GEOGRAPHIC VARIABILITY OF MONOTERPENES FROM CORTEX OF *PSEUDOTSUGA MENZIESSII*

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ABSTRACT

Cortical oleoresin from over 300 trees of *Pseudotsuga menziessii* (Mirb.) Franco has been collected in localities throughout the entire US–Canadian range of this species and analyzed for monoterpenoids. Striking chemical differences particularly in sabinene and α-pinene percentages between the 'pure' coastal var. *menziessii* and the inland var. *glauca* (Beissn.) Franco have been encountered. Intergradation between the two varieties was extensive in Canada, spanning the area between coastal ranges and the Canadian Rockies, but was less pronounced in central Oregon, where only a few chemically intermediate trees could be located. Less intensive, albeit definite, chemical differences were found between oleoresins collected in the northern inland (var. *caesia* Aschers. and Graebn.) and the southern inland (var. *glauca* Schneider) localities. California populations from Sierra Nevada appeared to represent a separate chemical race, closer to inland than to coastal variety. The variations found are discussed on the basis of evolutionary pressures exerted by Wisconsin glaciations.

INTRODUCTION

Douglas fir (*Pseudotsuga menziessii* (Mirb.) Franco) grows extensively throughout western North America from about the 55th parallel in British Columbia south to the US–Mexico border; it also appears sporadically in the mountain regions of northern and central Mexico (*Figure 1*)[1, 2]. *P. menziessii* is a typical middle elevation species, ranging from sea level to about 800 m in the north (Vancouver Island) and from 2400 to 2900 m in the south (southern Rocky Mountains).[3] Because of its commercial importance, many attempts have been made to plant it outside its natural range and consequently forests of this species can be found in many parts of the world, particularly throughout Europe[4a].

No intermediacy problems connected with other species seem to exist in the US–Canada parts of its range, as the only other *Pseudotsuga* species indigenous to this area is *P. macrocarpa* (Vasey) Mayr, which grows in the coastal mountains of southern California, with the respective ranges separated by a distance of about 30 km[5]. Furthermore, *P. macrocarpa* has been reported to hybridize with *P. menziessii* with difficulty[6], possibly because of a difference in chromosome numbers[6–8].

Proposals have been made to split *P. menziessii* into as many as ten separate

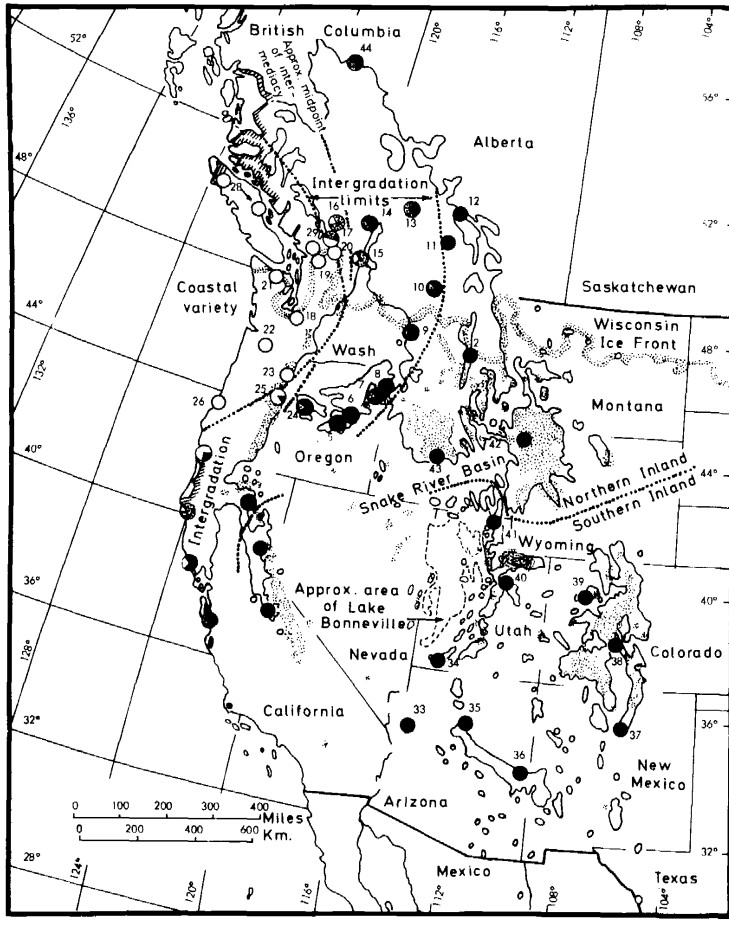


Figure 1. Range of *Pseudotsuga menziessii* and extent of the Wisconsin glaciations (gray). Dotted lines indicate separation of the varieties and chemical races of this species, while the rings indicate the populations sampled with approximate per cent of var. *glauca* (black) and intermediates (gray) indicated.

species[9], but separation into a few more or less well-defined varieties has gained a general acceptance. Some authorities, particularly in the US, Canada, and Great Britain recognize two varieties (or forms)—var. *menziessii*, indigenous to coastal British Columbia, western Washington, western Oregon and California, and var. *glauca* (Beissn.) Franco, found inland, in southwestern Alberta, central British Columbia, Montana, Idaho, eastern Washington and Oregon, and throughout the Rocky Mountains southward to Mexico[2, 3, 10–12] (*Figure 1*). In continental Europe, however, this species is commonly treated as three varieties —var. *viridis* Aschers. and Graebn. (synonymous with var. *menziessii*); northern inland var. *caesia* Aschers. and Graebn.; and southern inland var. *glauca* Schneider[4b, c, 13–15]. The northern

inland and coastal varieties come in contact in British Columbia and Northern Washington and Idaho and intergrade, which results in appearance of intermediate forms.

Apparently no single morphological characteristic is sufficient for distinguishing the varieties mentioned, although overall differences[4c, 10, 13, 16] are definite enough to warrant accepting the existence of at least var. *menziessii* and var. *glauca* (Beissn.) Franco. This distinction becomes important particularly in connection with the planting of *P. menziessii* in view of the intervarietal differences in longevity, growth-rate, size, frost and draught resistance, tolerance, cone producing age, fungus and insect resistance and wood and bark properties[4c-g, 10, 14, 16]. The higher specific gravity of the wood of var. *menziessii*[4d] and its higher permeability[4e] in preservative treatments, caused by a more symmetrical position of the torus within the bordering pit cavity[17], is of a considerable concern to the timber industry.

Although there is an extensive literature on the chemical composition of essential oil from various organs of *P. menziessii*[18] only a few attempts have been made to use this information as a taxonomic tool. The difference in odour between the essential oils of var. *menziessii* and var. *glauca*, secured either from cortex[4g], or from foilage[16] has been noticed before; in the latter case this difference has been explained by the presence of larger amounts of geraniol in the var. *menziessii* and of 'pinene' and bornyl acetate in the var. *glauca* oil. Hancock and Swan[19], working with wood obtained in British Columbia, found little intervarietal difference in terpenoid composition, while Hanover and Furniss[20], using samples secured in three different localities in central and northern Idaho and eastern Montana, reported significant geographic differences in some wood monoterpene components of var. *glauca*.

Working with cortical blister oleoresin obtained from ten trees of var. *menziessii* (collected in coastal ranges of central California), and var. *glauca* (collected in western Montana), we reported in 1965[21] remarkable differences in the majority of monoterpene components. The present study represents a continuation of this effort and is based on analyses of 302 oleoresin samples secured in 37 geographically different locations (*Table 1*; *Figure 1*). The area investigated covers the entire ranges of var. *menziessii* and var. *glauca* with the exception of Mexico, California and southern Oregon to be dealt with later. Methods of sampling and analysis were described by us before[22].

INTRASPECIFIC VARIATION IN MONOTERPENES

Differences between varieties *menziessii* and *glauca* (Beissn.) Franco which we encountered in our previous work were generally substantiated, and were found to involve nearly the entire spectrum of monoterpenes present (*Tables 2-6*). Accordingly, we separated the data on the basis of their geographic provenance and biological information into three categories—two representing allegedly pure populations (*Table 5A*), and the third covering the areas of reported or suspected intergradation (*Table 5B*). The last category covered mainly the central British Columbia, central and eastern Oregon and the Idaho Panhandle data; these were also found to be generally intermediate chemically, as expected. California Sierra Nevada populations were

Table 1. Collection Localities

Pop. No.	Number of samples	Empirical designation	Latitude	Longitude	Elevation (metres)
Pure Inland Varieties					
36	8	Springerville Arizona	34° 21.6′	109° 18′	2500
33	8	Hualpai Mnt. Arizona	35° 7.0′	113° 52.6′	1800
35	8	Flagstaff Arizona	35° 18.6′	111° 43.5′	2750
37	8	Santa Fe New Mexico	35° 45.7′	105° 47.1′	2600
34	8	Cedar Breaks Utah	37° 34.6′ 37° 35.6′	112° 53′ 112° 56′	2900 2500
38	8	Salida Colorado	38° 25.7′	106° 5.7′	2700
39	8	New Castle Colorado	39° 39.5′	107° 37.4′	2100
40	8	Price Utah	39° 53.6′	110° 45.6′	2600
41	8	Logan Utah	41° 46.1′	111° 37.3′	1900
43	8	Ketchum Idaho	43° 47.3′	114° 27.8′	2000
42	8	Yellowstone Wyoming	44° 38.9′	110° 56.2′	2250
6	8	Dixie Pass Oregon	44° 32.2′	118° 33.4′	1500
8	9	Wallowa Mnts. Oregon	45° 43.2′	117° 16.2′	1350
2*	4	Missoula Montana	46° 49.5′	113° 56.5′	1200
11	9	Invermere British Columbia	50° 28.6′	116° 2.1′	900
12	12	Banff Alberta	51° 11.6′	115° 36′	1800
Pure Coastal Variety					
26	8	Reedsport Oregon	43° 37.3′	124° 2.3′	300
23	8	Bear Paw Camp Oregon	45° 9.1′	121° 38.0′	900
22	8	Scaponia Camp Oregon	45° 52′	123° 9.6′	200
18	8	Mt. Rainier Washington	46° 56′	121° 56.2′	1100
21	8	Port Angeles Washington	48° 2.7′	123° 25′	500
19	8	Glacier Washington	48° 54′	121° 53′	500
29	10	Haney British Columbia	49° 17.5′	122° 33.5′	370
20	8	Hope British Columbia	49° 20.5′	121° 19′	600
28	8	Vancouver Island British Columbia	Collected in locations ranging from Jean's Landing (50° 28′; 127° 26.5′; 300 m) to Campbell river (50° 07′; 125° 24′; 30 m)		

Table 1 (continued)

Pop. No.	Number of samples	Empirical designation	Latitude	Longitude	Elevation (metres)
Intermediate Populations					
5	9	Seneca Oregon	44° 4.9′	118° 57.5′	1450
24	8	Prineville Oregon	44° 21.6′	120° 28.5′	1300
25	9	Suttle Lake Oregon	44° 25′	121° 44.6′	1100
7	8	Union Oregon	45° 8.9′	117° 44.2′	1000
9	8	Heyburn St. Park Idaho	47° 21.4′	116° 46.1′	750
10	8	Bonner's Ferry Idaho	48° 51′	116° 22′	600
15	8	Hedley British Columbia	49° 15′	120° 00′	750
17	8	Alexandra Bridge British Columbia	49° 43′	121° 24′	300
16	9	Lytton British Columbia	50° 15′	121° 30′	300
14	6	Monte Creek British Columbia	50° 37.5′	119° 55′	750
13	8	Revelstoke British Columbia	51° 00′	118° 11′	600
44	9	McLeod Lake British Columbia	55° 00′	123° 1.9′	700

found to be chemically different from either of the two varieties, but in better accord with var. *glauca* than with var. *menziessii* chemistry (*Table 4*); as this is at variance with the commonly accepted var. *menziessii* status of the California populations it was decided to defer the statistical treatment of this material until later, after expanding it to include oleoresin samples from additional geographic locations, and analyses for higher boiling chemical constituents. In the present treatment we have also deemphasized the southern Oregon and coastal California populations which exhibit a chemically intermediate status between var. *menziessii* and the Sierra Nevada race.

Tests for normality indicated that data from pure coastal (var. *menziessii*) populations conformed to the Gaussian distribution with the exception of a small deviation in the case of limonene (*Table 7*). In pure inland material, only α-pinene and total terpene content of the oleoresin exhibited normal distributions, while myrcene and limonene deviated slightly; β-pinene, β-phellandrene, 3-carene and terpinolene deviated strongly, however. Construction of the distribution diagrams revealed a considerable skewing towards higher values in these four cases (*Figure 2*). As this skewing could have resulted from inadvertent inclusion in the total data of a few populations having abnormal means, the presence of skewing within individual populations was checked by statistical methods (see *Note* on page 423). Results indicated that data skewing was significant within individual populations too,

Table 2. Monoterpene composition of a var. *menziessii* population, collected at Scaponia Camp, Oregon (Pop. No. 22)*

Sample No.	α-Thujene	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene	Total Terpenes
2373	1.2	24.5	0.2	10.6	4.9	34.9	2.8	1.3	0.9	18.7	42.1
2374	2.7	16.2	0.3	2.2	17.6	35.0	3.4	1.4	1.4	19.8	31.0
2375	1.1	17.5	0.3	14.4	18.9	28.7	3.2	1.0	1.3	13.6	40.0
2376	1.0	11.5	0.2	9.3	20.2	35.4	2.8	1.4	1.1	17.1	36.7
2377	3.4	21.8	tr	3.8	20.4	30.7	2.7	0.8	0.8	15.6	31.1
2378	1.8	9.0	0.1	8.2	22.2	34.0	2.6	2.5	0.9	18.7	26.9
2379	1.0	11.2	0.3	25.4	16.0	26.0	2.2	2.3	1.8	13.2	34.3
2380	1.0	22.5	0.2	12.3	15.5	28.9	2.4	2.0	0.7	14.5	33.4
Mean	1.7	16.8	0.2	10.8	17.0	31.7	2.8	1.6	1.1	16.4	34.4

Table 3. Monoterpene composition of a var. *glauca* population, collected in Hualpai Mountains, Northwestern Arizona (Pop. No. 33)

Sample No.	α-Thujene	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene	Total Terpenes
2515	—	57.5	0.8	1.5	8.7	tr	6.6	23.8	tr	1.1	22.2
2516	—	56.0	0.8	1.3	21.4	tr	6.0	11.3	0.3	2.8	18.7
2517	—	43.2	0.9	1.1	5.9	tr	10.0	38.0	0.2	0.6	24.4
2518	—	66.6	1.1	1.5	6.5	—	7.4	16.9	tr	tr	15.6
2519	—	63.7	1.3	1.4	6.5	tr	5.3	21.4	tr	0.5	22.3
2520	—	45.9	0.3	1.0	10.1	—	9.5	32.2	tr	0.8	22.4
2521	—	58.5	0.6	1.2	6.5	tr	6.8	26.4	tr	tr	19.2
2522	—	49.2	1.2	1.7	4.3	tr	9.1	34.5	tr	tr	21.9
Mean	—	55.1	0.9	1.3	8.7	tr	7.6	25.6	0.1	0.7	20.8

Table 4. Monoterpene composition of a population collected near Yosemite National Park, East-central California (Pop. No. 32)

Sample No.	α-Thujene	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene	Total Terpenes
2507	—	58.4	0.7	14.8	15.8	0.3	1.9	3.5	1.0	3.6	20.4
2508	—	51.8	0.3	22.2	4.6	tr	3.9	14.2	tr	3.0	20.6
2509	—	50.6	0.6	34.2	2.2	tr	2.8	7.0	2.3	0.4	23.6
2510	—	67.0	0.8	14.8	2.0	tr	3.2	8.4	0.5	3.2	22.1
2511	—	42.8	0.7	24.3	12.4	0.5	3.8	12.0	1.6	1.8	21.4
2512	—	55.2	0.6	33.9	2.7	0.5	1.8	2.6	0.6	2.1	26.4
2513	—	51.9	0.4	23.9	—	—	5.0	11.2	2.0	5.3	20.6
2514	—	66.3	0.5	26.7	1.9	—	1.5	2.2	0.8	tr	23.4
Mean	—	55.5	0.6	24.4	5.2	0.15	3.0	7.6	1.1	2.4	22.3

Table 5A. Variability of the cortical turpentine on the population level. Pure populations

Pop. No.	α-Thujene	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene	ΣS^2	Total terpenes Means	S^2
Coastal populations (var. *menziessii*), arranged in order of increasing latitude.													
26	0.9	23.0	0.4	7.0	14.5	33.0	2.8	2.2	1.0	15.3	190.8	33.4	12.0
23	1.5	16.4	0.2	6.6	20.1	30.9	3.1	1.9	1.5	16.9	131.9	30.5	13.8
22	1.7	16.8	0.2	10.8	17.0	31.7	2.8	1.6	1.1	16.4	134.4	34.4	25.1
18	1.1	17.0	0.1	8.1	21.8	31.3	2.6	1.5	0.7	16.0	183.2	33.5	8.0
21	1.1	27.8	0.3	12.7	16.4	23.7	2.7	2.4	1.2	11.7	338.4	34.1	5.0
19	1.9	21.9	0.2	6.0	20.4	25.9	2.7	2.0	1.2	15.0	278.2	31.1	10.2
29	0.7	30.6	0.3	11.5	20.4	18.1	2.8	3.4	1.2	11.0	351.1	30.3	11.0
20	1.1	24.3	0.2	10.2	17.1	28.3	2.6	3.0	1.0	12.4	203.8	30.2	17.9
28	0.9	25.9	0.2	12.5	17.5	23.6	2.9	2.9	1.6	12.1	150.7	31.9	11.6
Southern Inland populations, collected below 42°30′													
36	—	46.4	1.6	3.8	1.5	tr	9.2	35.0	0.8	1.7	219.2	30.5	61.9
33	—	55.1	0.9	1.3	8.7	tr	7.6	25.6	0.1	0.7	185.5	20.8	7.9
35	—	40.3	1.0	1.4	4.4	0.4	10.7	40.9	0.1	0.8	158.2	25.0	9.3
37	—	49.8	1.4	8.6	4.9	—	6.4	22.7	3.0	3.2	324.2	24.6	33.4
34	—	42.7	4.0	5.8	12.5	tr	6.8	25.5	1.9	0.8	279.9	23.1	5.3
38	—	40.9	1.8	4.8	6.2	—	9.4	35.1	1.6	1.4	303.1	23.9	26.1
39	—	52.9	2.6	4.4	4.1	—	7.5	27.2	1.0	0.4	252.2	25.9	10.6
40	—	44.3	3.5	5.1	5.5	—	7.8	31.4	1.7	0.6	251.2	25.0	8.2
41	—	44.0	2.4	3.3	8.7	—	8.5	31.0	0.8	1.3	162.3	25.2	17.9
Northern Inland populations, collected above 42°30′													
43	—	68.8	1.2	4.4	9.0	—	3.0	6.9	0.8	5.8	455.1	29.4	22.8
42	—	68.6	1.7	4.2	5.8	—	4.1	12.2	0.6	2.9	209.2	31.9	23.4
6*	—	58.4	1.1	4.8	6.6	0.6	5.4	17.8	1.2	4.2	245.9	40.5	46.1
8*	—	59.5	1.1	10.4	6.1	0.5	4.1	12.7	3.4	3.3	200.4	36.0	74.3
2	—	75.9	0.6	8.4	0.9	—	2.3	8.6	2.4	0.9	186.9	n.det.	—
11	—	71.4	1.3	8.6	3.3	0.4	3.0	7.8	2.6	1.5	64.9	38.1	22.7
12	—	68.8	2.0	12.1	1.6	0.2	2.3	7.2	4.1	2.0	122.1	40.5	23.0

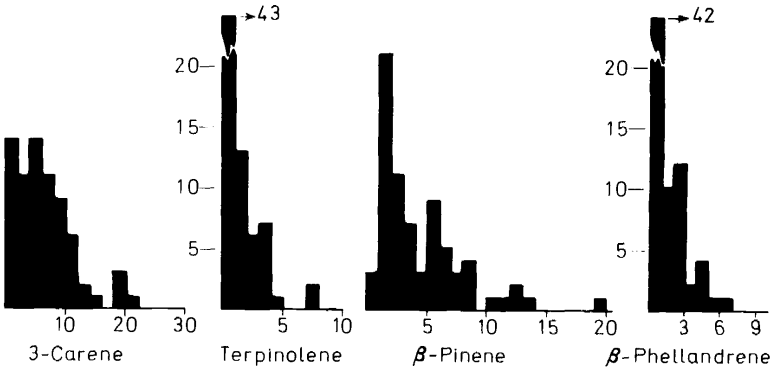


Figure 2. Strongly nonparametric distributions encountered.

which suggested a simple genetic control of terpene levels.† We found similar situations with other species.

As in most cases the terpene data either conformed to or deviated slightly from normal distributions, parametric statistical methods could usually be employed for the data analysis. With strongly deviating sets (comprising mostly quantitatively secondary compounds) nonparametric methods were used along with parametric treatment.

The significance of the differences between the var. *menziessii* and var. *glauca* was examined using the *t*-test, as well as the nonparametric *U*-test (*Table 6*). The probable unbiased, minimal error ('$P_{\%}^{min}$—see Appendix A) in assigning trees to one or the other variety on the basis of terpene percentages was also computed and the corresponding values are given in the same table. Statistics were calculated separately for the Canadian and northern US part of the respective ranges, because significant variations connected with latitude were indicated for either variety. Intervarietal differences usually appeared significant on a level as low as 0·0005. Particularly with α-pinene and sabinene, but also with α-thujene, terpinolene, and 3-carene '$P_{\%}^{min}$ was rather small and the differences substantial enough for use in the variety assignment of the individual trees. α-Pinene and sabinene thus afford a strikingly efficient way for distinguishing the above two varieties—more so than any morphology-based method available at the present time.

Analyses of variance for the pure coastal and for the inland data are given in *Tables 8* and *9*. Coastal data appeared rather uniform with variability on the population level connected chiefly with the sabinene family of terpenes (sabinene, α-thujene, and terpinolene). Applying Duncan's multiple range test to the population means indicated no clustering of any consequence.

† This argument is no more than suggestive in our case. Where data means are much smaller than standard deviations (a situation common in chemosystematics), the normality of distributions would require the presence of negative terpene percentages. As this is impossible, the respective terpenes would assume close-to-zero values and distort the otherwise normal distributions to skewed or bimodal ones.

Table 5B. Variability of the cortical turpentine on the population level
Intermediate populations

		α-Thujene	α-Pinene	Camphene	β-Pinene	3-Carene
[Oregon]						
5	Means	—	61.93	0.91	8.52	5.07
	Ranges	—	39.8–79.8	1.6–0.5	15.6–3.2	9.3–tr
24	Means	—	49.17	1.80	7.10	3.47
	Ranges	—	30.8–63.0	0–4.2	3.7–17.8	0–10.6
25	Means	1.54	20.95	0.32	11.12	19.62
	Ranges	0.9–2.1	12.2–35.1	0–0.5	1.0–19.7	7.5–32.6
7	Means	—	63.18	1.38	5.31	6.13
	Ranges	—	41.0–75.5	0.6–2.4	2.6–11.7	0.4–14.6
Canada/No. Idaho						
9	Means	—	68.88	1.35	6.93	2.78
	Ranges	—	60.2–78.8	0.7–2.5	2.7–15.9	0.7–5.9
10	Means	—	73.33	1.32	4.57	4.48
	Ranges	—	69.3–79.3	0.8–2.3	3.3–7.2	0.1–3.3
15	Means	0.58	50.76	0.47	12.67	5.90
	Ranges	0.6–1.1	26.0–71.5	0–0.9	1.8–25.1	0.4–18.1
17	Means	1.40	35.07	0.47	11.58	14.75
	Ranges	0–2.5	22.8–45.0	0.2–1.0	4.0–37.9	8.6–30.2
16	Means	2.25	41.53	2.29	13.20	10.23
	Ranges	0–7.6	8.5–59.6	0–5.7	2.7–20.7	4.4–19.8
14	Means	—	66.11	0.96	9.21	4.45
	Ranges	—	45.6–76.9	0.6–1.1	3.1–20.2	0.1–7.4
13	Means	0.38	65.37	1.32	8.56	7.28
	Ranges	0.8–2.3	55.1–81.1	0.4–3.0	2.1–20.2	2.6–15.8
44	Means	0.4	67.4	0.83	7.04	4.01
	Ranges	0–1.5	59.1–75.0	0.5–1.4	2.7–13.4	0–11.1
Mean Range for Coastal Pop.		0.4–2.1	10.8–35.5	tr–0.6	2.4–20.3	9.1–26.1
Mean Range for Northern inland Pop. 11, 12, 42, 43		—	51.2–79.2	0.9–3.1	3.5–13.8	0.8–14.1

Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolenes	Total terpenes
0.75 2.0–0.0	4.47 10.0–2.2	11.26 30.8–4.9	2.93 5.5–0.2	3.35 6.3–0.7	33.63 38.8–29.4
1.71 0–12.4	7.10 2.6–12.6	23.06 10.4–41.5	2.23 0–5.5	4.27 0–11.1	30.07 22.1–34.4
23.67 3.4–33.8	3.58 2.8–4.9	3.15 0.2–5.4	1.46 0.9–2.4	15.43 6.9–21.8	30.30 21.2–39.6
1.15 0.2–3.6	4.87 2.3–8.8	13.75 5.6–30.3	1.28 0.2–4.3	2.98 0.5–6.2	39.11 29.1–46.6
1.35 0.3–7.1	3.58 2.6–5.0	10.15 6.2–17.3	1.78 0.2–5.6	3.16 0.5–8.8	38.71 28.7–44.5
1.42 0.3–5.7	3.12 2.2–4.0	8.28 4.3–13.4	0.91 0.3–2.0	2.43 0.7–4.9	36.67 31.6–41.7
6.02 0–13.9	3.95 1.9–5.2	9.11 2.1–15.3	4.30 0.6–11.4	6.16 1.4–9.4	30.25 20.0–44.7
20.33 1.2–35.5	2.46 1.6–4.3	2.67 0.5–9.9	0.88 0.5–1.6	10.40 3.6–15.8	29.02 23.3–39.4
12.76 0.3–34.4	3.82 1.4–7.6	7.03 0.5–12.2	1.02 0.2–4.0	6.46 0–20.0	31.46 28.1–39.1
2.03 0.5–8.6	3.60 2.2–5.7	8.91 5.0–18.4	2.55 0.1–9.3	2.16 0.6–3.5	34.60 30.0–42.9
3.23 0.2–14.8	2.07 0.7–2.8	3.45 0.5–5.6	2.27 0.2–8.4	4.62 2.0–7.3	32.47 25.2–39.1
8.14 0.2–15.0	2.08 1.1–2.5	4.16 2.4–6.9	1.60 0.2–5.2	3.96 0–7.1	36.13 30.8–41.0
16.9–37.0	1.9–3.4	0.4–5.4	0.6–2.3	8.9–18.4	27.4–37.0
0–0.4	1.7–4.9	3.5–14.6	0.4–5.1	1.0–5.5	26.6–41.1

Table 6. Significance of the differences between coastal and inland data

Terpene	Canadian data				United States data			
	t	df	X	$P^{min}_{\%}$	t	df	X	$P^{min}_{\%}$
α-Thujene	11.1*	41	6.81*	5.0	10.5*	31	6.81*	3.4
α-Pinene	21.6*	61	6.81*	0.25	15.9*	27	5.53*	0.65
Camphene	5.4*	21	6.28*	16.0	7.2*	22	5.50*	11.0
β-Pinene	0.1^{NS}	26	0.22^{NS}	49.0	$2.2^{0.05}$	51	$1.94^{0.05}$	38.0
3-Carene	13.4*	63	6.70*	4.60	5.9*	30	4.48*	18.5
Sabinene	18.0*	41	6.81*	0.35	25.8*	31	5.60*	<0.01
Myrcene	0.5^{NS}	35	0.03^{NS}	47.0	1.20^{NS}	23	0.92^{NS}	41.0
Limonene	5.5*	29	5.07*	21.0	4.2*	20	5.29*	15.0
β-Phellandrene	4.2*	23	3.64*	24.0	$2.2^{0.05}$	22	3.49*	48.0
Terpinolene	19.0*	55	6.81*	0.75	18.7*	46	5.51*	1.5
Total terpenes	5.3*	29	5.02*	18.0	1.82^{NS}	37	1.47^{NS}	40.0
α-Pinene + Limonene	24.4*	62	—	0.11	22.2*	46	—	0.01

* Significance on 0.0005 level, NS not significant on < 0.05 level. Other levels indicated. Comparison was made separately for Canadian populations (19–21, 28, 29, versus 11, 12), and northern United States populations (18, 22, 23, 26, versus 2, 42, 43). $P^{min}_{\%}$ indicates the probability of making a wrong decision in assignment of a tree; X represents a statistic recommended by Goldstein[37] for establishing the significance level in the two sample rank test (U-test); using parametric approximation for N > 20.

Table 7. Normality of individual distributions*

Terpene	var. *menziessii*		var. *glauca*	
	Chi-square	df	Chi-square	df
α-Pinene	24.3	15	11.7	10
β-Pinene	24.1	11	56.8	7
3-Carene	6.8	4	88.6†	8
Sabinene	5.5	6	—	—
Myrcene	1.7	2	24.6†	4
Limonene	23.4†	5	28.1†	10
β-Phellandrene	6.1	1	105.4†	4
Terpinolene	5.1	4	88.7†	5
Total terpene	1.9	2	9.4	4

* See Ref. 36a and *Note* on page 423.
‡ Deviation significant on 99 per cent level.

Table 8. Analysis of variance for coastal populations‡

Terpene	Total, SS^2	df = 71 $\bar{S}_t^2$	Populations, SS^2	df = 8 $\bar{S}_p^2$	Individuals, SS^2	df = 63 $\bar{S}_i^2$	F $\bar{S}_p^2/\bar{S}_i^2$
α-Thujene	33.8	0.48	9.86	1.23	23.94	0.38	3.24†
α-Pinene	6683.1	94.13	1671.68	208.96	5011.42	79.55	2.63*
Camphene	323.5	4.56	0.43	0.05	323.12	5.13	0.01
β-Pinene	3145.4	44.30	435.20	54.40	2710.24	43.02	1.26
3-Carene	2673.2	37.65	369.92	46.24	2303.23	36.56	1.26
Sabinene	5360.4	75.50	1561.60	195.20	3798.76	60.30	3.24†
Myrcene	28.4	0.40	1.66	0.21	26.74	0.42	0.49
Limonene	279.7	3.94	27.52	3.44	252.22	4.00	0.86
β-Phellandrene	32.7	0.46	4.67	0.58	28.03	0.44	1.32
Terpinolene	992.6	13.98	330.98	41.36	661.70	10.50	3.94†
Total terpenes	892.8	12.57	188.16	23.52	704.59	11.18	2.10*

‡ After appropriate adjustment for unequal sample size.
* $F_{0.05} = 2.10$
† $F_{0.01} = 2.82$

Table 9. Analysis of variance for inland data**

Terpene	Total, SS^2	df = 119 $\bar{S}_t^2$	Varieties, *northern* vs. *southern* SS^2	df = 1 $\bar{S}_v^2$	Populations, SS^2	df = 13 $\bar{S}_p^2$	Individuals, SS^2	df = 105 $\bar{S}_i^2$	F $\bar{S}_v^2/\bar{S}_p^2$	$H_‡^†$ V/P	F $\bar{S}_p^2/\bar{S}_i^2$	H§ P/i
α-Pinene	25995.4	218.45	12032.80	12032.80	2103.88	161.84	11858.72	112.94	74.35†	11.1	1.43	28.1
Camphene	248.2	2.09	15.60	15.60	80.72	6.21	151.86	1.44	2.51	2.4	4.31†	31.4
β-Pinene	2307.0	19.39	303.48	303.48	782.92	60.22	2210.66	21.05	5.04*	3.1	2.86†	45.6
3-Carene	3798.8	31.92	24.48	24.48	960.00	73.85	2814.30	26.80	0.33	0.3	2.76†	41.7
Myrcene	1196.0	10.05	640.44	640.00	122.28	9.41	433.23	4.12	68.06†	11.1	2.36†	29.0
Limonene	21393.2	179.77	12146.16	12146.16	1969.20	151.48	7277.88	69.31	80.18†	11.1	2.19*	32.2
β-Phellandrene	404.6	3.40	28.80	28.80	148.16	11.40	227.68	2.16	2.53	2.1	5.28†	44.6
Terpinolene	510.9	4.29	133.44	133.44	134.24	10.33	243.21	2.31	12.92†	5.7	4.47†	33.3
Total terpenes	7504.1	63.06	3900.00	3900.00	986.56	75.89	2617.52	24.92	51.39†	9.4	3.05†	34.4

* $F_{0.05} = 4.67$ for df = 1/13; $F_{0.05} = 1.82$ for df = 13/105

† $F_{0.01} = 9.07$ for df = 1/13; $F_{0.01} = 2.33$ for df = 13/105

‡ Significance for nonparametric 'H' values at df = 1. $H_{0.1} = 2.71$, $H_{0.05} = 3.84$, $H_{0.025} = 5.02$, $H_{0.01} = 6.63$

§ Same for df = 13. $H_{0.05} = 22.4$, $H_{0.01} = 27.7$, $H_{0.005} = 29.8$

** Appropriately adjusted for unequal sample sizes

Note: Data skewing within individual populations was ascertained by computing the upper and lower median-based half-ranges for each terpene and each population (only populations with $N = 8$ samples were used), and examining by t-test the significance of the difference in means between the upper and lower sets of half-ranges separately for each terpene. Differences were significant on the 97.5% level with β-pinene and β-phellandrene, and on the 99.5% level with 3-carene and terpinolene from var. *glauca*.

Examining the changes in population means with latitude indicated, however, a significant increase in α-pinene and decreases in sabinene and terpinolene from south-to-north (rank correlations and significance levels: α-pinene, +0.717/0.975; sabinene, −0.800/0.99; terpinolene, −0.717/0.975); limonene, too, appeared to increase from south to north (+0.686/0.975), although the low F value of *Table 8* casts doubt on the reality of this change.

Turpentine variability of the individual populations was described by variance sums (ΣS^2 —see Appendix A) (*Table 5A*). The values obtained varied between 131 and 351, with localities more towards the centre, (Nos. 19, 21, 29) exhibiting higher variability ($t = 6.09$, sign. 0.01).

Results were different with inland material. Preliminary computations showed a strong contribution to the total variability from the variability population-to-population. Applying Duncan's multiple range test to the population means indicated a pronounced clustering into two groups, collected below and above 42°30′ latitude, respectively. This paralleled well the geographic delineation of the northern and southern inland varieties of Douglas fir, as suggested by Schenck[13] and used today by other workers in Germany[4a] (var. *caesia* Aschers. and Graebn. and var. *glauca* Schneider). Adding another tier to the analysis of variance (using nested design) indicated that this segregation was most pronounced with α-pinene, myrcene, limonene, and total terpene content of the oleoresin; it was also considerable with terpinolene, and small but significant with β-pinene. However, in spite of the high significance the differences separating the northern from the southern inland variety appeared less pronounced than those separating the coastal var. *menziessii* from the northern inland variety—either in Canada or in Oregon. Thus, $'P_{\%}^{\min}$ for northern–southern separation amounted to 7.7 per cent with limonene, 9.2 per cent with myrcene, 13.6 per cent with α-pinene, 24.5 per cent with total terpene content, and above 30 per cent with the rest. Within northern populations, slight affinity with the southern material was suggested in eastern Oregon samples (lower α-pinene in conjunction with higher limonene and myrcene—sign. level 0.0005/U-test) and in Idaho/Wyoming samples (lower β-pinene/β-phellandrene and total terpene contents, and higher 3-carene—sign. level 0.0005/U-test). This apparently influenced very little the sharpness of the break between northern and southern inland varieties and might represent an independent development.

As with coastal data, turpentine variability was described by variance sums (*Table 5A*). Geographically more marginal populations, Canadian No. 11 and 12 in the north, and Arizona No. 33, 35 and 36 in the south, tended to exhibit lower variabilities again ($t = 2.88/0.05$ and $t = 2.56/0.05$, respectively).

To test the results of the analysis of variance in view of the nonparametric, skewed distributions noticed with several monoterpenes, the distribution-free H-test was applied separately to data from northern and southern inland varieties, as well as to the combined population means. However, in no case did the aberrant distributions make themselves known (*Table 6*) and the results paralleled well the analysis of variance statistics; thus parametric approach proved sufficiently robust in our case.

As mentioned, the populations from central British Columbia and northern Idaho were found to be chemically intermediate between the coastal var. *menziessii* and inland var. *glauca* (Beissn.) Franco. Their respective analyses

are summarized in *Table 5 B*, together with the means and the mean ranges for the data from pure populations for comparison. In the same *Table* are given data for several populations from eastern Oregon in which a few intermediates were encountered. In a further statistical treatment we used terpene data to subdivide the individual trees sampled, into three empirically set categories—two categories represented pure individuals, and the third covered the chemical intermediates. The assumed distribution of intermediates was calculated by averaging means and standard deviations of the two pure distributions and the intervals defining the three categories were set so that the possible error in assigning a tree to a category was 5 per cent or less, otherwise the tree was designated as 'indefinite'. The calculations were performed separately for Canada and Oregon populations using Canadian and Oregon–Idaho–Montana data for computation of pure distributions. Only the two most significant terpene differences were considered: content on sabinene, and content on the α-pinene–limonene sum. As the latter two terpenes correlated strongly negatively and were higher in the inland varieties, the use of their sum reduced their inland variations and the crucial intervarietal differences were brought into focus. This is demonstrated by the more favourable $'P_{\%}^{\min}$ and *t*-values (*Table 6*).

The results obtained (*Table 10* and *Figure 1*) indicate a definite chemical gradient in the central British Columbia, spanning the area between the centre of coastal ranges and the southern part of the Canadian Rockies (*Figure 1*) but including their northern part. The intermediacy appears to be much weaker in central Oregon, however, where only a few clearly intermediate individuals could be located.

As discussed previously[23a], regression and correlation statistics of natural product data offer a way for speculating on biosynthetic pathways leading to these materials; the relevant statistics obtained in this work are listed in *Table 11*. However, interpretation of these is beset with several difficulties, one of which is connected with the non-Gaussian distribution of several data sets. This appears of little consequence in our case, however, as with the exception of the low significance 3-carene–terpinolene correlation, parametric and nonparametric treatments paralleled satisfactorily with each other.

Potential error connected with the geographic variability seems more serious. Use of correlation data for biosynthetic purposes assumed exclusion of any gene segregation and sampling from a completely randomized gene pool. The latter assumption does not hold if several populations are used in calculations, as evolutionary pressures can favour the appearance of certain terpenes together, independently of their biosynthetic relationships[23a, b]. The influence of this effect can be minimized by recalculation of the correlation statistics, using data from a reduced geographic area; we employed this method in our earlier publications[23b]. A more efficient way, which completely eliminates the geographic segregational factors, lies in the analysis of covariance and in statistics based on within sum-of-squares and cross-products. Application of this method resulted in elimination of several smaller correlations, with little effect on others (*Table 11*). Particularly significant appears to be the elimination of all but one correlation involving total terpene content of the oleoresins on one side and the percentages of individual terpenes in their total on the other. We had usually encountered this in previous

Tanle 10. Assignment of individual trees from intermediate populations

Collection locality	According to sabinene				According to α-pinene + limonene				
	Coastal	Indefinite (Interm. or coastal)	Intermediate	Inland	Coastal	Indefinite (Interm. or coastal)	Intermediate	Indefinite (Interm. or inland)	Inland
Canada									
Intervals (%)	100–20.1	20.1–8.2	8.2–1.1	1.1–0	0–39.8	39.8–47.4	47.4–67.7	–	67.7–100
17 Alexandra Bridge	4	2	2	1	3	4	1	0	0
16 Lytton	3	2	2	2	2	2	4	0	1
15 Hedley	0	3	2	3	1	0	5	0	2
13 Revelstoke	0	2	2	4	0	0	3	0	5
44 McLeod Lake	0	5	2	2	0	0	3	0	6
14 Monte Creek	0	0	1	5	0	0	2	0	4
United States									
Intervals (%)	100–21.1	–	21.1–1.1	1.1–0	0–33.4	–	33.4–62.2	62.2–64.4	64.4–100
25 Suttle Lake	6	0	3	0	7	0	2	0	0
24 Prineville	0	0	1	7	0	0	1	0	7
5 Seneca	0	0	1	8	0	0	0	0	9
6 Dixie Pass	0	0	0	8	0	0	0	1	7
7 Union	0	0	2	6	0	0	0	0	8
8 Wallowa Mnts.	0	0	0	9	0	0	1	0	8
9 Heyburn Park	0	0	2	6	0	0	0	0	8
10 Bonner's Ferry	0	0	2	6	0	0	0	0	8

Table 11. Correlation/regression analysis of inland and coastal data*

Independent variable	Dependent variable	r_t	r_w	r_s	a_t	b_t	s_t
Inland material (var. *glauca*); $df_t = 119$; $df_w = 105$; $r_{w,1\%} = 0.249$							
α-Pinene	3-Carene	−0.329	−0.443	−0.372	12.56	−0.126	5.36
α-Pinene	Myrcene	−0.876	−0.964	−0.879	16.55	−0.188	1.54
α-Pinene	Limonene	−0.876	−0.808	−0.881	65.66	−0.795	6.50
β-Pinene	β-Phellandrene	+0.952	+0.699	+0.913	−0.63	+0.399	0.57
Limonene	Myrcene	+0.980	−0.963	+0.977	1.14	+0.232	0.64
Coastal material (var. *menziessii*); $df_t = 71$; $df_w = 63$; $r_{w,1\%} = 0.318$							
Sabinene	Terpinolene	+0.718	+0.646	—	5.77	+0.309	2.62
Sabinene	α-Pinene	−0.641	−0.566	—	42.02	−0.715	7.50
Sabinene	β-Pinene	−0.484	−0.468	—	19.78	−0.371	5.86
α-Pinene	Terpinolene	−0.751	−0.673	—	20.70	−0.290	2.48
β-Pinene	Terpinolene	−0.520	−0.475	—	16.97	−0.292	3.22
Camphene	α-Pinene	+0.396	+0.442	—	18.35	+18.00	8.97

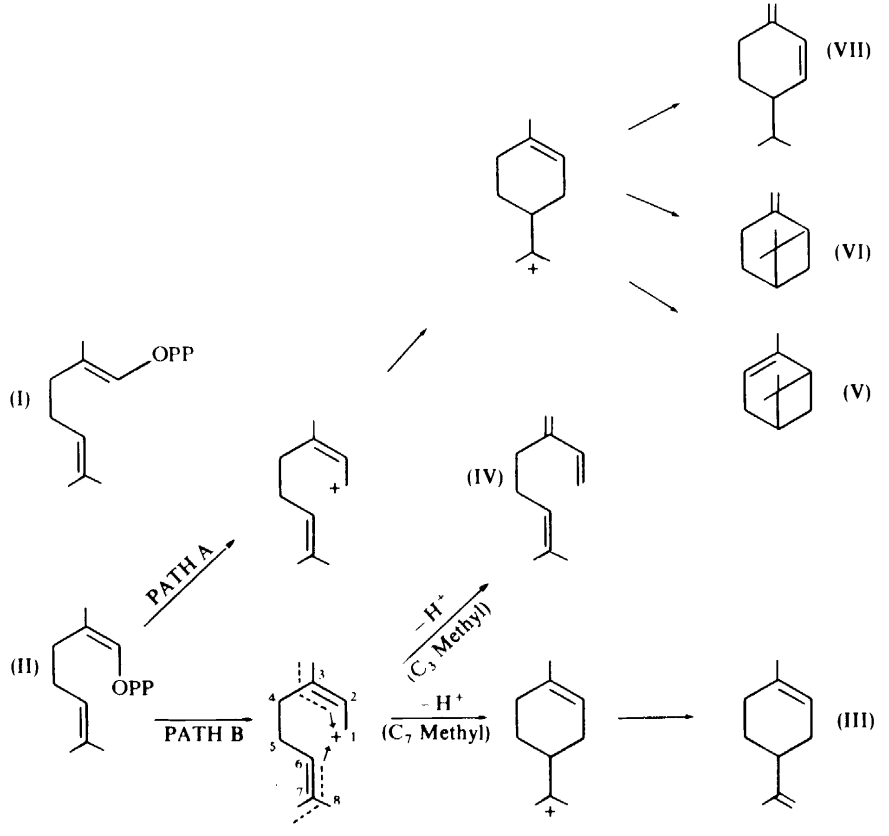
Significant on 1% level, but explaining less than 15% of variability: Inland—β-pinene/myrcene ($r_w = -0.268$), β-pinene/3-carene ($r_w = -0.306$), 3-carene/total terpenes ($r_w = -0.375$), and 3-carene/terpinolene ($r_w = +0.353$). Coastal—β-pinene/3-carene ($r_w = -0.328$), α-pinene/myrcene ($r_w = -0.360$), and α-pinene/3-carene ($r_w = -0.376$). Significant on 5% level: Inland—β-pinene/limonene ($r_w = -0.231$), Coastal—sabinene/α-thujene ($r_w = +0.311$) and 3-carene/total terpenes ($r_w = -0.291$).

* r_t—correlation coefficient based on total data. r_w—correlation coefficient based on 'within' sum-of-squares and cross-products. r_s—rank correlation. a_t, b_t—regression coefficients. s_t—standard error of estimate. Significance was assessed on the basis of 'within' data. Terpenes were identified by their relative retention volumes, with the exception of limonene and myrcene from var *glauca* samples where identification was substantiated by spectroscopic means—ir spectrum for limonene and by uv spectrum for myrcene; the latter is characteristic, as among monoterpenes myrcene represents the only one possessing a monoalkylated diene system (ethanol, $\lambda_{max} = 224$ nm). This substantiation was deemed necessary in view of the curious correlation of the two compounds mentioned in the text. The two terpenes were isolated by preparative GLC, using monoterpene hydrocarbon fraction and 0.65 cm o.d. × 10 m. 1% OV 17 on Chromosorb G 100–120 mesh column ($T = 100°C$, flow rate, He: 50 ml min^{-1}; Varian Autoprep Model 770, thermoconductivity detector), following rough prefractionating of 2 g of oleoresin on Al_2O_3 and distillation at reduced pressure.

work, and we believe it suggests independent biosynthesis of the common monoterpene precursor[23a].

Strong positive correlations were exhibited by sabinene–terpinolene–α-thujene and by β-pinene-β-phellandrene terpene sets. These correlations appear common in conifers and were previously discussed. Surprisingly, limonene (III) and myrcene (IV) (Scheme 1) also correlated strongly in the inland samples, while no correlations between this pair were met in our previous work on pines and firs. According to our previously postulated rules, this correlation implies that in Douglas fir cortex limonene and myrcene should be biosynthetically closer to each other than to α-pinene, β-pinene, and β-phellandrene to which they are negatively correlated[23a].

Geranyl-(*trans*)(II) and neryl-(*cis*)(I) pyrophosphates (Scheme 1) are generally considered as precursors of the monoterpenoids and have been identified as products of mevalonic acid metabolism in *Pinus radiata*[24]. Of the two, the *cis* compound alone is in a position to lead directly to limonene and other cyclics. Experimenting with model phosphates, Cramer and Rittersdorf[25] and Haley, *et al.*[26] demonstrated the predominant formation of acyclic compounds from the geranyl-compounds while monocyclics containing smaller amounts of acyclics resulted from neryl-derivatives. In no case have



Scheme 1. Biosynthetic pathways to monoterpenes

the bicyclics been isolated from either reaction mixture apparently requiring some enzymatic involvement. Thus, the strong correlation of limonene and myrcene could suggest that, in the case of Douglas fir, both compounds are predominantly produced from the same neryl pyrophosphate precursor, while in other conifers myrcene is produced from geranyl- and limonene from neryl-pyrophosphate.

The interpretation of the particularly close relation between myrcene and limonene versus α-pinene and other cyclic terpenes is, however, difficult on the basis of the simple Ruzicka carbonium-ion relationships. A removal of a proton from a C-3 or C-7 methyl attached to a C-2 or C-6 double bond in the neryl carbonium ion, respectively, followed by an electron flow through the double bond, represents a common feature in the formation of both limonene and myrcene (Scheme 1). Perhaps a more attractive explanation could be offered, however, by considering the special enzymatic environment (Path A), apparently required to produce bicyclic α-pinene (V), β-pinene (VI), and monocyclic β-phellandrene (VII) (biosynthetically related to bicyclics)[23a], terpenes never identified in mentioned model experiments. By controlling the rate of the metabolism of intermediate II along Path A this 'special environment' would affect the material flow towards limonene and myrcene along Path B and lead to a positive correlation between both terpenes.

PHYLOGENETIC IMPLICATIONS OF MONOTERPENE VARIATION

The tertiary and quaternary paleobotanical history of western America has been treated by several authors[27–29]. Up to the late Tertiary the Arcto-Tertiary Flora, which included Douglas fir, was apparently continuously distributed throughout the western part of the continent and only during the late Miocene or Pliocene separated into the coastal and inland parts by the appearance of an arid intermountain region of today's Nevada, southern Oregon and Idaho, and central Oregon, Washington and British Columbia. During the ensuing glacial periods of the Pleistocene, Douglas fir was periodically eliminated from the northern part of today's range, which it reinvaded after the withdrawal of ice; concurrently in ice-free areas its lower altitudinal limit fluctuated in the same rhythm, with the total area supporting this species increasing during the pluvial periods. The latter expansions apparently were never so large as to allow substantial fusion of the western and eastern parts of the Douglas fir range, which remained largely separated at all times[30]. The chemical, morphological, and other characteristics differentiating the present day's var. *menziessii* and var. *glauca* (Beissn.) Franco could have developed at any time since their original separation in late Miocene. Halliday and Brown[31] in discussing the quaternary history of *P. menziessii* postulated two Wisconsin glacial refugia for this species—one in the Pacific coast region of the US and the other in the Rocky Mountains of the US—and expressed the opinion that the formation of the two varieties ('forms') of this species may well relate to these two centres of dispersion. This certainly represents one of the possibilities and does not conflict with our chemical results, particularly with the large differences found between the coastal var. *menziessii* and the inland var. *glauca*. The

more uniform character of chemical data for the coastal variety is in agreement with a glacial refugium of moderate size west of the glaciated Cascades and south of the ice in the north, while with the inland variety the much higher variability population-to-population points to a larger glacial distribution covering a wider variety of habitats. This separation agrees also with the observations connected with other species, e.g., with the chemical differences found between the *Abies lasiocarpa* populations from the same two regions[22].

After the withdrawal of Wisconsin ice, northward expansion into the Canadian Rockies and the Pacific coastal ranges took place from both locations; this resulted in species distribution found today (*Figure 1*), with both varieties meeting in central British Columbia, northern Idaho, and north-central and north-eastern Washington. Intergradation of the two varieties in these areas has been reported in a number of instances and can be explained by the postglacial hybridization. However, little has been done to more or less accurately delineate the ranges for pure varieties or to determine the extent of this intergradation. Little[1] as well as Hosie[2] separate the two varieties by a line cutting across Fraser River near the town of Hope and extending northward along the centre of the coastal ranges. Hosie mentions the existence of a few var. *menziessii* populations in the wetter parts of the interior, while Frothingham[10] writes about intergradation in northern Idaho and northwestern Montana. Floehr[4b], on the basis of botanical data of Schenck[13] and Jahn[15] restricts the intergradation region to the north-central Washington and the corresponding area in south-central British Columbia along Fraser River (roughly south of 50°30′). To our knowledge nobody has mentioned any other possible regions of intergradation, such as Ochoco and neighbouring mountains in central Oregon.

Our results on the intermediate populations in British Columbia, Washington, Oregon, and Idaho suggest that gene exchange is much more intensive in the Canadian than in the northwestern US part of the range of this species. Separation of the two varieties by Little[1], Hosie[2], as well as Floehr[4b] appears correct only as far as denoting the westernmost extent of the intergradation area—which seems to be more extensive than heretofore assumed, reaching as it does beyond Revelstoke and including the northern part of the Canadian Rockies. This is not too surprising in view of the well-known western winds prevailing in this area and the fact that Douglas fir pollen can be wind-transported over long distances (pollen of this species has been identified about 200 miles east of the nearest Douglas fir populations in Alberta[32]). Intergradation in central Oregon, Washington, and northern Idaho seems to be less important with the line separating the two varieties running east of the Cascades through the first two states (*Figure 1*). The influence of the coastal variety is definitely identifiable throughout eastern Washington and Oregon, and northwestern Idaho on the basis of sabinene and α-pinene–limonene percentages, although the var. *glauca* chemical characteristics usually prevail. In the Cascades some indication of the eastern influence was noticed in a few trees; more work in this area is indicated.

In Europe, populations of the eastern inland var. *glauca* of Douglas fir are now commonly treated as two separate varieties: northern var. *caesia* and southern var. *glauca* Schneider, with respective ranges not too definitely

delineated; the Canadian, northern Idaho, and eastern Montana populations are generally recognized as var. *caesia*, while Colorado and New Mexico populations are classified as var. *glauca*. Populations from the remaining localities are designated either as belonging to one or the other pure variety or as intermediates—with a high geographic variability and absence of any good distinguishing marks stressed by all authors[4a, b, 13–15].†

In view of the above, the rather sharp chemical separation of the northern and southern inland varieties is surprising. The dividing line in North America approximately follows the Snake River basin (42°30′ latitude); this is close to the geographic division proposed by Schenck[13] (39° latitude). However, it is difficult to give a completely satisfactory paleobotanical explanation for this separation. The Colorado, New Mexico, and central Arizona forests were apparently connected during the Wisconsin glaciation[34]. They were also probably linked with the northern Utah forests, as Douglas fir grew much lower at this time. This connection could be responsible for the relatively uniform nature of the Douglas fir from inland southwestern US At the same time chemical data suggest a considerable barrier to gene flow between these southern and the more northern populations which resulted in formation of var. *caesia* as a separate unit. It is possible that this barrier consisted of several features acting together, namely the presence of Lake Bonneville, which covered the largest part of what is now western Utah (Great Salt Lake represents a small remnant of Lake Bonneville), the existence of extensive ice sheets covering the area of today's Yellowstone Park, Grand Tetons, Wind River, and Salmon River mountains, and[35] the arid Snake River drainage basin, which due to its low elevation (450–900 m), was well below the lower altitudinal limit expected here for Douglas fir even during Wisconsin glacial. The glaciations in the north most likely also split Douglas fir into several ranges remaining separated from each other through periods of time, thus allowing small chemical differences to develop, between inland Canadian and more southern central Idaho material. The Canadian Douglas fir possibly arose from one such marginally isolated populations—probably a rather small one, as suggested by the low chemical variability.

In studies to be reported later in more detail, the California Sierra Nevada populations were found to be closer overall to var. *glauca* than to var. *menziessii* (*Table 4*). At the same time the populations in the California coastal ranges formed a south-to-north chemical cline with the southernmost populations chemically more similar to those of Sierra Nevada. The intermediate status of one of the central Oregon populations in the Cascades (No. 25) mentioned previously could also relate well to this intergradation and represent its extension into central Oregon.

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† Without going further into nomenclatural intricacies, it must be remarked that the name var. *caesia* was first applied by Schwerin to Douglas fir populations of the interior British Columbia, i.e., to the region of intergradation of the var. *menziessii* and var. *glauca* and not to populations from Canadian Rockies, Idaho and Montana[33].

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APPENDIX A—STATISTICAL ANALYSIS

Clustering of population means was examined, using the new Duncan's multiple-range test[38]. The nonparametric H-test was used for analysis of variance where data strongly deviated from normal distributions. Total variability of turpentine composition was described for individual populations by variance sums (ΣS^2)—obtained by summing the variances of all individual terpenes in a population—and the significance of differences between value sets obtained was tested by the t-test.

Given two normal distributions with means $M_a < M_b$, standard deviations, S_a and S_b, and data numbers, $N_a = N_b$, the fraction of N_a falling within an interval $X_k - X_l$, expressed in per cent of the fraction of $N_a + N_b$ falling within the same interval, is given by:

$$ {}^{l}_{k}P^{a}_{\%} = \frac{100 \cdot {}^{l}_{k}A_a}{{}^{l}_{k}A_a + {}^{l}_{k}A_b} \tag{1} $$

where ${}^{l}_{k}A_{a,b}$ represent the areas for the two distributions, located within $X_k - X_l$. These areas can be computed using the cumulative normal distribution functions, by well known procedures[36c]. In assigning a tree to a certain variety on the basis of terpene percentages, T, falling within a certain interval ($X_k \geq T \geq X_l$), ${}^{l}_{k}P^{a,b}_{\%}$ represents the probability of correct assignment to a variety V_a or V_b, or the probable error in assignment to a variety V_b or V_a, respectively.

With two partially overlapping distributions the optimal intervals $X_0 - X$, and $X - X_{100}$, for assigning trees to varieties V_1 and V_2 on the basis of terpene percentages were defined by the condition:

$$ P^{\min}_{\%} = \frac{({}^{X}_{0}A_b + {}^{100}_{X}A_a)}{2} 100 = \text{minimum} \tag{2} $$

with the quantity $P^{\min}_{\%}$ designated as the minimal probable error. However, for simplicity of calculation, we used in our work the quantities ${}'P^{\min}_{\%}$ and ${}'X$, instead, calculated on the basis of equations 3 and 4:

$$ {}'X = \frac{(M_b - M_a) S_1}{S_a + S_b} + M_a \tag{3} $$

$$ {}'z^{\min} = \frac{M_b - M_a}{S_a + S_b} \tag{4} $$

Expressions 3 and 4 follow from the conditions of ${}'^{X}_{0}A^{b}_{\%} = {}'^{100}_{X}A^{a}_{\%}$, i.e., ${}'z_a = {}'z_b = {}'z^{min}$, and common X, with ${}'P^{min}_{\%}$ directly obtainable from ${}'z^{min}$ using a cumulative distribution table (${}'P^{min}_{\%} = 100{}'^{X}_{0}A^{b}$). These conditions involve the stipulation of equal probable errors in assignment to a variety V_b or V_a, i.e., no bias in tree identification, and in our case gave the values sufficiently close to X and $P^{min}_{\%}$.

The intermediate populations from British Columbia (No. 13–17, 44) were treated separately from intermediate US populations (5–10, 24, 25) in tree assignment. The respective distribution parameters for the pure data were established using coastal 19–21, 28, 29 and northern inland populations 11, 12 for Canada and coastal 18, 22, 23, 26 and northern inland populations 2, 42, 43 for US. Distributions of the 'intermediate' data were approximated by averaging. The intervals for tree assignment were set on the basis of expression 3, if $P^{min}_{\%} \leqslant 5\%$. Otherwise two limits, X_k and X_l were calculated for each two overlapping distributions, so that ${}^{k}_{0}P_{\%}$, ${}^{100}_{l}P_{\%} \leqslant 5\%$ within intervals X_0–X_k and X_l–X_{100}, and with data falling within interval X_k–X_l designated as 'indefinite', i.e., possibly belonging to either variety (*Table 10*).

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Note added in proof. After this paper was submitted, a publication by E. von Rudloff on the variability of the needle essential oil of the same species within the Canadian part of its range, came to our attention (*Canad. J. Botany*, **50**, 1025, 1972). While no major discrepancies between his and our chemosystematic conclusions were apparent, his finding of a biosynthetic 'anomaly' (actually several) which 'can not be explained readily by his approach' (our mathematical methods[23a]), deserves a comment. Besides giving no supporting statistical data, von Rudloff derives his anomalies from comparison of coastal and inland essential oils, while our methods apply to variability within evolutionary undiversified populations (or to data mathematically reduced to the same). This has been pointed out in this and in our earlier publications. Most likely the von Rudloff's anomalies have their basis in different selective pressures which led to coastal/inland essential oil diversification, and do not relate to biosynthetic relationships.