean values from 10 trees of the Mesatchie Lake population (Vancouver Island, 48° 45'N) with those from Elbe (Washington, 47° 0'N) and those from Cave Junction (southern Oregon, 42° 08'N). The reduction in relative percentage of β -pinene (peak 7a) and corresponding increase of sabinene (peak 7b), α -terpinene (peak 10a) γ -terpinene (peak 14), terpinolene (peak 15) and 4-terpinenol (peak 21) is clearly visible. The typical terpenes of the Rocky Mountain variety were almost completely absent. Minor changes in the relative amounts of the acyclic terpenes citronellol, citronellyl acetate and geranyl acetates (peaks 23, 25 and 26) as well as of α -pinene (peak 5), limonene (peak 11b), and α -terpineol (peak 22) were also recorded. Since the tree-to-tree variation in these coastal areas is as high as or higher than that recorded previously for the Haney and Hope populations, the latter changes may be too small to be significant, but they do lend further support that a cline or clustering exists in the coastal populations. The relatively high variability in coastal populations can be considered as an expression of inherent genetic variability (rich gene pool) that may be the basis of successful adaptation of the Douglas fir

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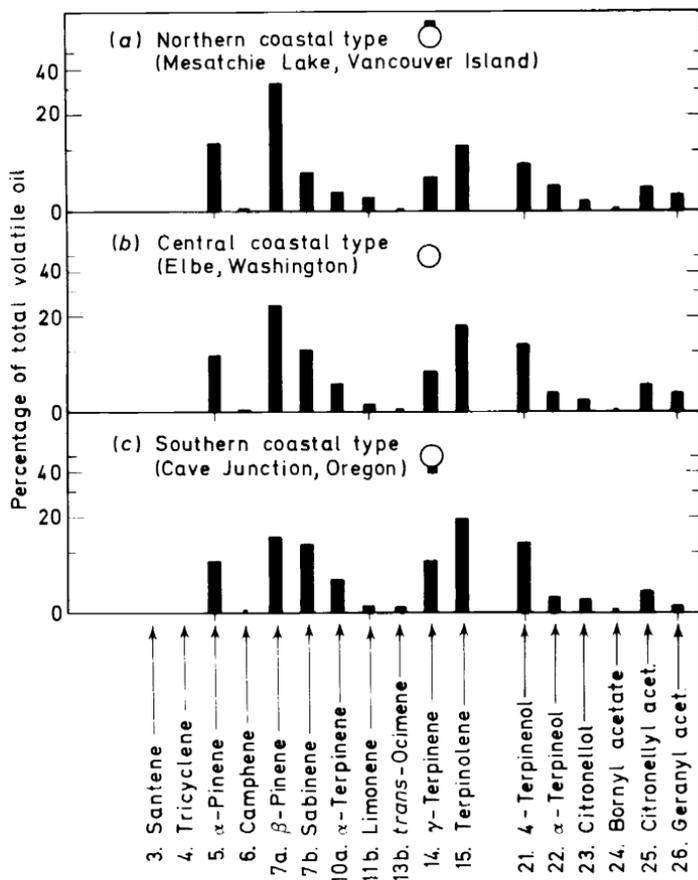


Figure 1. Bar-histograms of the terpene patterns (means from 10 trees): (a) Northern coastal type. (b) Central coastal type, (c) Southern coastal type.

to the widely different ecological conditions. The possible biosynthetic relationships of the characteristic terpenes was already discussed²³.

Re-analysis of the 3-year old provenance samples (Kemano, Lonesome Lake, Owl Creek, Thasis, Olalla; see Figure 2) from coastal populations growing at the Cowichan Lake Forestry Nursery showed that the relative amounts of terpenes differed from those of mature trees from similar populations. This may be because the total foliage of these seedlings had to be used to obtain sufficient amounts of plant material (10 to 30 g) for satisfactory analysis. As shown previously, the ratios of terpenes differ markedly in the oil of the leaves and twigs. Thus the weight of leaves and twigs and the yields of oil from these different parts may differ from those of older trees and hence result in significantly different terpene compositions. However, comparison of the mean percentages of β -pinene and sabinene of these provenance samples (e.g. Kemano 42.5, 2%; Thasis 37, 2.5% and Olalla 32.5, 4%,

respectively) confirms the north-south cline. Re-examination of the volatile oils from the leaves, twigs and buds of more mature trees (10 to 50 year old) confirmed that the differences in terpene percentages of that of the leaves alone and that of the combined foliage are small. In the coastal variety only the difference in percentage of 3-carene was outside the experimental error and in the Rocky Mountain variety those of santene and limonene. Hence, in

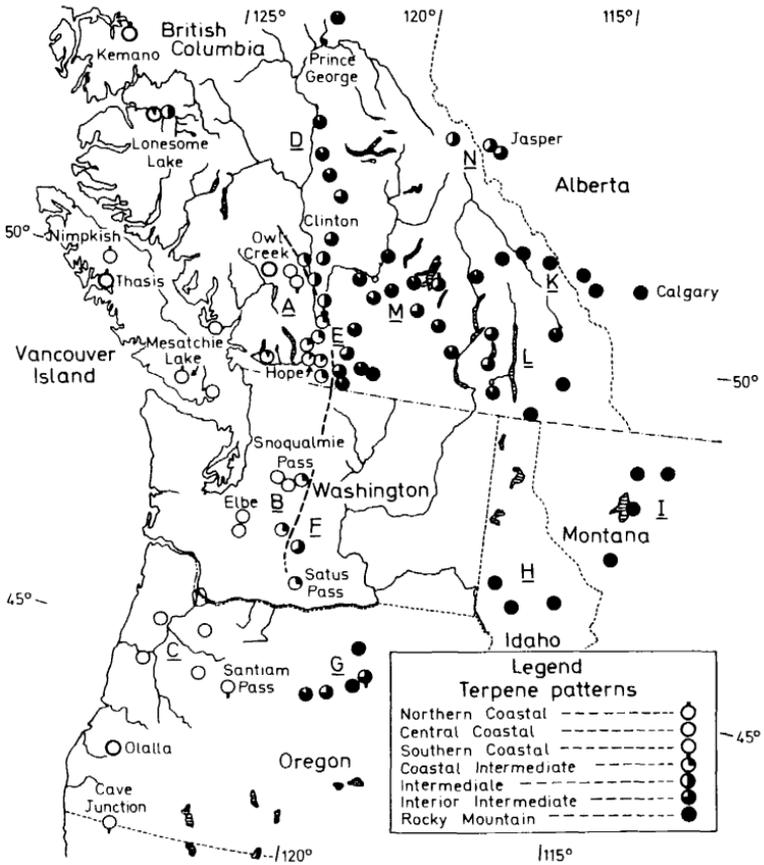


Figure 2. Map showing sites of collection and terpene patterns found. A to N refer to areas discussed in the text. The heavy broken line --- denotes the division between coastal and interior Douglas fir as based on terpene patterns. Provenances (coastal) shown as heavy open circles.

most studies the tedious procedure of separating leaves from twigs and buds can be omitted when more mature trees are sampled. In the present study both the combined foliage and the leaves alone were steam-distilled and the recovered oils were analyzed separately.

New sample collections ranging from the northern limits of the Douglas fir in central British Columbia (north of Summit Lake, 54° 30'N) along the

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Fraser River valley and the Cascades into Oregon as far south as Cave Junction (42° 08'N) and across the Cascades (Santiam Pass) to eastern Oregon, Idaho and Montana were made to complement those made previously. On the western slopes of the Cascades in British Columbia (Owl Creek-Pemberton-D'Arcy-Lillooet; area A, Figure 2) and in Washington (Snoqualmie-Elbe-Morton, area B) either coastal terpene patterns (see Figures 1a or 1b) or the coastal intermediate pattern (see Figure 3a) were

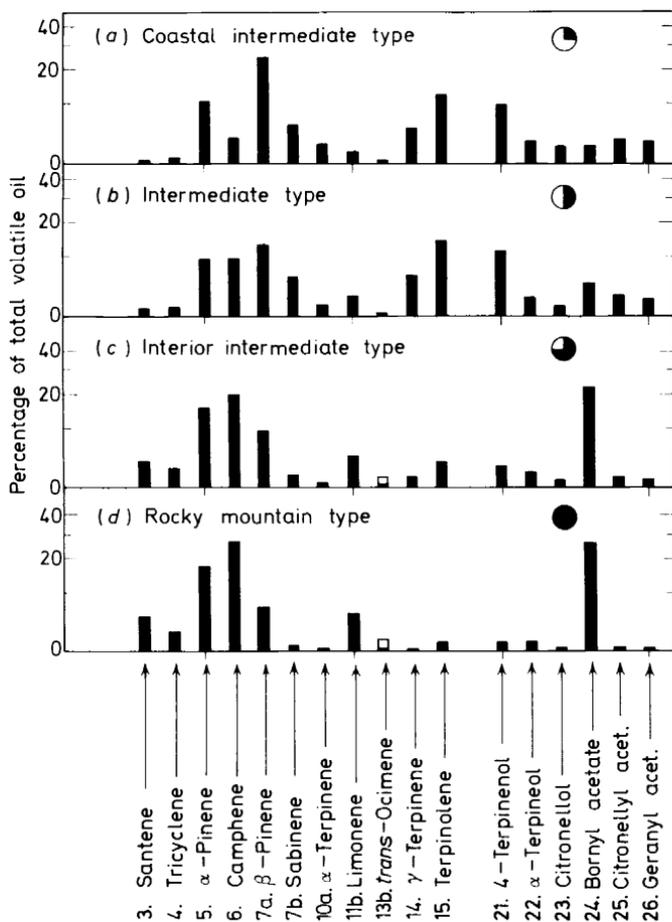


Figure 3. Bar-histograms of the terpene patterns (means from 10 trees): (a) Coastal intermediate type, (b) Intermediate type, (c) Interior intermediate type, (d) Rocky Mountain type.

recorded. In the samples from northwestern Oregon (area C) mainly the central coastal terpene pattern was recorded. However, out of the 10 trees of each population, 1 showed the northern and 2 or 3 the southern pattern. Along the divide and the eastern slopes of the Cascades rather variable

patterns ranging from the coastal intermediate to intermediate (*Figure 3b*) and even interior intermediate (*Figure 3c*) were found. Trees from the dry-belt have predominantly the terpene pattern of the interior intermediate type (Clinton, north to Prince George area D, east of Manning Pass, area E and in Washington east of the Snoqualmie and White Passes, area F). At Satus Pass in southern Washington there was a stronger tendency to the coastal intermediate and in Oregon no intermediate patterns were recorded in the transect from Foster to Santiam Pass. In fact, the southern coastal pattern predominated at Santiam Pass. Hence, it appears that the overlap of the coastal and Rocky Mountain varieties in the Cascades ends in southern Washington or northern Oregon. The heavy broken line in *Figure 2* shows the line of division as obtained with chemical data. It extends farther south than that shown in the range map of Fowells³⁰.

The samples from eastern Oregon (Ochoco and Malheur National Forests, area G, *Figure 2*), had either the interior intermediate or the true Rocky Mountain pattern (*Figure 3d*). However, one tree from the Flowers Gulch Summit had a new intermediate pattern with only 2% β -pinene and over 16% sabinene. This would indicate that southern coastal type genes which are characterized by high sabinene amounts (*Figure 1c*) are present in this part of Oregon. Since Douglas fir does not grow in the semi-arid region east of Santiam Pass to the Ochoco National Forest (Redmond-Prineville area) the bridge across which gene interchange could take place remains to be found. It is noteworthy, that in many of the trees sampled in the Ochoco-Malheur National Forest districts, relatively high amounts (4-9%) *trans*-ocimene were recorded. The trees sampled further east in Idaho (Kooskia-Syringa, area H), and Montana (north of Missoula, Flathead Lake, west and east of Glacier National Park, area I), had either the interior intermediate (near Kooskia) or the typical Rocky Mountain patterns. The tendency towards high percentages of *trans*-ocimene diminished and the means from the Rocky Mountain areas in Montana were similar to those recorded previously for those from the Calgary-Banff-Golden transect (area K). It cannot be ruled out that a southern Rocky Mountain terpene pattern with significant amounts of *trans*-ocimene exists. This will be studied further by us.

New samples from south-eastern British Columbia (Cranbrook-Nelson-Nakusp-Vernon, area L, M) gave fairly consistent results with the Rocky Mountain terpene pattern predominating near Cranbrook and Creston. A slight influx of the interior intermediate pattern was detected at Columbia Lake, near Nelson and east and west of the Monashee Pass (Needles-Cherryville) and interior intermediate patterns were found near Slocan and Nakusp. The Salmon Arm area (area M) was resampled and the tendency from interior intermediate to Rocky Mountain pattern was confirmed; the unusual pattern (intermediate with about 40% β -pinene) found previously in a single tree near Sycamous²³ was not encountered in any of these samples. The samples from the Clinton-Williams Lake-Prince George transect (area D) had either the interior intermediate pattern, or one between this and the Rocky Mountain type. Hence, the tendency towards the intermediate type found previously in the Tête Jaune Cache-Jasper area (area N) is somewhat unexpected. A complex situation was found in the areas between Lillooet, Pemberton and Sechelt (area A). Here the central coastal type predominated,

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and near D'Arcy a tendency towards the southern coastal type was recorded. In view of the northern coastal type being found on Vancouver Island, and the central coastal type (with a slight influx of the interior type) at Haney and Hope, a southern coastal type is unexpected. However, the situation may be complicated by the influx of interior types across the passes of the Cascades (near Lillooet and Hope). In this respect it is noteworthy that Bramhall, in his study of permeability of Douglas fir heartwood³⁴, also found unexpectedly low penetration values (similar to those of interior intermediates) in this area. Such areas, as well as possible correlations with wood permeability and other anatomical, genetical or morphological characteristics require further studies.

CONCLUSIONS

The results obtained with leaf oil analysis indicate that several chemical variants or 'races' may exist in each variety. The quantitative data obtained appear to be particularly useful in classifying the various intermediate forms between the coastal and Rocky Mountain variety, but geographic variations within each variety appear to be differentiated as well. The full range of the Douglas fir must be surveyed and correlation with some useful biological character, such as nuclear characteristics³⁵, must be sought to obtain acceptable classification beyond the *menziesii-glauca* division.

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