Changes in Composition of Volatile Terpenes in Douglas Fir Needles During Maturation

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The compositions of the volatile terpenes in Douglas fir new tip growth and in year old needles were investigated over a growing season using a simultaneous steam distillation-extraction method and direct vapor analyses. The results demonstrate the almost complete absence of acyclic oxygenated monoterpenes and cis-ocimene in the new tip growth as it first appears, with the gradual increase in concentration of these components as the growth matures. The cyclic oxygenated monoterpenes are immediately present in the new growth in amounts equal to those in year old growth and show little seasonal variation in either young or old growth. The effects of fertilization with urea vs. gypsum on the volatile terpene compositions and the possible relationship of the terpene compositions of the new tip growth to the greater palatability of this growth to browsing ruminants are discussed briefly.

Most of the major and several of the minor constituents in the essential oil from the mature needles of Douglas fir have been identified (Sakai et al., 1967) as part of a larger project concern with food palatability preferences of foraging ruminants. The possible relationship of the individual constituents of the Douglas fir needle oil to acceptance of different food materials by browsing animals has been investigated (Oh et al., 1967) with the observation that monoterpene alcohols and carbonyl compounds appear to have an inhibitory effect. It has been observed that animals browsing on Douglas fir have strong preference for the young growing tips relative to the mature needles. A study of the differences in composition of the volatile terpenes in the new growth relative to the composition in mature needles and the changes in composition as the growth matured was thus of interest in the investigation of the relative palatability of the foliage to browsing animals, as well as from possible biosynthetic aspects.

The biosynthesis of terpenes has been the subject of many investigations in recent years (Loomis, 1967; Richards and Hendrickson, 1964), with various mint species in particular being used extensively in the studies (Burbott and Loomis, 1967; Hefendehl, 1967; Hefendehl et al., 1967; Malingré, 1966). The incorporation of 2-^{14}C mevalonic acid into monoterpenes has been investigated (Hefendehl, 1966; Sandermann and Bruns, 1962, 1965; Sandermann and Schweers, 1962) but the level of incorporation has always been extremely low. Seasonal variations in volatile terpenes have been studied in a variety of plant species (Attaway et al., 1967; Juvonen, 1966; Rudloff, 1962; Rudloff and Hefendehl, 1966; Rudloff and Underhill, 1965; von Schantz and Norri, 1968).

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The isolation and concentration techniques normally used may well alter the quantitative as well as the qualitative composition of an essential oil relative to the composition of the terpenes present in the plant material. Direct injection techniques (Baerheim Svendsen, and Karlsen, 1966; Roberts, 1968; Rudloff, 1965), direct extraction with solvents (Kubeczka, 1966; Schratz and Wahlig, 1965), and a vacuum transfer technique (Kubeczka, 1966) have been proposed to minimize such changes. Another method to minimize artifact formation, the direct analysis of the vapor over a sample, was used in the present study. This paper presents the results of the analyses of the terpene compositions in mature and maturing Douglas fir needles using a simultaneous steam distillation-extraction technique and direct vapor analyses.

EXPERIMENTAL

Plant Material. Four Douglas fir trees, about 5 ft in height, located in the Pacific Coast Range west of Ukiah, Calif., were used for this study. Three of the trees had been fertilized a year previous to sampling, two with nitrogen (urea) and one with sulfur (gypsum), while the fourth tree was fertilized with urea earlier the same winter. Small branches were removed from each tree periodically during the growing season, starting shortly after the first appearance of new growth in the spring. The samples were wrapped in aluminum foil, transported to Davis in an ice chest, and kept in a refrigerator until they were analyzed (maximum of 2 days). The needles were taken from the stem just prior to analysis, the 1-year old needles used being those immediately adjacent on the branches to the new growth needles.

Small-Scale Steam Distillation-Extraction Analyses. A simultaneous steam distillation-extraction procedure was employed using an apparatus (Figure 1) similar to that of Likens and Nickerson (1964). The distillate-return arm for the extracting solvent was 1 cm higher than the distillate return arm for the aqueous phase. Before starting a run, the U-tube separator section of the apparatus was charged with
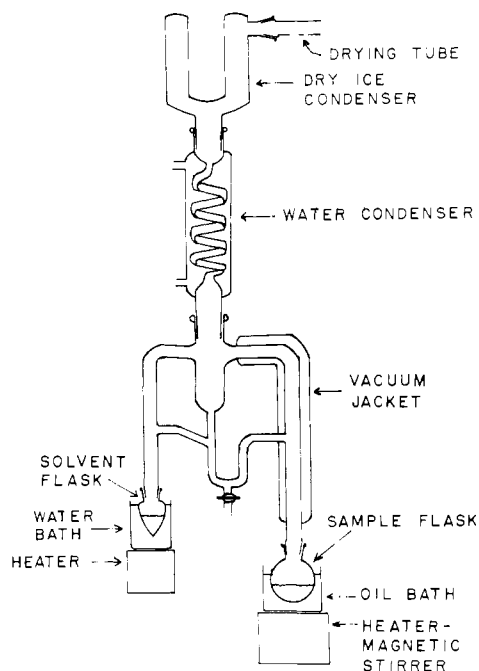
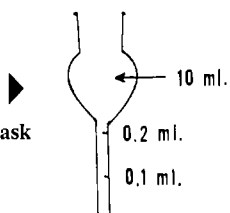**Figure 1.** Small scale steam distillation-extraction apparatus
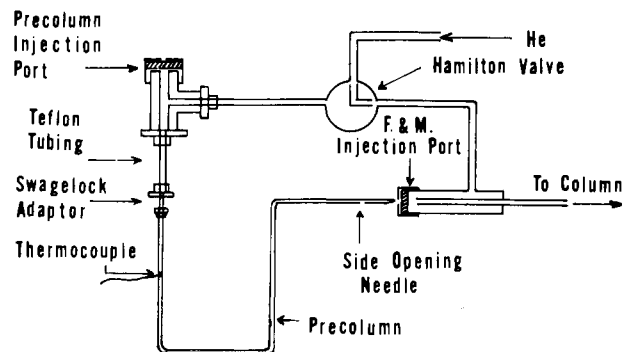

Figure 2. Concentration flask

Figure 3. Precolumn injection arrangement

3 ml of water and 2 ml of *n*-pentane. A 2.0-g sample of needles was placed in a mortar, quickly frozen, and ground to a powder under liquid nitrogen, and the pulverized needles washed into a 100-ml round-bottomed flask with 50 ml of distilled water. The sample flask, after addition of a stirring bar, was connected to the apparatus and 8 ml of *n*-pentane was placed in the solvent flask. The run was started by placing a water bath, preheated to 50° C, around the solvent flask and a silicone oil bath, preheated to 130° C, around the sample flask. The steam distillation-extraction was run for exactly 1 hr from the time the heating was started, with the sample stirred magnetically and the heating baths maintained at the temperatures indicated throughout the period. On completion of the run, the pentane in the U-tube separator section was added to the solvent flask, the combined pentane extracts

Figure 4. Gas chromatograms of essential oils from young and old Douglas fir needles (Tree 1)

500-ft × 0.03-in. i.d. Carbowax 20M column; temperature isothermal at 75° C for 5 min then programmed at 2° C per min to 145° C and run isothermal thereafter; chart speed, 20 in. per hr; range 10, attenuations as indicated
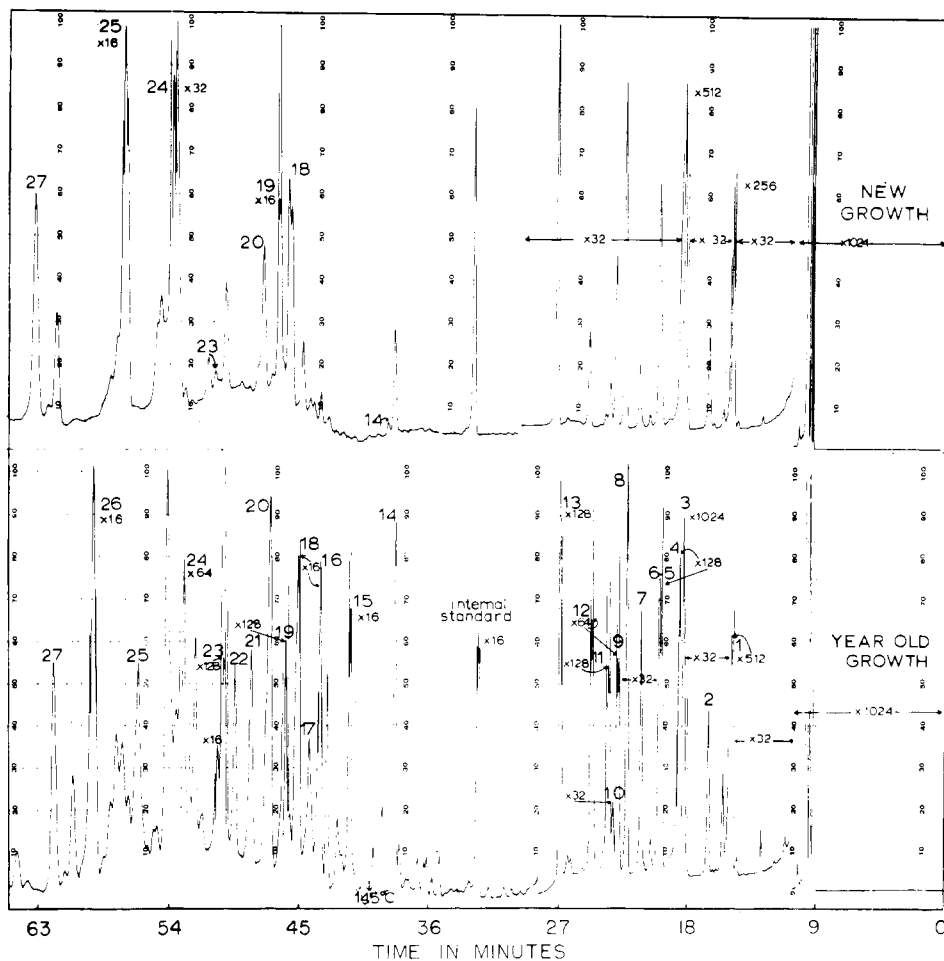
Table I. Changes in Composition of Douglas Fir Needle Oil[a]

Peak No.	Component	Needle Age[d]	Tree 1[b] Collection Date — May 3	May 14	May 30	June 12	June 26	July 24	Tree 2[b] Collection Date — May[c] 3	May 14	May 30	June 26	July 24
			Peak Heights[e]						Peak Heights[e]				
1	α-pinene	young	25.3	19.5	22.2	10.8	24.9	30.6	...	13.7	15.0	29.8	13.6
		old	31.2	29.8	37.3	26.7	40.7	43.5	13.5	20.4	11.0	21.5	17.2
2	camphene	young	1.1	0.9	0.9	0.5	1.1	1.3	...	0.6	0.7	1.3	0.6
		old	1.2	1.2	1.6	1.2	1.9	1.8	0.60	0.9	0.6	1.0	0.8
3	β-pinene	young	66.9	59.0	67.5	37.3	83.6	103.3	...	37.2	42.4	94.2	42.5
		old	92.0	92.2	111.5	90.6	135.0	141.0	44.5	66.6	36.9	71.8	60.6
5, 6	3-carene + myrcene	young	2.9	3.2	4.5	2.2	4.9	6.0	...	1.6	2.3	4.3	2.2
		old	9.5	8.6	8.9	8.6	12.5	11.3	1.5	2.4	1.7	3.9	2.8
7	unknown	young	0.8	0.5	0.5	0.3	0.5	0.4	...	0.1	0.1	0.1	0.0
		old	2.0	1.1	0.9	1.3	0.6	1.0	0.0	0.0	0.0	0.0	0.0
8	limonene	young	4.2	3.6	3.5	1.7	3.2	3.8	...	2.3	3.9	5.2	4.0
		old	3.1	2.9	3.6	3.5	3.3	4.1	1.4	1.9	2.8	4.0	4.2
9	β-phellandrene + 1,8-cineole + 2-hexenal	young	2.1	1.9	2.5	1.3	3.0	3.4	...	1.0	1.2	2.7	1.1
		old	3.4	3.1	3.7	3.6	2.4	4.5	1.2	2.0	1.2	1.9	1.7
10	ethyl caproate + unknown	young	0.6	0.3	0.4	f	f	f	...	0.3	0.6	0.7	0.9
		old	0.6	0.7	1.4	f	f	f	2.5	0.3	0.8	1.0	0.7
11	cis-ocimene	young	0.1	0.6	1.5	1.2	2.2	3.4	...	f	0.3	0.8	f
		old	6.8	6.7	6.4	6.5	9.5	8.8	0.1	f	0.3	0.8	f
12	γ-terpinene	young	1.1	0.8	0.9	0.6	1.0	1.0	...	0.1	0.1	0.2	0.1
		old	4.1	2.4	2.2	3.1	1.7	2.6	0.7	1.2	0.1	0.2	0.3
13	terpinolene	young	5.0	4.2	4.7	2.5	4.1	3.5	...	0.9	1.0	0.9	0.4
		old	12.3	7.6	4.9	8.7	3.2	5.9	0.5	0.6	0.6	0.7	0.6
14	citronellal	young	0.0	0.0	0.1	0.2	0.4	0.7	...	0.1	0.0	0.1	0.5
		old	0.7	0.8	0.5	0.9	0.8	0.7	0.3	0.7	0.4	0.5	0.7
15	linalool	young	0.0	0.0	0.1	0.2	0.3	0.4	...	0.0	0.7	0.4	0.9
		old	1.1	1.4	0.7	2.5	0.8	1.7	0.9	0.7	2.0	0.2	0.8
16	unknown	young	0.1	0.1	0.3	0.3	0.5	0.6	...	0.1	0.1	0.1	0.3
		old	1.2	1.2	0.9	1.5	1.3	1.3	0.4	1.3	0.2	0.2	0.3
17	fenchyl alcohol	young	0.2	0.3	0.2	0.2	0.3	0.2	...	0.1	0.1	0.1	0.1
		old	0.3	0.1	0.3	0.3	0.4	0.3	0.1	0.1	0.2	0.2	0.3
18	bornyl acetate + sesquiterpene hydrocarbon	young	0.9	0.6	0.8	0.9	1.2	1.3	...	0.3	0.4	0.6	0.7
		old	1.3	1.2	1.1	2.0	1.6	1.9	0.3	0.7	0.4	0.6	1.0
19	terpinen-4-ol	young	1.5	1.0	1.1	1.3	0.7	1.2	...	0.2	0.1	0.1	0.1
		old	7.5	3.9	2.4	5.7	3.4	3.0	0.2	0.2	0.1	0.1	0.2
20	β-caryophyllene	young	0.5	0.5	0.5	0.6	0.6	0.7	...	0.2	0.1	0.1	0.2
		old	0.7	0.6	0.6	0.9	0.8	0.8	0.2	0.4	0.1	0.1	0.1
21	unknown	young	0.0	0.0	0.1	0.1	0.2	0.5	...	0.0	0.0	0.0	0.0
		old	0.5	0.4	0.6	0.8	0.7	0.9	0.2	0.2	0.0	0.1	0.1
22	terpene alcohol	young	0.3	0.4	0.3	0.3	0.3	0.4	...	0.2	0.2	0.2	0.2
		old	0.4	0.4	0.2	0.2	0.4	0.3	0.1	0.2	0.1	0.1	0.1
23	citronellyl acetate	young	0.1	0.4	2.3	3.1	5.9	7.6	...	0.0	0.3	1.7	5.9
		old	6.9	7.2	6.5	10.8	8.9	11.2	?	4.6	3.1	3.8	7.1
24	α-terpineol	young	4.4	5.6	3.4	4.1	6.0	4.4	...	2.2	3.1	2.2	1.9
		old	5.0	3.6	5.3	6.2	5.8	4.0	?	1.8	2.9	2.8	2.9
25	sesquiterpene hydrocarbon	young	2.5	1.7	0.8	0.7	0.7	1.1	...	1.5	1.3	1.0	1.3
		old	0.4	0.3	0.2	0.6	0.4	0.6	0.2	0.1	0.1	0.2	0.4
26	citronellol + geranylacetate	young	0.0	0.0	0.5	1.3	3.0	3.9	...	0.0	0.1	0.7	3.3
		old	1.6	1.5	1.3	2.8	2.0	3.2	0.7	1.0	1.1	1.3	2.9
27	terpene alcohol	young	0.7	0.6	0.4	0.4	0.3	0.5	...	0.6	0.4	0.4	0.4
		old	0.4	0.5	0.2	0.5	0.4	0.4	0.3	0.5	0.1	0.2	0.3

[a] Isolated by steam distillation-extraction method; analyzed on 500 ft × 0.03 in. Carbowax 20M column, column oven isothermal at 75° C for 5 min, then programmed at 2°/min to 145° C. [b] Tree 1 was fertilized with nitrogen (urea); tree 2 with sulfur (gypsum). [c] No new growth available yet. [d] Young = new tip growth; old = 1 year old growth. [e] Listed peak heights were calculated relative to the peak height of the internal standard all at range 10, attenuation 32. [f] Peak observed as a shoulder only.

dried over anhydrous Na_2SO_4, and the pentane removed through a micro Vigreaux column using the concentration flask (shown in Figure 2) heated with a water bath at 50° C. After removal of the pentane, liquid was allowed to drain back down into the top of the flask up to approximately the 0.1 ml mark, 50 μl of a standard solution of n-tetradecane in n-heptane (100 μl per 100 ml) was added as an internal standard and the volume brought up to the 0.2 ml mark with n-heptane. After thorough mixing, a 0.50 μl sample was injected onto the 500-ft × 0.03-in. Carbowax 20M column for analysis.

Direct Vapor Analyses. A precolumn technique was used for the introduction of the vapor samples, since the direct injection of a large volume gas sample into the capillary column was not feasible. The precolumn arrangement,

shown schematically in Figure 3, consisted of a section of the stainless steel capillary tubing (12-in. × 0.03-in. i.d.) with a side opening needle sealed to the exit end, all coated with Carbowax 20M in the normal manner. The precolumn was connected to an injection port by means of a short section of Teflon tubing to permit direct electrical heating of the column. For the analysis, a 2.0-g sample of needles, cut into small pieces, was placed in a 300-ml infusion flask under a nitrogen atmosphere and allowed to equilibrate at room temperature for 1½ hr. To analyze for monoterpene hydrocarbons, a 9-ml gas sample was withdrawn into a 10-ml Hamilton gastight syringe [the syringe was filled and emptied five times to equilibrate the sample in the syringe (Kepner et al., 1964)] and 1 ml of saturated vapor over n-tridecane as a reference standard was also pulled into the syringe. The gas sample was

Table II. Direct Vapor Analyses of Douglas Fir Volatiles. Monoterpene Hydrocarbon Region[a]

Peak No.	Component	Needle Age[c]	Tree 1[b] Collection Date May 3	May 14	May 30	June 12	June 26	July 24	Tree 2[b] Collection Date May 14	May 30	June 26	July 24
			Peak Height[d]						Peak Height[d]			
1	α-pinene	young	572.8	531.0	643.8	656.0	896.0	771.5	400.0	384.2	448.0	313.5
		old	648.0	448.0	541.0	502.4	467.0	416.0	83.4	188.8	297.5	264.0
2	camphene	young	16.0	14.4	16.0	15.6	22.4	19.2	10.8	11.6	10.6	9.6
		old	15.0	9.0	14.2	11.2	11.8	11.2	3.8	5.6	8.0	7.8
3	β-pinene	young	809.5	800.0	985.0	992.0	1343.0	1165.0	624.8	678.5	781.0	577.0
		old	794.0	620.5	816.0	694.4	730.0	710.0	259.0	320.0	461.5	476.8
4	sabinene	young	44.0	50.0	95.0	72.0	44.0	81.8	19.2	e	e	e
		old	193.6	146.4	125.6	150.0	60.0	66.0	3.4	e	e	e
5	3-carene	young	17.2	24.6	24.8	39.2	25.2	1.6	22.8	11.6	1.6	...
		old	12.8	10.0	12.8	9.6	2.8	4.4	5.6	20.0	4.0	...
6	myrcene	young	26.0	28.4	42.0	58.8	56.0	64.8	18.8	30.0	34.4	26.0
		old	54.4	42.8	52.4	54.8	45.2	42.8	7.2	5.6	14.0	11.8
7	unknown	young	1.1	1.4	1.6	0.6	0.5	0.3	0.4	0.1	...	...
		old	1.4	1.5	0.8	...	0.2	0.3	0.1	...	...	...
8	limonene	young	27.6	22.1	18.0	17.0	18.2	17.3	18.0	33.8	22.8	21.3
		old	10.1	8.4	10.3	9.5	8.9	9.3	3.5	9.1	12.4	13.5
9	β-phellandrene + 1,8-cineole + 2-hexenal	young	13.6	12.2	16.3	15.7	20.5	18.2	18.7	9.8	11.8	7.8
		old	12.0	10.3	12.0	10.9	10.0	9.9	3.6	4.0	5.7	5.6
10	ethyl caproate + unknown	young	1.0	0.7	0.5	0.0	0.3	0.0	0.3	0.3	0.2	0.2
		old	0.5	0.1	0.1	0.0	0.0	0.1	...	0.5	0.1	...
11	cis-ocimene	young	0.5	2.3	8.6	10.0	10.8	12.2	0.1	1.9	3.0	2.4
		old	18.7	16.0	17.4	16.7	14.5	15.5	0.1	0.9	1.0	0.8
12	γ-terpinene	young	1.4	1.6	1.8	1.5	0.9	1.0	0.3	0.1	0.2	0.3
		old	2.2	1.7	1.5	1.7	0.8	1.2	1.7	0.2	0.1	0.1
13	terpinolene	young	14.8	12.6	15.1	11.3	8.6	10.1	2.7	0.6	...	0.3
		old	17.5	15.0	12.3	15.3	5.6	6.1	0.3	0.1	...	0.1

[a] 500 ft × 0.03 in. Carbowax 20M column, column oven isothermal at 75° C. [b] Tree 1 was fertilized with nitrogen (urea); tree 2 with sulfur (gypsum). [c] Young = new tip growth; Old = one year old growth. [d] All peak heights were calculated at range 10, attenuation 8. [e] Peak observed as a shoulder only.

slowly (5 ml per min) passed into the precolumn cooled in a dry ice bath. The needle of the precolumn was inserted into the injection port of the gas chromatograph, the dry ice trap removed, and the carrier gas flow diverted through the precolumn, which was then heated electrically to 140° C in a few sec time and maintained there for 3 min. The carrier gas flow was then diverted back through the gas chromatograph injection chamber and the chromatogram developed isothermally at 75° C. For the direct vapor analysis of the oxygenated monoterpenes, a 50 ml gas sample (no internal standard) was injected by the same procedure and the chromatogram developed isothermally at 135° C.

Gas Chromatography. An F & M Model 810 gas chromatograph with dual flame ionization detection was used. The instrument was fitted with two 500-ft × 0.03-in. i.d. stainless steel capillary columns coated with Carbowax 20M + Igepal (20 to 1) and SF 96(50) + Igepal (20 to 1), respectively; injection temperature, 170° C (liquid injections) or 130° C (direct vapor injections); detector temperature, 210° C; column temperature, programmed from 75° to 145° C (liquid injections) or isothermal at 75° or 135° C (direct vapor injections); He, H₂, and air flows were 9, 23, and 300 ml per min, respectively.

RESULTS AND DISCUSSION

Because the results of the analyses of the three trees fertilized with urea were very similar, data for only one of the three (tree 1, fertilized 1 year prior to analysis) and data for the tree fertilized with gypsum (tree 2) are presented. In considering the changes in composition of the volatile terpenes during maturation, only foliage samples from the same tree are compared in order to avoid differences that might exist between trees. The relatively small size of the trees investigated tended to minimize variations in composition due to the loca-

tion of the foliage sample on the tree (Juvonen and Lako, 1967) but necessitated the collection of small foliage samples and the use of extremely sensitive methods of analysis. The amounts of the individual components were measured in terms of peak heights, since the peaks from the capillary columns are very sharp and the changes rather than the absolute amounts of the components are of most significance in this study. The changes in composition of most of the monoterpene hydrocarbons and the oxygenated monoterpenes, the major components of mature needle oil, were followed, but the compositions of most of the sesquiterpene components, present in relatively small amounts, were not investigated.

The analyses of the essential oil samples obtained by the simultaneous steam distillation-extraction technique are given in Table I. Figure 4 presents typical chromatograms for the new and old growth essential oils isolated from foliage from the first collection from the tree fertilized with nitrogen. The direct vapor analyses of the monoterpene hydrocarbons and of the oxygenated monoterpenes in equilibrium with the needles are given in Tables II and III, with typical chromatograms for these analyses illustrated in Figures 5 and 6, respectively. The data in each table are internally consistent in that peak heights were calculated to a common reference basis for each analytical method, and can be used to interrelate the relative amounts of the various components and the changes in a given component over a growing season. However, the calculated peak heights cannot be cross related between tables to compare absolute amounts of components by the different methods of analysis. Included in this study are four monoterpenes (1,8-cineole, cis-ocimene, β-phellandrene, and sabinene) not identified in the earlier study (Sakai et al., 1967) but subsequently identified by their mass spectra and Kovats indices.

The same major changes in volatile terpenes in the maturing

Table III. Direct Vapor Analyses of Douglas Fir Volatiles. Oxygenated Monoterpene Region[a]

Tree 1[b]
Collection Date

Peak No.	Component	Needle Age[c]	May 3	May 30	June 12	June 26	July 24
					Peak Height[d]		
14	citronellal	young	0.3	3.5	19.0	14.2	30.8
		old	...	...	16.7	19.4	25.6
15	linalool	young	0.0	1.9	2.5	2.6	3.6
		old	...	...	7.2	8.5	13.8
16	unknown	young	0.8	4.8	5.0	5.2	5.2
		old	...	...	12.3	11.1	8.4
17	fenchyl alcohol	young	0.1	e	e	e	e
		old	...	...	e	e	e
18	bornyl acetate + sesquiter-	young	4.3	9.6	12.8	8.0	9.2
	pene hydrocarbon	old	...	...	8.1	8.7	8.6
19	terpinen-4-ol	young	3.2	5.0	5.6	4.6	3.4
		old	...	...	7.2	5.6	4.4
20	β-caryophyllene	young	3.4	8.2	8.1	5.8	4.6
		old	...	...	5.8	5.4	4.2
21	unknown	young	0.2	0.5	0.4	0.2	0.2
		old	...	...	2.1	2.3	3.4
22	terpene alcohol	young	1.1	1.8	1.4	0.7	...
		old	...	...	...	0.4	...
23	citronellyl acetate	young	0.2	12.5	13.8	12.6	20.0
		old	...	...	18.3	21.4	23.8
24	α-terpineol	young	3.1	9.6	9.4	6.3	4.2
		old	...	...	3.9	3.4	2.4
25	sesquiterpene hydrocarbon	young	3.5	2.5	2.4	1.3	1.4
		old	...	...	0.9	0.8	0.7
26	citronellol + geranyl-	young	0.0	1.4	4.1	3.5	7.0
	acetate	old	...	...	2.6	2.8	4.9
27	terpene alcohol	young	0.6	0.6	0.5	0.5	0.5
		old	...	...	0.6	0.5	0.5

[a] 500 ft × 0.03 in. Carbowax 20M column, column oven isothermal at 135° C. [b] Tree 1 was fertilized with nitrogen (urea). [c] Young = new tip growth; Old = one year old growth. [d] All peak heights were calculated at range 1, attenuation 32. [e] Peak observed as a shoulder only.

needles were observed whether the simultaneous steam distillation-extraction technique or direct vapor analysis was used (Tables I, II, and III), indicating that the heating associated with the steam distillation process was not causing major artifact formation. If artifacts are produced in these analyses, they must be minor components which were not followed in this study, or they must be components which are formed during both procedures—either under the low heat conditions of stripping off the solvent, or enzymatically as a result of injury to the plant tissues during processing of the needle samples.

The temperature programmed chromatograms of the first method did not separate β-pinene and sabinene adequately, and 3-carene and myrcene appeared as a single peak (Figure 4 and Table I). α-Pinene and β-pinene, the two major volatile terpenes present in the oils isolated by the steam distillation method, showed slight seasonal increases which were comparable in both young and old growth, with the amounts always greater in the old than the new growth (Table I). In the direct vapor analyses the amounts of α-pinene and β-pinene were greater in the young than in the old growth, with a slight seasonal increase shown in the new growth and decrease in the old growth (Table II). This difference is undoubtedly the result of the different methods of isolation, steam distillation vs. direct vapor measurements, of the volatile components from the plant material, and suggests the possibility that the surface structure of the new growth may be more permeable to terpenes than that of mature growth. The large amounts of α- and β-pinenes in the needles result in the formation of two artifact peaks (Figure 5, peaks 1a and 3a) in the direct vapor chromatograms which are the direct result of the backflow of a few percent of the total sample into the dead space of the chromatograph injector chamber during the injection from the precolumn (Lunteren et al., 1967). When the He flow is diverted back through the chromatograph injection chamber,

this backflow sample is injected onto the column several minutes later than the regular sample, and results in artifact peaks for the two components present in sufficient quantities to be observed on the chromatogram.

The amount of sabinene in the year old needles showed a marked seasonal drop (Table II), while the content in the young needles increased slightly as they matured. The direct vapor analyses for 3-carene (Table II) demonstrated a sharp decrease in amount in both young and old needles near the end of the period studied. The acyclic monoterpene hydrocarbon, cis-ocimene, was essentially absent in the new growth and increased strongly as the needles matured, while the amount in the old needles was essentially constant over the period studied (Tables I and II). A second acyclic monoterpene hydrocarbon, myrcene, which could be followed under the conditions of the direct vapor analyses (Table II), also showed a definite seasonal increase in the maturing new growth. No significant differences between young and old needles or changes during maturation were observed for camphene, β-phellandrene, 1,8-cineole, limonene, γ-terpinene, or terpinolene.

The changes in terpene compositions which are of most significance in this study are those involving the oxygenated monoterpenes. The acyclic oxygenated monoterpenes previously identified in mature Douglas fir needles (Sakai et al., 1967), citronellal, citronellol, citronellyl acetate, geranyl acetate, and linalool, are completely or almost completely missing in the new growth as it first emerges in the spring (Tables I and III). These compounds increase in amounts as the needles mature, but show essentially no seasonal variation in the mature needles. In contrast, the cyclic oxygenated monoterpenes previously identified (Sakai et al., 1967), fenchyl alcohol, bornyl acetate, terpinen-4-ol, and α-terpineol, are all immediately present in the new tips at concentrations approximately equal to those in the mature needles, and show

Figure 5. Gas chromatograms of direct vapor analyses of young and old Douglas fir needles (Tree 1). Monoterpene hydrocarbon region

500-ft × 0.03-in. i.d. Carbowax 20M column; precolumn injection of sample; temperature isothermal at 75° C; chart speed, 20 in. per hr; range 10, attenuations as indicated
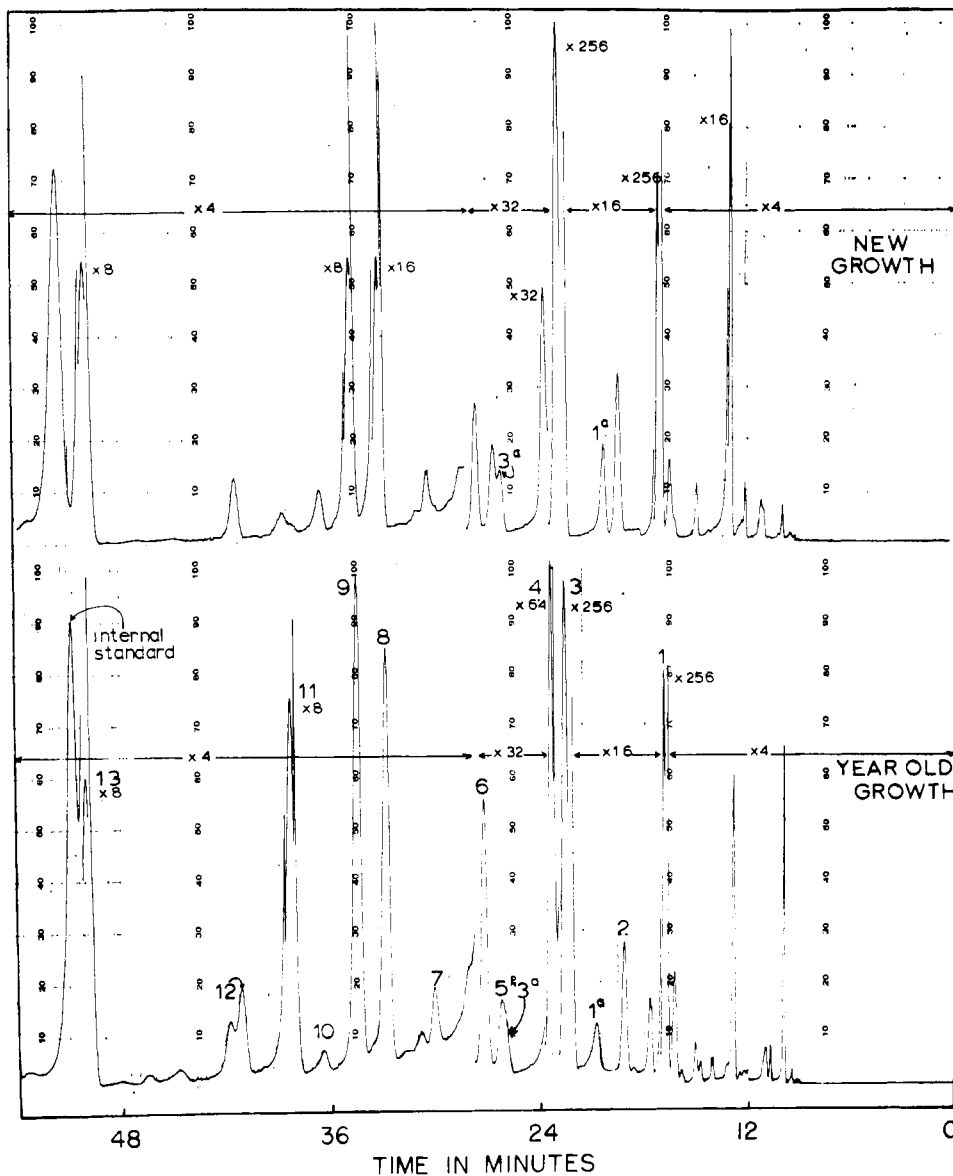
Figure 6. Gas chromatogram of direct vapor analysis of young Douglas fir needles (Tree 1). Oxygenated monoterpene region

500-ft × 0.03-in. i.d. Carbowax 20M column; precolumn injection of sample; temperature isothermal at 135° C; chart speed, 20 in. per hr; range 1, attenuations as indicated
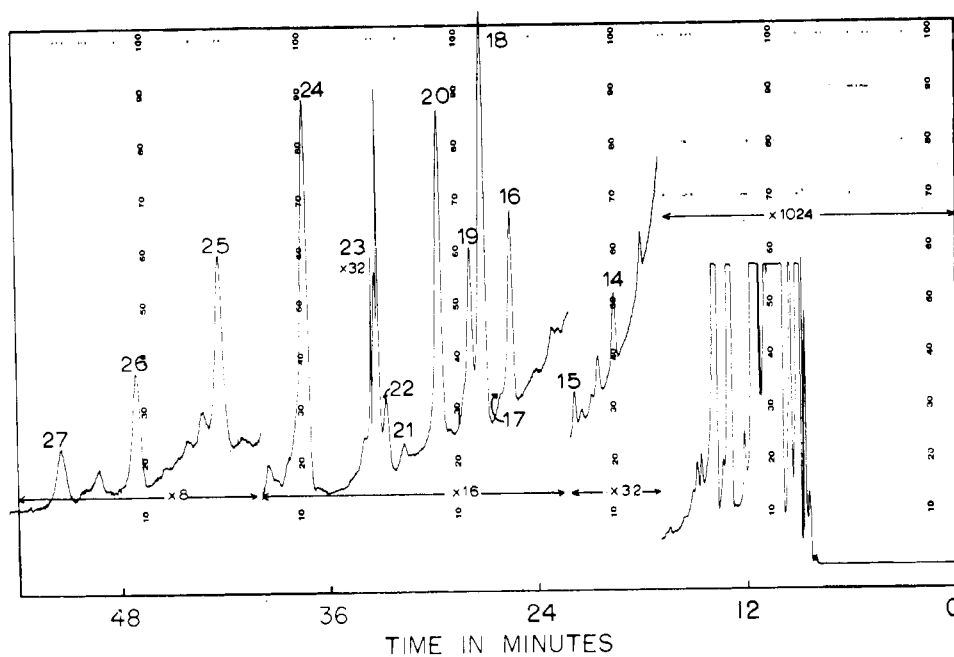
essentially no seasonal variation in either the year old or new growth foliage. The experimental results further suggest by analogy that the unknown compounds of peaks 16 and 21 are most likely acyclic, and those of peaks 22 and 27 are most likely cyclic oxygenated monoterpenes.

Experiments with model compounds (Cramer and Rittersdorf, 1967; Loomis, 1967; Miller and Wood, 1964; Rittersdorf and Cramer, 1967, 1968) suggested the likelihood of neryl pyrophosphate rather than geranyl pyrophosphate as the main precursor of cyclic monoterpenes. Beytia *et al.* (1969) obtained evidence for the formation of neryl pyrophosphate by the enzyme system of *P. radiata* seedlings in tracer experiments with $2\text{-}^{14}C$-mevalonic acid, and suggested that neryl pyrophosphate is most likely the precursor of cyclic monoterpenes in this system. The almost complete lack of acyclic monoterpenes and the immediate appearance of cyclic monoterpenes in the young growing tips of Douglas fir foliage suggest the possibility that the immediate precursor of the monoterpenes in the new growth is most likely neryl pyrophosphate, with geranyl pyrophosphate becoming important as the needles mature.

In essential oil samples isolated from very young Douglas fir foliage, Peak 25 (Table I and Figure 4) is a very large peak compared to the size of the peak from mature needles. The concentration of terpenes contributing to this peak diminishes rapidly in the first three weeks of growth of the new needles. The terpenes in this peak were isolated from needles collected about 1 week after first appearance of the new growth, and were shown by infrared and glc to be a mixture of sesquiterpene hydrocarbons. Insufficient material was obtained to permit characterization of the sesquiterpene hydrocarbons present. The components of this peak are under further investigation to determine whether the transient component or components in the very young tips may possibly be precursors in the formation of additional sesquiterpene components.

The effect of fertilizing Douglas firs with urea (tree 1) *vs.* gypsum (tree 2) is quite marked. The foliage of tree 2 was not as green or healthy as that of tree 1, and the new growth was approximately 2 weeks later in developing. The total level of volatile terpenes in tree 2 was much lower than in tree 1 (Tables I and II). The vapor analyses (Table II) gave much larger amounts of monoterpene hydrocarbons in the young foliage than in the year old needles. The steam distillation-extraction analyses (Table I) demonstrated the same lack of acyclic oxygenated monoterpenes in the new growth of tree 2 as was observed for tree 1, with the corresponding cyclic components again present immediately in the new growth.

The significance of the results of this investigation with respect to the food chain habits of browsing ruminants is not easily ascertained. The young growing tips of Douglas fir foliage, which are the most palatable to animals, are shown to have lower total concentrations of monoterpene alcohols and carbonyl compounds than the mature needles. More work is needed to determine whether the lower amounts of these components, which have been shown by Oh *et al.* (1967) to be highly inhibitory to the functioning of the rumen microorganisms of deer and sheep, is a significant factor in the greater palatability of the new growth to the browsing animals. The balance of nutrients *vs.* inhibitors (Longhurst *et al.*, 1968) is also very likely a factor in the differences in palatability of the new and the old growth.

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