MONOTERPENE CONCENTRATION IN DOUGLAS-FIR IN RELATION TO GEOGRAPHIC LOCATION AND RESISTANCE TO ATTACK BY THE DOUGLAS-FIR BEETLE

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The concentration of monoterpenes in *Pinus monticola* Dougl. has been shown to be genetically controlled (Hanover, in preparation). Genetic control of terpene concentration has been implied, also, from analyses of parents or interspecies hybrids in other species (Bannister et al. 1959; Williams and Bannister 1962; Smith 1964, and Forde 1964). Evidence that genes regulate the formation of plant terpenes has stimulated the use of the terpenes as an aid in population studies. Bannister et al. (1962) showed significant variation in α-pinene and β-pinene between three populations of *Pinus radiata* in California. Also, Forde and Blight (1964) found that *Pinus muricata* Don in California could be classified into three different groups according to chemical composition. The monoterpene composition of *Pseudotsuga* has not been defined adequately although Cvrkal and Janak (1959) included *Pseudotsuga douglasii* Lindl. among several conifers they examined.

We were particularly interested in measuring the monoterpenes in wood oleoresin of *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco to determine the variation in monoterpenes (1) between individual trees, and (2) between groups of geographically separated trees. We also wished to

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determine the relation between monoterpenes and resistance to attack by the bark beetle, *Dendroctonus pseudotsugae* Hopk. Finally we studied seasonal variation of monoterpenes, viscosity of resin, and the relation between monoterpenes and several tree characteristics.

Materials and Methods

Monoterpenes were sampled in 1964 at three localities in Idaho and Montana. The three localities (fig. 1) are sufficiently isolated to permit some genetic divergence between populations in each area. Oleoresin was sampled from groups of trees that survived attack by the bark beetles during 1962 and also from unattacked trees within Salmon River (A, Valley County) and Flathead Lake (B, Lake County) locations. At Moscow Mountain (C, Latah County) only unattacked trees were sampled. The 94 sample trees ranged in age from 81 to 184 years; diameter at breast height ranged from 24.9 to 76.7 cm; the last 10-year radial growth ranged from 1.4 to 41.6 mm; and crown class ranged from suppressed to dominant. Tree measurements did not appear to differ significantly between localities.

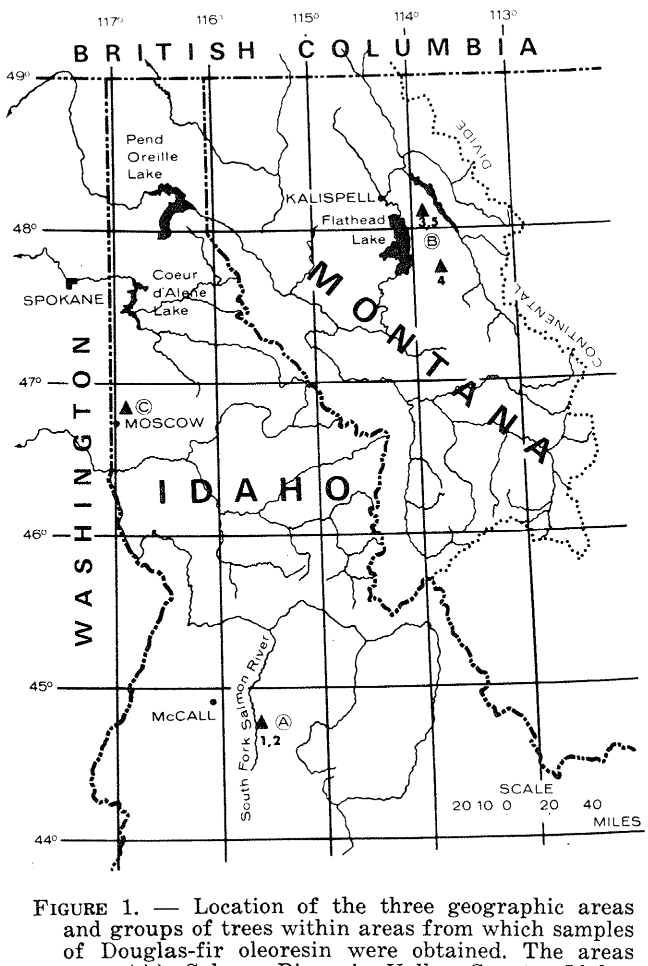

FIGURE 1. — Location of the three geographic areas and groups of trees within areas from which samples of Douglas-fir oleoresin were obtained. The areas are: (A) Salmon River in Valley County, Idaho; (B) Flathead Lake in Lake County, Montana; and (C) Moscow Mountain in Latah County, Idaho.

Differences in monoterpenes between attacked (resistant) and unattacked trees were tested by statistical analysis. When no significant differences occurred between groups, both types of trees within each area were combined for tests of population differences.

Oleoresin samples were collected during the latter part of the bark beetle flight period, between June 6 and 19, 1964. Fifteen trees of the Flathead Lake population were sampled again on October 14, 1964, to determine seasonal variation in their monoterpenes. Samples were collected and analyzed as follows: One day prior to collection of oleoresin, each tree was chopped to expose a cross-sectional portion of the wood. A 30-microliter sample of oleoresin that exuded from the wood resin ducts was drawn into a calibrated glass capillary tube. The tube of oleoresin was kept in a sealed centrifuge tube under refrigeration until analyzed soon afterwards.

Monoterpenes were analyzed qualitatively and quantitatively by gas-liquid chromatography. The oleoresin sample was dissolved in 50 microliters of acetone and a 2-microliter aliquot injected into an F&M Model 500[2] gas chromatograph with a flame ionization detector. The chromatograph column was 1/4″ X 6′ stainless steel packed with 10-percent polypropylene glycol on 60-80 mesh Diatoport W-AW. Temperatures were: injection port, 195 C; column, 95 C; detector, 185 C. Helium flow rate was 165 ml per minute at the injection port.

Monoterpenes were tentatively identified by comparing relative retention times of the unknowns with those of known compounds. Identity was also determined by the addition of known monoterpenes to the plant samples to enhance the corresponding peaks. This procedure utilized both the polar polypropylene glycol column and a nonpolar Apiezon-L column packing. Agreement between two such columns is considered sound qualitative determination. With Apiezon-L, temperatures were: injection port, 195 C; column, 135 C; detector, 195 C. Helium flow rate was 35 ml per minute. Monoterpene concentration was expressed as a percent of total oleoresin by use of standard curves relating peak area (disc integrator values) and monoterpene concentration. Because no curve was available for the unknown monoterpene, this component of the oleoresin was omitted in determining total concentrations of the monoterpenes.

[2] *Use of trade name is for identification and does not constitute endorsement by the Forest Service.*

Terpenes Detected and Their Concentrations

Seven terpenes were detected in the Douglas-fir wood oleoresin (fig. 2). Six of these were tentatively identified, in the order of their retention times, as α-pinene, camphene, β-pinene, myrcene, 3-carene, and limonene. The remaining compound, number 7, has not been identified. In the 94 trees of this study, α-pinene was always the predominant monoterpene. It ranged from 10.4 to 52 percent of the oleoresin content, exclusive of the unknown. Other monoterpenes were present in the following concentrations: β-pinene, trace to 16.2 percent; myrcene, 0.0 to 4.2 percent; 3-carene, 0.0 to 7.0 percent; and limonene, 0.3 to 8.4 percent. Because camphene was always present in but minute amounts, it was excluded from statistical analyses.

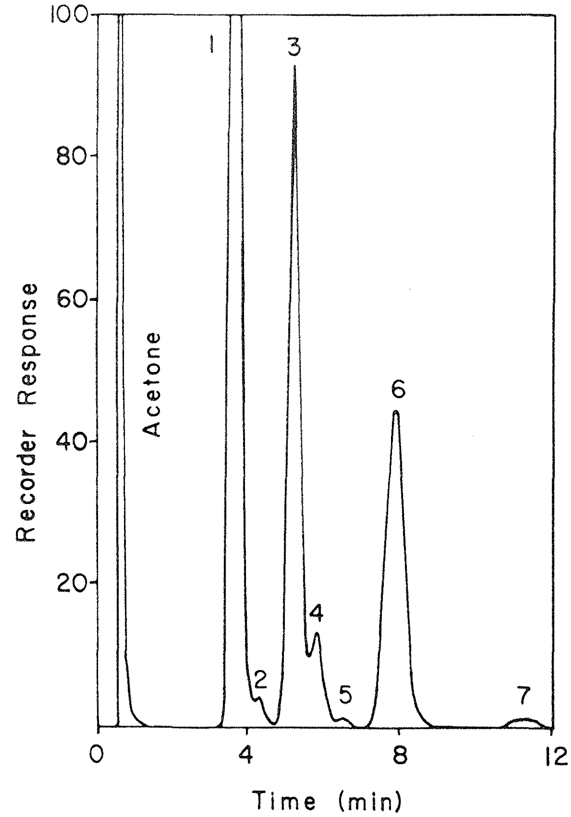

FIGURE 2. — Typical chromatogram of monoterpenes in wood oleoresin of *Pseudotsuga menziesii* var. *glauca* dissolved in acetone (see text).

Monoterpenes and Resistance to Douglas-Fir Beetle

Monoterpenes did not differ qualitatively between trees that survived attack and those that were unattacked (table 1). However, some quantitative differences occurred. At Flathead Lake, concentration of 3-carene differed significantly between trees that resisted attack (groups 3 and 4) and unattacked trees (group 5), while concentration of the unknown monoterpene differed between groups 3 and 5. These two monoterpenes also usually were more highly concentrated in unattacked trees at Salmon River and Moscow Mountain, but the resistance of trees at Moscow Mountain is not known.

The data in table 1 do not directly compare beetle-resistant and susceptible trees. Several problems prevent such a comparison. For example, death after attack was used as proof of susceptibility. Lack of oleoresin exudation pressure, and physiological changes accompanying death made resin sampling impossible or undesirable. Also, the trees that survived may have been attacked by too few beetles to kill them; or the unsuccessful attacks may have disrupted the physiology of the sample trees and affected their monoterpenes. Furthermore, probably not all the unattacked trees in this study were susceptible to beetle attack. The beetle characteristically attacks groups of trees without evident selection. Our inventories of these groups have shown that each contained some survivors. Twenty-two percent of 740 trees attacked in the Flathead Lake area were still alive when examined in 1963, 1 year after attack. Thus, any group of unattacked trees selected at random for sampling is likely to contain some trees that would resist beetle attack. Therefore, our sample of unattacked trees is probably confounded by the presence of resistant trees in the sample.

Table 1.--Mean concentrations of monoterpenes in oleoresin from trees that resisted attack and unattacked trees

Geographic area and group	Number of trees	Resisted (R) or unattacked (U)	Percent of oleoresin and standard error for-						Unknown (integrator units and standard error)
			α-pinene	β-pinene	Myrcene	3-carene	Limonene	Total	
Salmon River									
A 1	25	R	28.2±1.08	2.1±0.3	0.8±0.1	0.1±0.1	2.4±0.2	33.6±1.1	10.6±1.5
A 2	10	U	26.7±2.43	3.6±1.0	0.5±0.1	0.6±0.4	2.5±0.4	33.9±3.2	14.0±6.0
Flathead Lake									
B 3	11	R	21.7±1.5	7.1±1.2	0.9±0.1	0.1±0.1	2.9±0.1	32.6±0.8	4.5±1.9
B 4	15	R	25.1±1.0	6.9±1.2	0.9±0.2	0.4±0.2	3.2±0.4	36.5±1.4	11.5±2.6
B 5	22	U	22.1±1.2	4.7±0.6	0.4±0.7	1.6±0.5	2.9±0.3	31.6±1.1	17.0±3.3

Unfortunately, we know no suitable method of testing resistance or susceptibility of Douglas-fir to bark beetle attack. Until such a method is available, biochemical or similar comparisons of trees will be inconclusive.

Seasonal Variation

Concentration of α-pinene and β-pinene increased significantly between June and October (table 2). In some other species, concentration of monoterpenes has remained constant between seasons (Smith 1964; Mirov 1961). However, Bannister et al. (1959) found that the ratio of α-pinene to β-pinene in resin samples from *Pinus radiata* varied seasonally (seasons not specified) but to a lesser degree than between trees. Analyses of variance between and within seasons showed that the seasonal variation in amounts of α-pinene and β-pinene in Douglas-fir was also significantly less than the between-tree variation. However, the relative concentrations of monoterpenes — especially α-pinene — in individual trees did change slightly between seasons. Concentrations of the three other identified monoterpenes were relatively constant between June and October, but the error in measuring low concentrations could mask small seasonal differences. Monoterpenes in *Pinus monticola* (Hanover, in preparation) show a seasonal variation pattern, similar to that of Douglas-fir. Therefore, in comparing monoterpene content of individual trees or in studying monoterpenes in relation to physiological traits, it is desirable to restrict sampling of oleoresin of these species to a uniform time of the year.

Monoterpenes and Tree Measurements

Simple correlation coefficients of all monoterpenes with 10-year radial growth, diameter, and age were computed for 94 trees by locality. Only combined data for all localities are presented in table 3. Highest positive correlations existed between β-pinene and limonene, and 3-carene and the unknown. Within localities, strong positive correlations also existed between β-pinene and limonene and between myrcene and limonene. Monoterpenes were not strongly correlated with tree diameter, age, or growth.

Although only 11 trees were involved at Flathead Lake (Group B-3) it is interesting that all of the identified monoterpenes were highly correlated ($P < 0.01$) either positively or negatively with one another in the group. This result was unique and may reflect a physiological response of trees that repelled bark beetle attack. Possibly, the so-called resistant trees of group 3 were more resistant to attack than the other "resistant" groups. Greater resistance of group B-3 may also be indicated by its low content of 3-carene and unknown terpene as compared with other groups (table 1).

Table 2.--Seasonal variation in the concentrations of five monoterpenes in individual Douglas-fir trees at Flathead Lake

	Percent of oleoresin for-											
Tree	α-pinene		β-pinene		Myrcene		3-carene		Limonene		Total	
	June	Oct.	June	Oct.	June	Oct.	June	Oct.	June	Oct.	June	Oct.
13	28.2	25.1	6.0	6.6	0.6	1.2	0.1	0.6	4.1	4.7	39.0	38.2
17	31.6	26.7	1.9	2.8	0.5	1.2	0.1	0.3	1.3	2.5	35.4	33.5
23	18.7	26.9	10.4	10.8	0.1	0.1	0.1	0.1	5.9	4.8	35.2	42.7
26	22.4	25.0	4.4	5.0	0.6	0.6	4.5	3.9	4.0	2.9	35.9	37.4
30	12.7	20.9	5.7	7.4	0.1	0.1	5.0	6.3	3.3	2.7	26.8	37.4
31	23.5	41.3	1.6	2.3	0.3	0.4	0.0	0.1	1.3	1.4	26.7	45.5
32	18.6	22.9	3.6	4.1	1.1	0.8	0.0	0.2	5.6	4.3	28.9	32.3
33	18.4	25.5	5.0	6.0	0.3	4.2	5.2	*	3.5	3.2	32.4	38.9
34	19.1	19.6	6.8	7.7	0.1	0.1	3.7	5.8	3.9	3.7	33.6	36.9
42	24.2	35.7	1.3	2.4	0.1	0.1	2.5	3.9	0.9	0.5	29.0	42.6
51	24.4	41.3	5.2	7.6	0.6	0.1	0.1	0.6	3.6	3.0	33.9	52.6
63	29.3	52.0	1.9	2.3	0.1	0.1	0.0	0.1	0.6	0.8	31.9	55.3
64	17.8	23.0	9.0	12.1	0.5	0.1	0.1	0.5	4.7	4.8	32.1	40.5
69	26.0	23.3	5.5	5.5	0.9	1.7	0.1	0.4	3.3	4.3	35.8	35.2
94	17.6	21.0	8.2	9.2	0.1	0.1	0.1	0.3	3.5	4.3	29.5	34.9
Average	22.2	28.7	5.1	6.1	0.4	0.7	1.4	1.5	3.3	3.2	32.5	40.2
Paired t-test	$P < 0.01$		$P < 0.01$		NS		NS		NS		$P < 0.01$	

* Not determined

Table 3.--Simple correlation coefficients for Douglas-fir monoterpenes and tree characteristics (93 d.f.).

Monoterpenes	β-pinene	Myrcene	3-carene	Limonene	Unknown	10yr. radial growth	Diameter	Age
α-pinene	-0.430**	0.222*	-0.188	-0.315**	0.030	-0.018	0.111	0.197
β-pinene	--	-.011	-.001	.657**	.058	-.005	-.172	-.226
Myrcene		--	-.386**	.378**	-.024	-.010	-.002	-.049
3-carene			--	.046	.557**	.102	-.242*	-.230*
Limonene				--	.170	.125	-.064	-.245*
Unknown					--	-.037	-.101	-.091
Total						.077	-.034	-.059

* $P < .05$, $r = .202$
** $P < .01$, $r = .264$

Table 4.--Quantitative variation in Douglas-fir monoterpenes associated with geographic location of trees [1/]

Geographic area	Percent of oleoresin and standard error for-						Unknown (integrator units and standard error)
	α-pinene	β-pinene	Myrcene	3-carene	Limonene	Total monoterpenes	
A-Salmon R.	27.8±1.0	2.6±0.3	0.70±0.08	0.27±0.13	2.41±0.19	33.7±1.2	11.6±2.0
B-Flathead L.	[22.9±0.7	[5.9±0.5	0.66±0.07	[2/] 0.86±0.24	[3.01±0.21	33.4±0.7	12.4±1.9
C-Moscow Mtn.	21.9±1.4]	5.4±0.8]	0.55±0.17	1.10±0.61	4.01±0.59]	33.0±1.6	13.1±4.5

1/ Bracketed values differ from unbracketed values at the 0.05 or lower probability levels.

2/ Differs significantly ($P < .05$) from Salmon River only.

Oleoresin viscosity varied considerably in samples collected for the analyses. However, "very viscous" and "very fluid" resin did not appear to differ in monoterpene composition or content.

Geographic Differences

If monoterpenes in Douglas-fir are controlled genetically, one could expect an evolution of population differences for these compounds. Such differences might be detectable between populations that are widely separated geographically or by some other barrier to natural crossing. The three populations sampled in this study differed significantly in certain components of their monoterpene composition (table 4). The Salmon River population is distinguished from both Flathead Lake and Moscow Mountain populations by having comparatively higher levels of α-pinene but lower levels of β-pinene, 3-carene and limonene. However, mean total concentration of monoterpene was remarkably constant for all localities.

If these results are representative, they support our hypotheses that the Flathead Lake and Moscow Mountain populations have diverged but little, or that they are more closely related to one another than to the Salmon River population. Further speculation about their evolutionary patterns, based upon monoterpene data, would require more sampling than is reported here. As pointed out by Bannister et al. (1962), interpretation of statistical analyses of population data assumes random sampling and homogeneous variances within the sampled populations. We lack proof of this. Also, we assume a lack of environmental influence on the traits analyzed. Nonetheless, the present study indicates that monoterpenes may be useful in more intensive study of insect resistance, genetics, and physiology of Douglas-fir.

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