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BIOSYNTHETIC IMPLICATIONS OF TERPENE CORRELATIONS IN *PINUS CONTORTA*

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Abstract—Foliar monoterpenes were analysed in 10 clones of lodgepole pine (*Pinus contorta*) and open-pollinated seedlings from the 10 original ortets. Correlation analysis revealed three groups of terpenes, those with camphane or pinane skeletons, mono- and acyclic monoterpenes and sesquiterpenes. Relationships between terpenes were more obvious in results based on absolute data than relative percentage data. The correlations did not differ greatly in progeny from trees which had different terpene patterns. The data are consistent with monoterpene synthesis via groups of related enzymes stabilizing a carbonium ion transition state in which similar gross conformations of the active sites of the enzymes determine the structure of the carbon skeleton.

INTRODUCTION

Chemical analyses have been used to augment morphological characters in taxonomic and genetic studies of forest tree species in which intergradation of morphological characters makes differentiation of individuals and populations difficult. Terpenes appear to possess the structural variety, physiological stability, general distribution in conifers, and ease of analysis, which make them particularly suitable for such studies [1].

Foliar terpenes of lodgepole pine have been used in studies of geographic variation within the species in wild populations [2, 3]. Wild populations of lodgepole have been divided into four subspecies, *contorta* (coastal), *latifolia* (Rocky Mountain and intermountain), *murryana* (Sierra Nevada) and *bolanderi* (Mendocina White Plains) [4]. On the basis of terpene patterns, populations could be differentiated into three main types containing: (a) > 50% β-phellandrene and < 10% β-pinene; (b) less β-phellandrene and 15–45% β-pinene; and (c) mainly α-pinene, camphene, bornyl acetate and β-pinene. This last type of terpene pattern was similar to that of Jack pine–lodgepole pine hybrids [2, 3]. Tree to tree variability within populations was high. While there appeared to be geographic differentiation of populations with respect to terpene pattern in southern British Columbia and Alberta, those from northern locations showed all three patterns and some intermediate types. Foliar terpenes were also examined in populations of the closely related Jack pine (*Pinus banksiana*) [5] They were diverse with respect to terpene patterns. Two major types were distinguished, one containing mainly α-pinene, bornyl acetate and related monoterpenes, plus cadinol and related sesquiterpenes. The other contained less of these terpenes and relatively more β-pinene. In additon, there were a number of minor types, with little geographic differentiation.

Cortical monoterpenes have been studied in plantations of lodgepole pine growing in Britain [6], Sweden and in wild stands in Canada [7]. As with foliar monoterpenes, cortical monoterpene patterns could be divided into three major types based on the relative amounts of α- and β-pinene and β-phellandrene, a Jack pine introgressive type containing high levels of the pinenes and camphene, and several minor types. In addition each pattern could be subdivided on the basis of 3-carene and limonene content. The monoterpene patterns were sufficiently distinct between provenances and constant within provenances to allow assignment of plantations of unknown origin to one of the original introductions. Differences in terpene patterns between different introductions from the same area were noted [6]. Terpene patterns in samples from wild stands were similar to those in European plantations originating from the same area, though geographic differences were less clear-cut. The pattern classified as 'north coastal' in plantations was found to extend south to Vancouver Island in wild trees, and the 'southern interior of British Columbia' pattern occurred in wild trees from 46° 43′ in Washington, U.S.A. to 54° 04′ in central British Columbia [7].

To assess the diagnostic value of terpene patterns, it is necessary to know which are biosynthetically related [1, 8]. However, the biosynthetic pathways of conifer terpenes are poorly known. Inferences about their biosynthetic similarities have been drawn by examining the correlations between different terpenes in individuals taken from large populations [9, 10].

Compounds showing proportional relationships (high positive correlation, regression coefficient large and regression constant small), are assumed to be more closely related biosynthetically than those showing substitutional relationships (high negative correlation), and compounds showing either relationship are assumed to be closer biosynthetically than those showing an independent relationship (low correlation coefficient). Zavarin [9] and Martin *et al.* [10] noted that intermediate degrees of correlation are difficult to assess and that results can be affected by constraint due to expressing results as percentages, by biosynthetic differences in different populations, and by gene linkage.

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Forrest [6] estimated correlations between relative amounts of cortical monoterpenes of lodgepole pine with an adjustment for the constraint of a common sum. Correlations with β-phellandrene, often the major monoterpene, frequently appeared to be largely due to constraint. Other correlations, which were less affected by constraint, varied somewhat in trees from different sources. The correlation between α- and β-pinene was high except in the Mendocino population (subspecies *bolanderi*), and the correlation between 3-carene and α-terpinolene was also unusually low in this population.

The present study was undertaken to determine correlations between foliar monoterpenes of lodgepole pine of known genetic relationship, to evaluate the effect of constraint caused by expressing results as percentages, and to assess the effect of common parentage on such correlations.

RESULTS

Single-linkage clustering of terpenes in all progeny (203 trees) by highest correlation coefficients based on absolute amounts of terpenes are shown in Fig. 1. The bicyclic monoterpenes formed one group of closely interrelated compounds, the monocyclic monoterpenes and sabinene, together with the acyclic monoterpenes myrcene and *cis*-ocimene a second, and the sesquiterpenes a third. The correlations shown with dashed lines represent the highest correlations between terpenes in different groups. The low correlation of 3-carene with other monoterpenes may have been due, in part, to its poor resolution from a compound tentatively identified as hex-2-en-l-al (cf. ref. [2]) which interfered with the quantitation of low levels of 3-carene.

The effect of expressing results on the basis of percentage of total terpenes was to greatly decrease the positive correlations and introduce large negative correlations. Table 1 demonstrates the size of the effect of constraint to a sum of 100 on unadjusted correlation coefficients based on percentage values. Not surprisingly, large negative correlations with β-phellandrene, which was frequently the major terpene, appeared when results were calculated on a percentage basis. The closeness of the interrelationships between the mono- and acyclic monoterpenes was lost, and only the clustering of tricyclene, camphene, α-pinene and bornyl acetate, and the cadinene group, were clear. In contrast to percentage data, results based on absolute values produced few negative correlations, none of which was significant at $P = 0.001$.

Terpene correlation coefficients within the 10 families are given in Table 2, along with the type of terpene pattern of the mother tree, as determined by analyses of the grafts. A number of terpene types occurred within the progeny of each mother tree. The grafts of mother tree 73 showed variable terpene patterns. Terpene inheritance will be discussed in a further paper [White E.E. and Nilsson J.-E., unpublished].

The major correlations between the mono- and acyclic terpenes varied little between families, irregardless of the terpene pattern of the mother tree or the frequency of different patterns in its progeny. The correlations between pairs of sesquiterpenes, which had the same pattern (cadinols > cadinene isomers 1 > cadinene isomers 2) in all trees, were also very constant.

The camphene group bicyclic terpenes were major terpene components in only one mother tree, tree 50, and in only 18 of the 203 seedlings, of which 14 were progeny of tree 50. In spite of their highly uneven distribution as major components in different families, their correlations were relatively high in all families. Families 09, 16, 73, 76 and 89 contained no C-type progeny (< 0.3 mg/10 g camphene and < 1.0 mg/10 g bornyl acetate). Nevertheless, the correlations within the camphene group were generally high in these families, while those between the camphene group terpenes and the monocyclic terpenes, as exemplified by the correlation between β-phellandrene and bornyl acetate, were low. Families 19, 30, 39 and 59 each contained one C-type tree, which may have contributed unduly to the camphene group correlations for these families. So few of the trees in family 59 contained measurable tricyclene that a correlation coefficient could not be calculated for tricyclene–camphene, and the very high bornyl acetate–camphene correlation in this family is the result of many very low values and one high one. Two-thirds of the trees in family 50 were C-type, and the correlations in this family represent correlations for trees containing high amounts of camphene group terpenes.

The correlation between α- and β-pinene was the lowest within the bicyclic terpene group. Correlations between β-pinene and all other terpenes were also low. The β-pinene–α-pinene correlation was the highest correlation for β-pinene in all families, except family 59 where the correlation between β-pinene and *cis*-ocimene was 0.711.

The correlations of individual terpenes with total amount of terpenes are given at the bottom of Table 1. Total terpenes tended to be high when the mono- and acyclic monoterpenes and sesquiterpenes were high, while the bicyclic terpenes were not strongly correlated with total terpene production. No terpene was negatively correlated with total terpene production.

DISCUSSION

The terpene groups revealed by their quantitative co-occurrence have obvious structural similarities which would make their synthesis by common routes plausible. Monoterpene biosynthesis has been proposed to proceed from geranyl pyrophosphate by the cyclization of an enzyme-stabilized cation [11–13]. The structural requirements, for close fit for the active site of an enzyme which would stabilize an ion that could cyclize to the bulky camphane or pinane skeletons, would not be the same as those of an enzyme stabilizing a cation which cyclized to the half-chair or flattened ring skeletons of the phellandrenes and limonene, or the L conformation of sabinene. A cation precursor to the acyclic monoterpenes could fit an active site similar to the one stabilizing the monocyclic type of precursor. The correlations observed could be explained by synthesis via common or closely related enzyme-stabilized cationic transition states, with the gross structure of the enzyme's substrate binding site determining the type of terpene skeleton produced. Smaller modifications of the active site, or differences in allosteric sites, could determine the final structure of the terpene.

The effect of constraint, caused by expressing results as percentages, was to obscure possible biosynthetic relationships and produce the impression that the terpenes varied at each other's expense. While this is true for percentage data, it was not the case for the absolute data as no significant negative correlations were found between absolute amounts of terpenes. Terpene production ap-

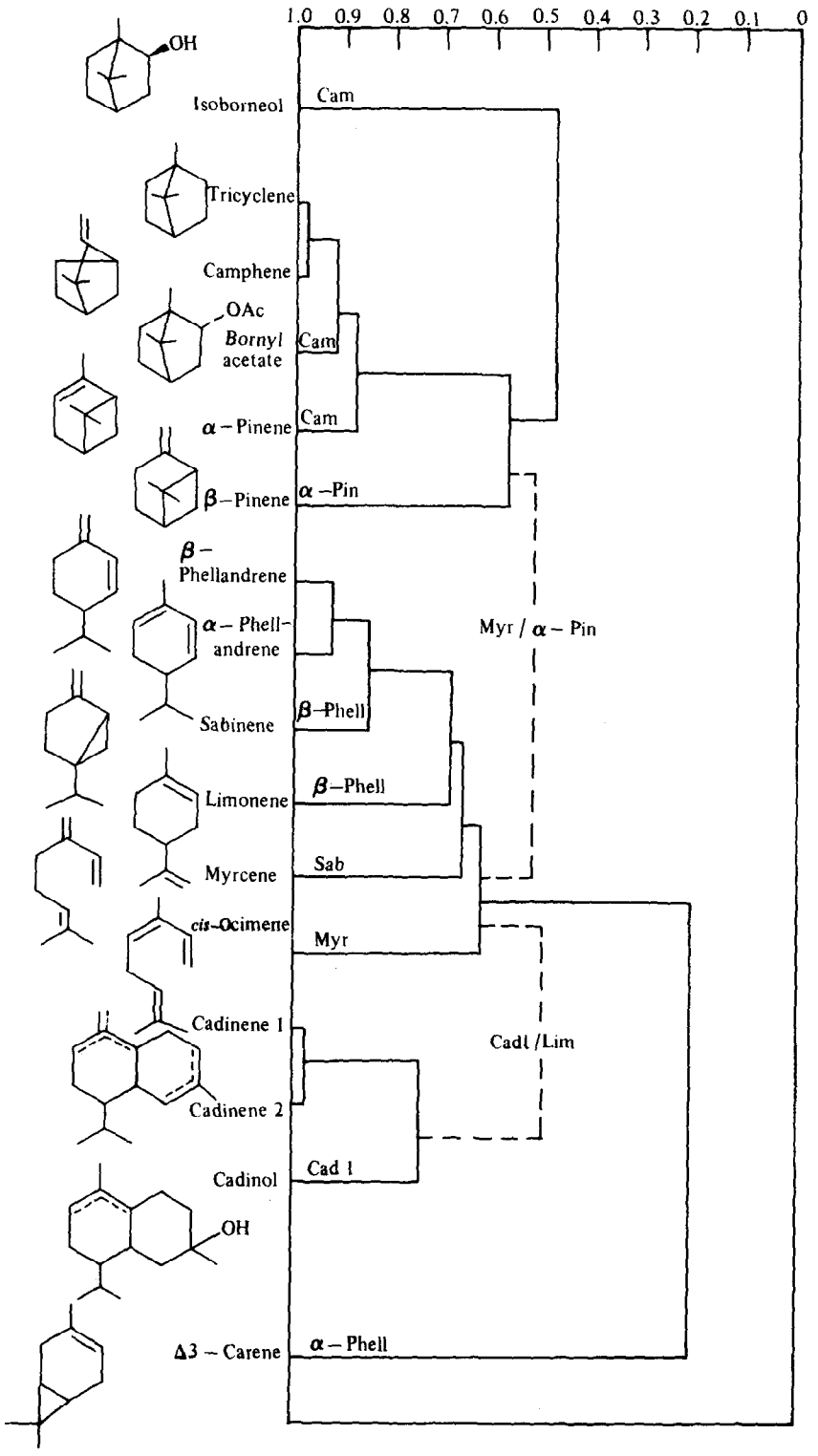

Fig. 1. Single linkage clustering of terpenes by highest correlation coefficients. Trees in all families. Correlations based on absolute values. All correlations significant at $P = 0.001$ except 3-carene–α-phellandrene. The correlations shown with dashed lines represent the highest correlations between terpenes in different groups, but are not highest correlations for those terpenes.

Table 1. Comparison between correlation coefficients based on absolute amounts of terpenes (upper figure) and percentage of total terpenes (lower figure)

	Tricyclene	α-Pinene	Camphene	β-Pinene	Sabinene	3-Carene	Myrcene	α-Phellandrene	Limonene	β-Phellandrene	*cis*-Ocimene	Bornyl acetate	*iso*-Borneol	Cadinenes 1	Cadinenes 2	Cadinols
α-Pinene	0.8685	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	0.8339	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Camphene	0.9814	0.8806	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	0.9563	0.8822	—	—	—	—	—	—	—	—	—	—	—	—	—	—
β-Pinene	0.2810	0.5736	0.3100	—	—	—	—	—	—	—	—	—	—	—	—	—
	0.1268	0.3872	0.1733	—	—	—	—	—	—	—	—	—	—	—	—	—
Sabinene	0.1562	0.3035	0.0998	0.1690	—	—	—	—	—	—	—	—	—	—	—	—
	−0.0833	−0.2082	−0.1166	−0.3217	—	—	—	—	—	—	—	—	—	—	—	—
3-Carene	−0.0480	0.0315	−0.0600	0.1151	0.1044	—	—	—	—	—	—	—	—	—	—	—
	−0.1260	−0.0298	−0.1048	−0.0800	−0.2718	—	—	—	—	—	—	—	—	—	—	—
Myrcene	0.4009	0.5344	0.3661	0.3607	0.6712	0.1553	—	—	—	—	—	—	—	—	—	—
	0.0684	−0.0184	0.0678	−0.1347	0.0153	−0.0350	—	—	—	—	—	—	—	—	—	—
α-Phellandrene	−0.1299	0.0469	−0.1951	0.1206	0.8212	0.2071	0.5890	—	—	—	—	—	—	—	—	—
	−0.3750	−0.4532	−0.3997	−0.3847	0.3540	0.1400	0.0348	—	—	—	—	—	—	—	—	—
Limonene	0.0474	0.2173	0.0171	0.2362	0.6839	0.1628	0.5575	0.6793	—	—	—	—	—	—	—	—
	−0.2070	−0.1951	−0.1986	−0.1974	−0.0483	0.2390	−0.0531	0.2605	—	—	—	—	—	—	—	—
β-Phellandrene	−0.1372	0.0268	−0.2065	0.0330	0.8618	0.1235	0.5880	0.9332	0.0236	—	—	—	—	—	—	—
	−0.5164	−0.6322	−0.5766	−0.6222	0.4227	−0.1525	0.0805	0.5689	0.1387	—	—	—	—	—	—	—
cis-Ocimene	0.1184	0.2882	0.0969	0.2680	0.5845	0.1759	0.6346	0.5744	0.1264	0.5632	—	—	—	—	—	—
	−0.1169	−0.1317	−0.1070	−0.1887	0.1942	−0.1551	0.0912	0.2449	0.2141	0.2223	—	—	—	—	—	—
Bornyl acetate	0.9048	0.8166	0.9199	0.3048	0.1083	−0.0425	0.3449	−0.1802	0.0522	−0.1876	0.1221	—	—	—	—	—
	0.8516	0.8005	0.8869	0.1480	−0.1648	0.0029	−0.0262	−0.4182	−0.1910	−0.5727	−0.1065	—	—	—	—	—
iso-Borneol	0.4787	0.4500	0.4847	0.1848	0.1145	−0.0538	0.2380	0.0394	0.1510	0.0089	0.1105	0.3674	—	—	—	—
	0.4163	0.3341	0.4348	0.0070	−0.0655	−0.1562	−0.0220	0.1868	−0.1387	−0.2512	−0.0693	0.3073	—	—	—	—
Cadinenes 1	0.1002	0.1803	0.0746	0.1505	0.4123	0.0299	0.4045	0.4256	0.0183	0.4399	0.2979	0.0666	0.3247	—	—	—
	−0.0548	−0.2276	−0.0794	−0.2276	−0.0355	−0.1495	−0.1654	−0.0106	−0.1938	−0.1310	−0.0514	−0.1083	0.2122	—	—	—
Cadinenes 2	0.0873	0.1637	0.0620	0.1482	0.3879	0.0180	0.3817	0.4000	0.0115	0.4206	0.2767	0.0525	0.2950	0.9848	—	—
	0.0525	−0.2280	−0.0790	−0.2214	−0.0434	−0.1417	−0.1738	−0.0225	−0.1960	−0.1210	−0.0642	−0.1029	0.1726	0.9550	—	—
Cadinols	0.0955	0.2103	0.0899	0.1730	0.4467	−0.0219	0.4063	0.3806	−0.0174	0.3913	0.3018	0.0942	0.1708	0.7457	0.7251	—
	−0.1231	−0.1925	−0.110	−0.1309	−0.1752	0.1241	−0.0942	−0.2943	0.0444	−0.4124	−0.1942	−0.0793	−0.0191	0.4137	0.3920	—
Total terpenes	0.3461	0.5803	0.3144	0.5535	0.7960	0.1389	0.7799	0.7203	0.7151	0.7244	0.6297	0.3181	0.2985	0.6475	0.6235	0.6635

Trees in all families.

Table 2. Correlation coefficients between absolute amounts of terpenes in 10 open-pollinated lodgepole pine families

Family	Terpene type of mother tree	Mono- and acyclic monoterpene group						Bicyclic terpene group				Sesquiterpenes	
		β-Phell–α-Phell	Sab–β-Phell	Lim–β-Phell	Myr–Sab	Myr–Ciso	β-Phell–BoAc	Tri–Cam	BoAc Cam	α-Pin–Cam	α-Pin–β-Pin	Cad 1–Cad 2	Cad 1–(Cadl)
09	A β-Phell ⩾ α-Pin > β-Pin	0.975	0.945	0.934	0.890	0.591	0.590	0.857	0.570	0.845	0.689	0.994	0.824
16	B β-Phell > β-Pin > α-Pin	0.787	0.885	0.784	0.848	0.910	−0.263	0.970	0.990	0.884	0.682	0.943	0.665
19	A β-Phell ⩾ α-Pin > β-Pin	0.958	0.819	0.644	0.844	0.642	−0.320	0.986	0.998	0.979	0.903	0.995	0.821
30	B β-Phell > β-Pin > α-Pin	0.967	0.960	0.872	0.245	0.170	0.529	0.404	0.542	0.808	0.764	0.968	0.593
39	A β-Phell ⩾ α-Pin > β-Pin	0.942	0.790	0.767	0.831	0.714	−0.179	0.987	0.995	0.959	0.165	0.984	0.816
50	C BoAc = α-Pin = β-Pin ⩾ β-Phell + Cam + Tri	0.889	0.782	0.828	0.525	0.708	−0.250	0.989	0.763	0.980	0.793	0.996	0.804
59	B β-Phell > β-Pin > α-Pin	0.951	0.822	0.573	0.740	0.626	0.055	—	0.999	0.949	0.427	0.949	0.682
73	B β-Phell = β-Pin or β-Pin > β-Phell	0.965	0.940	0.796	0.922	0.897	0.621	0.676	0.445	0.216	0.263	0.992	0.920
76	B β-Phell = β-Pin > α-Pin	0.972	0.950	0.541	0.925	0.807	0.921	0.683	0.794	0.821	0.892	0.995	0.626
89	B β-Phell = β-Pin > α-Pin	0.965	0.938	0.879	0.908	0.863	0.111	0.620	0.209	0.848	0.846	0.982	0.881

Correlations between terpenes in the same group, with β-phellandrene–bornyl acetate as example of correlations between members of different groups.

peared to be governed by the levels of precursor and activity of enzymes available for their synthesis. This, and the fact that the major correlations did not vary greatly in families with different frequencies of terpene types, would indicate that similar biosynthetic sequences were present throughout the trees sampled and that the differences in relative amounts of terpenes in different trees were due to steady state differences, that is differences in those factors which affect enzyme activity. These factors include slight alterations in the amino acid sequence of an enzyme which alter its efficiency without rendering it inactive. Poulouse and Croteau [12] suggest that α-terpene, α-thujene and α-terpineol may be produced by a single enzyme in *Thymus vulgaris* L. Alteration in the structure of such an enzyme could produce an isoenzyme which produced the same compounds in different ratios.

If two terpenes were the products of the same enzyme, even higher correlations could be expected than if they were products of the same biosynthetic pathway, or if they were produced by different pathways and the genes controlling the enzymes involved were linked. In this study, in the absence of data from controlled crosses, it is impossible to eliminate linkage as the cause of the correlations observed. However, the structural similarity of the terpenes which had high correlations, and the consistency of these correlations in trees of different parentage, makes biosynthetic similarity a more attractive hypothesis. It is likely that, if linkage was responsible, the gene combinations in different families would vary at random, and positive and negative correlations would be equally common.

Low correlations are expected if two terpenes are part of independent biosynthetic sequences, if they are part of the same sequence but the genes controlling the enzymes catalysing the synthesis or further metabolism of one member of the pair vary in frequency in the trees examined, or if they are part of the same sequence but one member of the pair is also synthesized by an alternate route. The low correlations between β-phellandrene and bornyl acetate would seem to illustrate the first alternative, terpenes synthesized by different sets of enzymes. The low correlations between α- and β-pinene in some families could result from either of the latter two alternatives. The second possibility, that they are terpenes synthesized by the same path with variable frequency of the enzymes affecting one member of the pair, seems less likely. In such a case a missing enzyme would cause a negative correlation between a terpene and another which represented a precursor or product. β-Pinene showed no strong negative correlations with any other terpene indicating build-up of another terpene due to a metabolic block, preventing the formation of β-pinene, or build-up of β-pinene at the expense of another terpene. The α- and β-pinene correlations may illustrate the third alternative, two terpenes with similar structures and modes of biosynthesis, one of which has an alternate mode of synthesis. Zavarin [9] proposed that β-pinene and the (−)-form of α-pinene are both produced by a cyclization of the 1-*p*-menthane-8-carbonium ion which involves interaction with the double bond, while the (+)-form of α-pinene is produced by direct attack of the positively charged C-8 at C-6. Whether the high correlations between α-and β-pinene involved only the (−)-form of α-pinene could not be determined in this study as the two forms of α-pinene were analytically indistinguishable. As the monoterpenes likely have a common C_{10} precursor, and as the evidence available indicates that both monoterpenes and sesquiterpenes are derived from mevalonate [14], complete independence of terpene biosynthesis is not envisaged. Degrees of biosynthetic independence may result from compartmentation of the enzymes involved [15, 16].

These data divide the camphene group bicyclic monoterpenes, the mono- and acyclic monoterpenes and sabinene, and the sesquiterpenes of lodgepole pine into three groups of compounds with related biosynthetic mechanisms. The data are consistent with monoterpene synthesis, via groups of enzymes with similar active sites stabilizing a cationic transition state, in which the gross conformation of the active sites of the enzymes determines the structure of the carbon skeleton, while smaller enzymatic differences determine the final structure, and factors which control enzyme concentration, compartmentation and kinetic properties determine the relative amounts of terpenes.

EXPERIMENTAL

Plant material. The trees sampled were from a clonal archive and a seedling seed orchard at Sör Amsberg near Borlänge, Sweden. Scions and seed had been collected from selected wild trees in Canada; trees numbered 09–39 S.–N. between Fort St. James and Mansen Creek; 73–89 along Parsnip Reach: 59 N. of Chetwynd, Pine Pass area: 50 near Wonowon, E. of the Rockies. At the time of sampling trees were 10-years-old from time of sowing or grafting. 10 first degree lateral twigs were collected in early February around the tree from the upper second and third branch whorls of seedlings, and three twigs were collected from grafts. Individual samples were placed in plastic bags which were transported and stored in heavy weight plastic bags at − 5° in the dark.

Terpene extraction and analysis. 10 g fr. wt of needles (1 g/twig, for seedlings, or 3.3 g/twig, for grafts) were weighed, immediately crushed in a mortar with liquid N_2, homogenized for 2 min in 100 ml cold *n*-pentane containing 8.57 mg *p*-cymene as an int. standard, placed in an ultrasonic bath at 5° for 1–2 hr, filtered [17] and the filtrate concd to *ca* 10 ml in a long-necked flask in a water bath at 40–45° [18]. Analytical error was calculated from nine replicate extractions of the same sample and duplicate extractions of trees from three different families. Grafts of each clone and some seedlings were extracted without added *p*-cymene. Separate 10 g samples were dried at 90° in a forced draft oven for 24 hr to calculate tissue dry wt.

Pentane extracts were analysed using GC on a 50 m × 0.3 mm WCOT column with SP-1000 stationary phase inserted into a glass precolumn of 3% SP-1000 on Chromasorb W, an inlet splitter, flame ionization detector and chromatographic data system. Operating conditions were: injector 200°, detector 230°, carrier gas N_2 at 1.5 ml/min, split ratio 1:55, sample vol. 1–2 μl, temp. programmed from 50° to 110° at 4°/min then isothermally at 150°. Identities of all major peaks were confirmed by GC/MS. Absolute amounts of each terpene were calculated by peak area relative to int. with a detection limit of 0.05 mg/10 g fr. wt.

The mean analytical error for individual terpenes was ± 0.1 mg/10 g fr. wt, and there was no tendency for this to be greater for more volatile terpenes. γ-Terpinene was monitored in all samples, as it has been shown to be a probable precursor to *p*-cymene [11], used as int. standard, which has been detected in lodgepole pine [2]. Any samples containing detectable γ-terpinene, and samples from each clone, were extracted without added int. standard. Only traces of natural *p*-cymene were present in these samples. The mean dry wt of foliage was 41.33 ± 1.8% and did not vary significantly between families. Analyses rep-

Table 3. Means and ranges for terpenes normally above the limit of detection

Terpene	Mean		Range	
	mg/10 g	% of terpenes	mg/10 g	% of terpenes
Tricyclene	0.9	0.3	0–1.2	0–3.1
α-Pinene	1.7	6.0	0–11.6	0–24.8
Camphene	0.3	1.1	0–4.8	0–10.9
β-Pinene	3.6	12.3	0–21.2	0–54.6
Sabinene	0.2	0.8	0–0.9	0–3.1
3-Carene	0.4	2.4	0–3.1	0–6.3
Myrcene	0.7	2.6	0–2.9	0–2.0
α-Phellandrene	0.3	1.0	0–0.9	0–1.1
Limonene	0.5	2.0	0–2.2	0–12.5
β-Phellandrene	11.7	45.1	0–32.4	0–67.3
cis-Ocimene	0.7	2.5	0–2.5	0–6.5
Terpinolene	0.6	0.2	0–0.5	0–2.5
Bornylacetate	0.7	2.2	0–13.0	0–37.3
Isoborneol	0.2	0.6	0–1.9	0–8.8
Cadinenes 1	1.1	4.0	0–4.9	0–11.6
Cadinenes 2	0.7	2.7	0–3.3	0–7.5
Cadinols	3.8	14.1	0–18.0	0–57.0
Total terpenes	28.1	100.0	0.1–73.9	—

Individual data for all trees and grafts.

licated at the beginning and end of the storage period were within the range of analytical error.

The means and ranges for terpenes regularly above the limit of detection are given in Table 3. In addition, α-terpinene, γ-terpinene, camphor, linalool and methyl chavicol were identified in some extracts but infrequently occurred in amounts > 0.5 mg/10 g and were not included in the statistical analysis. Hexanal, undecane and undecanone were quantified but showed no correlation with any terpene. Muurolene isomers, β-caryophyllene and γ-elemene were identified in some extracts and, together with three unidentified peaks, were occasionally present in appreciable amounts.

Correlations between terpenes were calculated within all clones, all half-sib families, all grafts combined and all seedlings combined on the basis of absolute amounts of terpenes, percentage of total monoterpenes, and percentage of all terpenes analysed.

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