Phytochemistry, 1970, Vol. 9, pp. 377 to 395. Pergamon Press. Printed in England.

ON THE GEOGRAPHIC VARIABILITY OF THE MONOTERPENES FROM THE CORTICAL BLISTER OLEORESIN OF *ABIES LASIOCARPA*[*]

EUGENE ZAVARIN[†], KAREL SNAJBERK[‡], THOMAS REICHERT[§], and ELAINE TSIEN[||]

Forest Products Laboratory, University of California, Richmond, California, U.S.A.

(*Received* 22 *July* 1969)

Abstract—Over 400 trees of *Abies lasiocarpa* from fifty localities covering its entire range have been examined for composition of the cortical monoterpenes. The existence of two chemical variants was demonstrated. One of these was distributed predominantly in the areas closer to the Pacific coast, and contained turpentine with large amounts of β-pinene and β-phellandrene and small amounts of limonene; the other, concentrated mainly in the U.S. Rocky Mountains, produced turpentine with large amounts of limonene and small amounts of the other two compounds. The frequency distribution diagrams for the monoterpenes were either poly-modal or skewed, thus suggesting control by few genes only. Strong positive correlation between β-pinene and β-phellandrene, and strong negative correlations between the sum of these compounds and limonene, was obtained.

INTRODUCTION

Abies lasiocarpa (Hook.) Nutt. is a North American fir, belonging to a group of three related species which include *A. balsamea* L. (Mill.) and *A. fraseri* (Pursh) Poir. *A. lasiocarpa* ranges from the Yukon Territory through British Columbia and western Alberta, to southern Oregon in the Cascades, and to Arizona and Colorado in the Rocky Mountains (Fig. 1).[1] Recently, it has been reported to occur on Prince of Wales Island[2] and near Anchorage[3] both in Alaska. In its extreme northern range it descends to sea level, but in the United States it is a high elevation species, usually growing in the northern Rockies between 5000 and 9000 ft, in the southern Rockies between 9500 and 11,000 ft, and in southern Cascades between 5000 and 7500 ft.

A. lasiocarpa represents usually a fairly small tree with a narrow, spire-like crown and smooth, exceptionally blistery, grey-white bark which becomes rough only when relatively old and then only near the base. Morphological characteristics separating *A. lasiocarpa* from the other firs of its group are either few or rather subtle or both, so that the taxonomic status of the three species cannot be regarded as completely settled. A proposal has even been made recently to regard all three as subspecies or varieties of *A. balsamea*.[4] Two intragradation problems exist with *A. lasiocarpa*: in Colorado and New Mexico the typical

* Part V in the series "Chemotaxonomy of the genus *Abies*".
† Forest Products Chemist.
‡ Assistant Specialist.
§ Research Assistant.
|| Laboratory Technician.

[1] H. A. FOWELS, *Silvics of Forest Trees of the United States*, Agr. Handbook No. 271, U.S. Dept. of Agr. Forest Service, p. 36 (1965).
[2] A. S. HARRIS, *Northwest S.* 39, 132 (1965).
[3] E. LEPAGE, *Am. Midland Naturalist* 46, 754 (1951).
[4] B. BOIVIN, *Naturaliste Canadien* 86, 219 (1959).

variety intergrades with *A. lasiocarpa* var. *arizonica* Merriam (a fir also growing in Arizona), which can be distinguished from *A. lasiocarpa* proper chiefly by a corky, thick bark (occasionally up to 0·5 in. thick); in central Alberta, *A. lasiocarpa* intergrades with *A. balsamea*, from which it differs in morphology largely by the presence of stomata on the upper surface of its leaves.

The present report is a continuation of the attempt to clarify the problems mentioned—i.e., those of intergradation and of taxonomic status—by the combined methods of quantitative analysis of pertinent morphological variables and of chemotaxonomic investigations, based on chemical analysis of terpenoids from the cortical blister oleoresin. To effectively attack these problems, it is obvious that a clear picture of the natural variability within "pure" species must be available. Because distinguishing morphological characters of the taxa under investigation are fairly well known, we decided to limit the quantitative analysis of these primarily to the regions of intergradation. Chemical variability between individual trees and between geographically separated groups (populations) of trees has been demonstrated often enough for pines, but practically nothing is known with respect to firs. Therefore, the present paper represents a study of this subject, in so far as terpenes from cortical blister oleoresin of *A. lasiocarpa* not growing in areas of intergradation are concerned.

The volatile fraction of *A. lasiocarpa* cortical blister oleoresin was investigated by Bickford *et al.* in 1934,[5] who identified β-pinene and β-phellandrene among its constituents and suspected the presence of limonene, bornyl acetate and "cadinene". In 1965, Zavarin and Snajberk[6] examined by GLC the terpene fraction of this material obtained from trees growing in British Columbia and reported its composition as 9 per cent α-pinene and traces of camphene; 28·5 per cent β-pinene; 6·5 per cent 3-carene; 1·0 per cent myrcene; 11·5 per cent limonene; 43·0 per cent β-phellandrene; and 0·5 per cent terpinolene. Variation between individual trees (based on three populations investigated) was not reported as high.

RESULTS AND DISCUSSION

In the present work, 416 trees from fifty locations were sampled covering the entire range of *Abies lasiocarpa* var. *lasiocarpa*, which is from near the Alaskan–Canadian border south to southern Oregon, Arizona and Colorado. Contrary to findings in our previous report, which was based on sampling in a rather limited area in British Columbia, this time an impressive variability in the monoterpene composition of the cortical blister oleoresin was found. β-Pinene, β-phellandrene, and limonene exhibited a particularly high degree of variability, with their percentages (total monoterpene basis) ranging from mere traces to 37·0, 66·5 and 91·5 per cent respectively (Table 1; Figs. 2–7). α-Pinene and 3-carene varied somewhat less. Myrcene was practically always present but in small amounts only, whereas camphene, α-thujene, sabinene, α-phellandrene and terpinolene represented trace constituents. Table 2 summarizes the results obtained with populations from near Lake Wenachee (Washington), near Missoula (Montana) and near Lander (Wyoming); each collection was made in an area of a few acres only. The variability between individual trees and between populations is very significant with the Lander population almost exclusively containing trees high in limonene, whereas the Wenachee material is high in β-pinene and β-phellandrene, and Missoula samples in between. This stresses again the importance of statistically sound approach in this type of work, even in cases where morphological evidence points to uniformity

[5] C. A. BICKFORD, S. C. CLARKE and E. C. JAHN, *Proc. Pacific Science Congress* V, 3941 (1934).
[6] E. ZAVARIN and K. SNAJBERK, *Phytochem.* 4, 141 (1965).

of a species. The percentage of monoterpenes in the oleoresin varied much less drastically than monoterpene composition (Fig. 2). The average value amounted to 29·3 per cent, and although the range was between 12 and 40 per cent, 96·9 per cent of the samples were between 22 and 38 per cent with a standard deviation of 3·9, and a distribution of values satisfactorily approaching the Gaussian curve.

Figures 3–7 show the frequency distribution diagrams for the individual monoterpenes. With α-pinene, 3-carene, and myrcene the distributions are markedly skewed to the higher values, while in the plots for β-pinene plus β-phellandrene (plotted as sum due to strong positive correlation, see later) and limonene a distinct polymodality is apparent. In the case of limonene, sharp maxima occur at 0 to 4 per cent and 22 to 32 per cent, and a broad maximum (possibly a doublet) between 58 and 86 per cent. The β-pinene plus β-phellandrene plot is more diffuse, with definite maxima at 2 to 6 per cent and 78 to 86 per cent and possible additional peak at 50 to 64 per cent. The general character of diagrams did not change when some populations, such as fifty samples collected near Lolo Hot Springs (Montana), were used singly in computations.

Deviations from the pure Gaussian curve by all six compounds, and particularly by the last three, could suggest that biosynthesis of monoterpenes in *A. lasiocarpa* cortex is controlled by few genes only. Similar behavior has been noted with several monoterpenes of *Pinus ponderosa*,[7] *P. contorta*, *P. coulteri* and *P. washoensis* xylem oleoresin[8] and *P. elliottii* cortical and xylem oleoresin.[9] Single gene control, or control by a few genes only, has been suggested by Forde[10] for the inheritance of α- and β-pinene in xylem oleoresin of *P. attenuata* × *P. radiata* hybrids, and by Hanover[11] for the 3-carene inheritance in the cortical oleoresin of *P. monticola* intraspecific hybrids.

Separation of *A. lasiocarpa* into several chemical strains according to the cortical terpene composition was further analyzed, assuming independent genetic control for the biosynthesis of limonene and β-pinene plus β-phellandrene. Four categories of trees were defined, based on the distribution diagrams: trees rich in limonene (L; >7 per cent); trees poor in limonene (l; <7 per cent); trees rich in β-pinene, β-phellandrene (P; >9 per cent); trees poor in β-pinene, β-phellandrene (p; <9 per cent). Assuming random hybridization within each popu-

TABLE 1. STATISTICS FOR THE VARIABILITY OF INDIVIDUAL MONOTERPENOIDS IN *A. lasiocarpa**

Monoterpene	Mean	Standard deviation	Modes	Range
α-Pinene	9·1	4·4	7·5	32·0–2·0
β-Pinene	15·9	9·1	1·0, 20·0, 27·0	36·0–0·0
3-Carene	10·3	6·65	8·0	43·4–0·0
Myrcene	0·8	1·2	0·25	5·5–0·0
Limonene	28·0	29·5	3·0, 29·0, 70·0	91·0–0·0
β-Phellandrene	35·1	21·3	2·0, 40·0, 54·0	66·5–0·0
Terpinolene	0·6	0·6	0·5	4·0–0·0
Total monoterpenes in oleoresin	29·4	3·9	29·5	39·8–12·2

* Concentration of individual compounds expressed on turpentine per cent basis; concentration of total monoterpenes expressed on oleoresin per cent basis.

[7] R. H. SMITH, U. S. Forest Service Res. Paper PSW-15 (1964).
[8] R. H. SMITH, *Forest Sci.* 13, 246 (1967).
[9] A. E. SQUILLACE and G. S. FISCHER, *Forest Sci.*, 13, 53 (1967).
[10] M. B. FORDE, *New Zealand J. Bot.* 2, 53 (1964).
[11] J. W. HANOVER, *Forest. Sci.* 12, 447 (1966).

TABLE 1—*continued*

LINEAR REGRESSION STATISTICS*

*Independent variable**	*Dependent variable*	Constant a (turpentine *basis*)	Slope b (turpentine *basis*)	Slope b (oleoresin *basis*)	Standard error of estimate (turpentine *basis*)
β-Pinene	α-Pinene	7·58	0·095	0·112	4·42
β-Pinene	3-Carene	13·2	−0·145	−0·087	6·54
β-Pinene	β-Phellandrene	−0·59	2·24	2·28	6·41
3-Carene	Terpinolene	0·16	0·036	0·038	0·60
Limonene	α-Pinene	10·4	−0·044	−0·045	4·18
Limonene	β-Pinene	24·1	−0·292	−0·288	2·87
Limonene*	3-Carene	9·19	0·063	0·052	6·69
Limonene	β-Phellandrene	54·3	−0·69	−0·68	6·55
Limonene	β-Phellandrene + β-Pinene	78·4	−0·98	—	8·41
β-Phellandrene	α-Pinene	7·50	0·076	0·074	4·96
β-Phellandrene	β-Pinene	1·68	0·41	0·40	2·73
β-Phellandrene	3-Carene	12·7	−0·033	−0·014	7·21
β-Phellandrene + β-Pinene*	α-Pinene	8·06	0·021	—	4·33
β-Phellandrene + β-Pinene*	3-Carene	13·8	−0·070	—	6·32
α-Pinene	Total turpentine content of oleoresin	31·0	−0·176		3·84
β-Pinene	Total turpentine content of oleoresin	28·4	0·0612		3·90
3-Carene	Total turpentine content of oleoresin	30·4	−0·0996		3·86
Limonene	Total turpentine content of oleoresin	29·8	−0·0145		3·88
β-Phellandrene	Total turpentine content of oleoresin	28·2	0·0349		3·84

* Generally variables with larger ranges were taken as independent variables.

CORRELATION COEFFICIENT MATRIX*

	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene	β-Phellandrene + β-Pinene	Terpinolene	Turpentine content of oleoresin
α-Pinene	0·18 (0·24)	−0·040	0·00	−0·30 (−0·33)	0·26 (0·28)	0·15	−0·040	−0·20
β-Pinene	—	−0·19 (−0·12)	−0·090	−0·95 (−0·89)	0·95 (0·96)	—	−0·10	0·14
3-Carene	—	—	0·16	0·23 (0·19)	−0·079 (−0·034)	−0·32	0·37 (0·39)	0·17
Myrcene	—	—	—	0·048	−0·18	—	0·078	−0·038
Limonene	—	—	—	—	−0·95 (−0·88)	−0·96	−0·020	−0·11
β-Phellandrene	—	—	—	—	—	—	−0·090	0·19
Terpinolene	—	—	—	—	—	—	—	−0·020

* With r = 0·098 and = 0·128 as correlation coefficients at the 5 % and 1 % levels of significance, respectively. Data in parentheses were computed on total oleoresin per cent basis.

Remark. On a few occasions non-linear relationships between variables were encountered towards curve ends (cf. Fig. 13). The correlation coefficients and regression constants were computed in these cases on the basis of *linear curve portions only.*

TABLE 2. COMPOSITION OF *Abies lasiocarpa* MONOTERPENES FROM VARIOUS POPULATIONS

Herb. No.	α-Pinene	Camphene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene	Terpinolene	Total terpenes
				Lake Wenachee population (No. 74, Washington)					
1331	9·9	0·0	23·9	2·3	0·0	1·1	62·5	0·0	32·3
1332	5·2	0·0	22·9	7·2	0·3	2·0	61·0	1·0	30·6
1333	11·4	0·0	22·9	6·3	0·6	1·9	55·1	1·4	25·3
1334	11·1	0·1	18·3	18·1	0·5	1·7	48·4	1·4	29·8
1335	7·8	0·8	24·6	9·5	0·3	1·3	54·7	0·7	31·0
1336	9·1	0·0	24·0	12·3	0·5	1·9	51·0	0·8	25·6
1337	5·0	1·1	16·7	5·2	0·3	20·9	49·4	0·9	23·5
1338	8·7	0·3	25·9	9·3	0·1	1·0	53·0	1·2	32·1
				Missoula population (No. 33, Montana)					
360†	4·5	0·0	4·5	24·5	2·0	55·0	8·0	1·5	32·0
361*	13·5	tr	10·5	30·0	1·5	18·5	24·0	1·5	29·8
362†	21·0	0·0	17·5	14·5	0·5	9·5	36·0	1·0	26·6
363	11·0	0·0	21·0	8·0	1·0	29·0	30·0	0·0	29·3
364	14·0	0·0	25·5	6·0	0·5	1·0	53·0	tr	27·4
365	10·5	0·0	28·5	4·5	0·5	1·0	55·0	0·0	32·2
366	8·0	tr	0·5	8·0	1·5	82·0	0·0	tr	30·0
420‡	6·5	0·0	14·5	15·5	0·5	24·5	37·0	1·0	27·3
318	16·5	tr	9·0	25·5	1·0	24·0	22·0	2·0	29·0
319	12·0	tr	20·0	16·0	1·5	1·9	48·0	1·5	37·7
				Lander population (No. 5, Wyoming)					
798	5·0	0·5	2·0	8·0	2·0	80·0	1·5	1·0	29·1
799	20·0	1·0	6·5	4·5	1·5	55·5	10·5	0·5	28·2
800	5·0	1·0	0·5	6·0	0·5	85·0	1·0	1·0	33·6
801	9·0	2·0	1·0	21·0	1·0	60·5	4·0	0·5	25·3
802	5·0	0·0	tr	14·0	1·0	77·5	1·5	1·0	33·5
803	7·0	tr	1·5	5·5	0·5	83·0	2·0	0·5	27·5
804	12·0	1·0	0·5	22·5	1·0	60·5	1·5	1·0	25·4
805	5·5	tr	0·5	7·5	0·5	84·0	1·5	0·5	26·6
806	6·0	tr	2·0	8·5	1·0	81·5	1·0	tr	24·0
807	12·0	tr	tr	10·0	0·5	75·5	2·0	0·0	22·4

* Contains 0·5% of sabinene.
† Contains traces of sabinene.
‡ Contains traces of α-phellandrene.

TABLE 3. TEST FOR INDEPENDENT INHERITANCE OF LIMONENE AND β-PINENE PLUS β-PHELLANDRENE

	Lolo Hot Springs		Other populations*		Total	
	Expected	Observed	Expected	Observed	Expected	Observed
LP	28	26	48	42	76	68
Lp	4	6	30	36	34	42
lP	16	18	16	22	32	40
lp	2	0	6	0	8	0

* Only populations having trees of all four categories were considered, i.e. populations 3, 4, 7, 18, 33, 43, 54, 55, 57, 59, 111 and 113. Lolo Hot Springs population (No. 54) is tabulated separately.

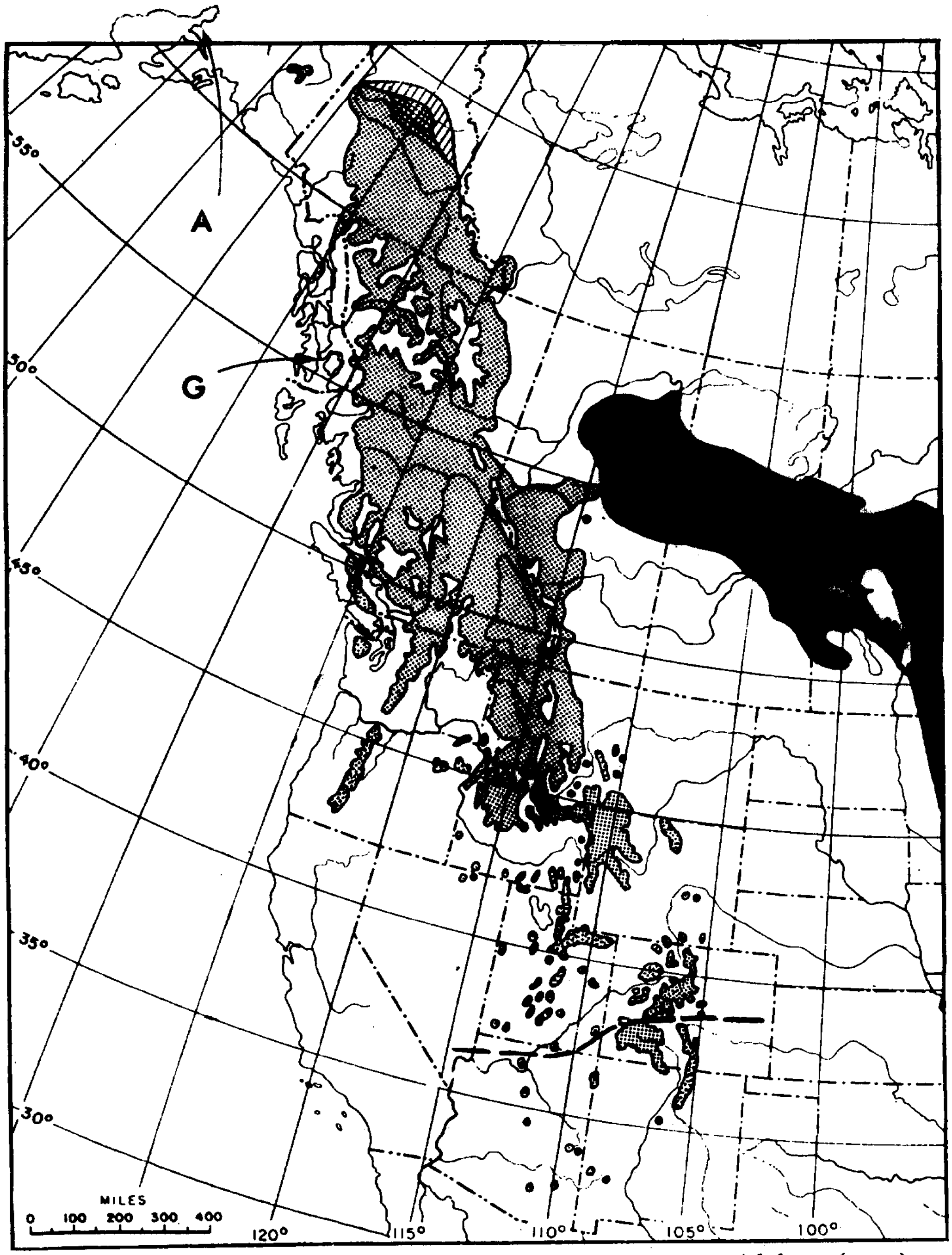

FIG. 1. RANGE OF *Abies lasiocarpa* (DOTTED) AND WESTERN PART OF THE RANGE OF *A. balsamea* (BLACK). Arrows point to stands of *A. lasiocarpa* near Anchorage (A) and on Prince George Island (G), Alaska. Dashed line designates approximate break of *A. lasiocarpa* into var. *lasiocarpa* and var. *arizonica.* Area in north (designated with squares) represents estimated extension of the *A. lasiocarpa* range on the basis of collections by Mr. Karel Snajberk; the striped area designates additional range for the same species, estimated on the basis of his conversations with inhabitants of the area. (Original map taken from *Silvics of Forest Trees of United States*, Agr. Handbook, No. 271, USDA.)

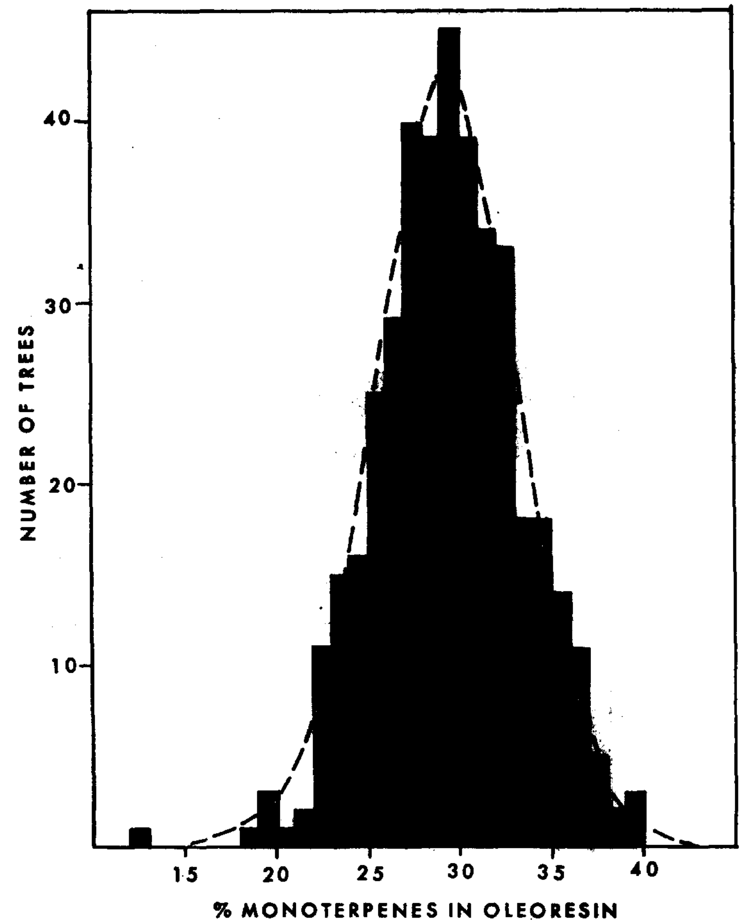

FIG. 2. PERCENTILE DISTRIBUTION OF THE MONOTERPENE CONTENT OF *Abies lasiocarpa* CORTICAL OLEORESIN. DASHED CURVE REPRESENTS GAUSSIAN DISTRIBUTION.

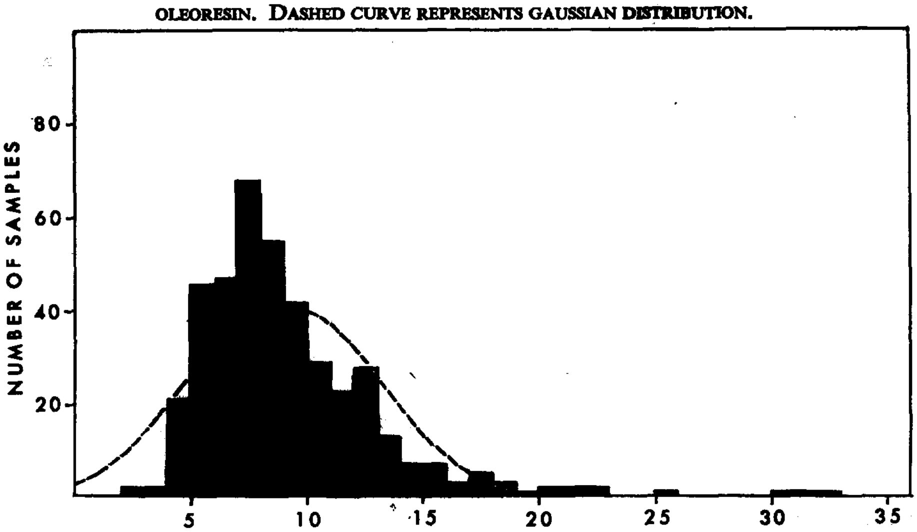

FIG. 3. PERCENTILE DISTRIBUTION OF α-PINENE. DASHED CURVE REPRESENTS CALCULATED GAUSSIAN DISTRIBUTION.

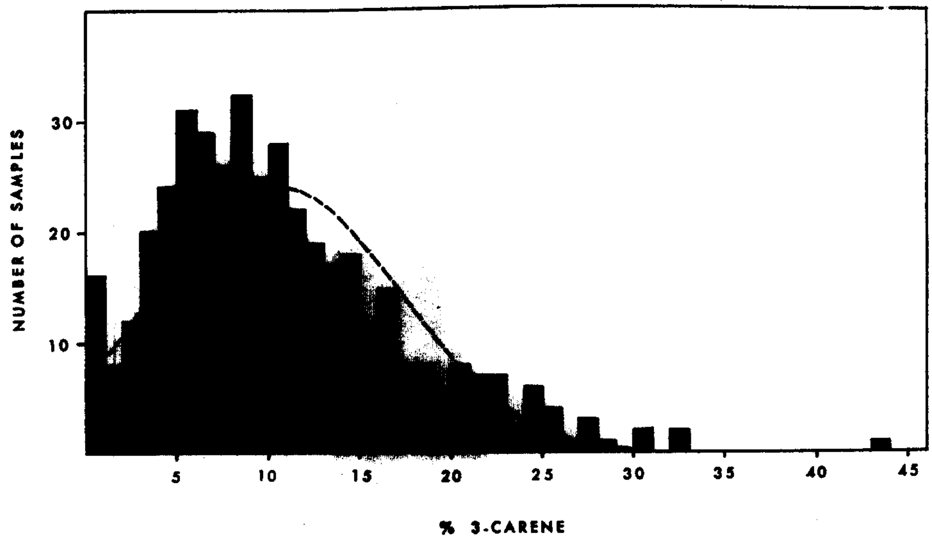

FIG. 4. PERCENTILE DISTRIBUTION OF 3-CARENE. DASHED CURVE REPRESENTS CALCULATED GAUSSIAN DISTRIBUTION.

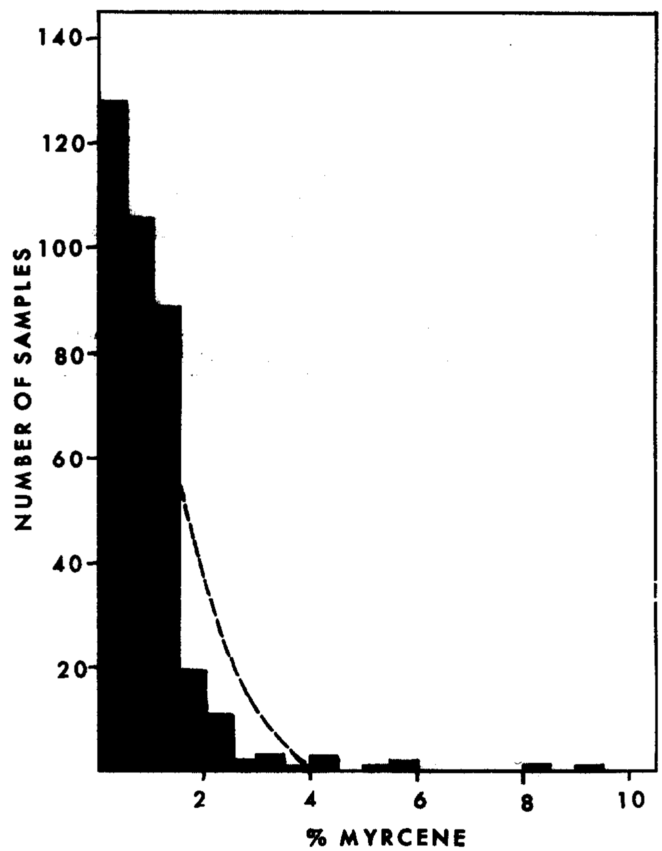

FIG. 5. PERCENTILE DISTRIBUTION OF MYRCENE. DASHED CURVE REPRESENTS CALCULATED GAUSSIAN DISTRIBUTION.

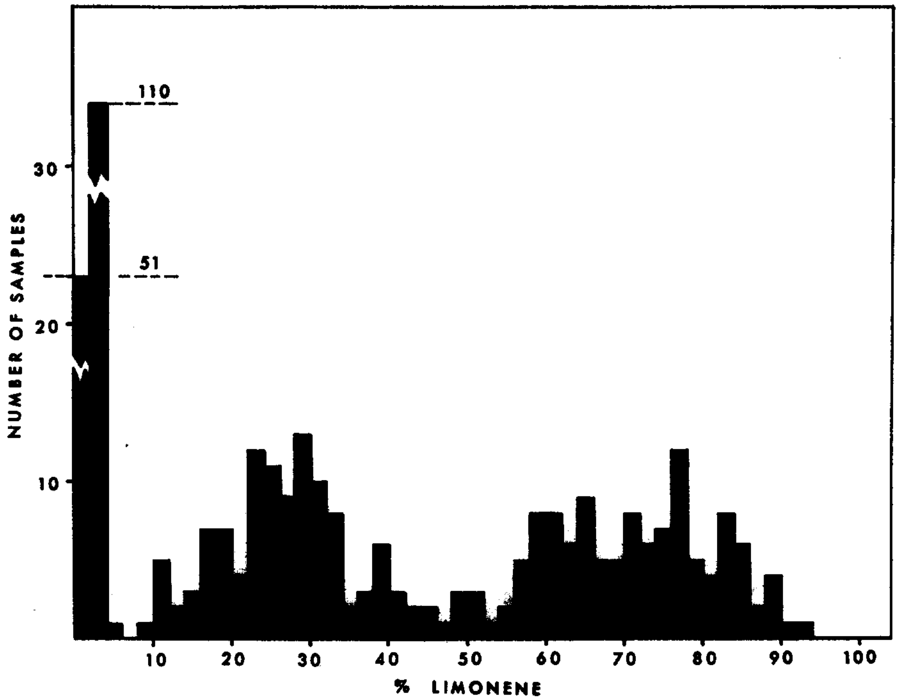

Fig. 6. Percentile distribution of limonene.

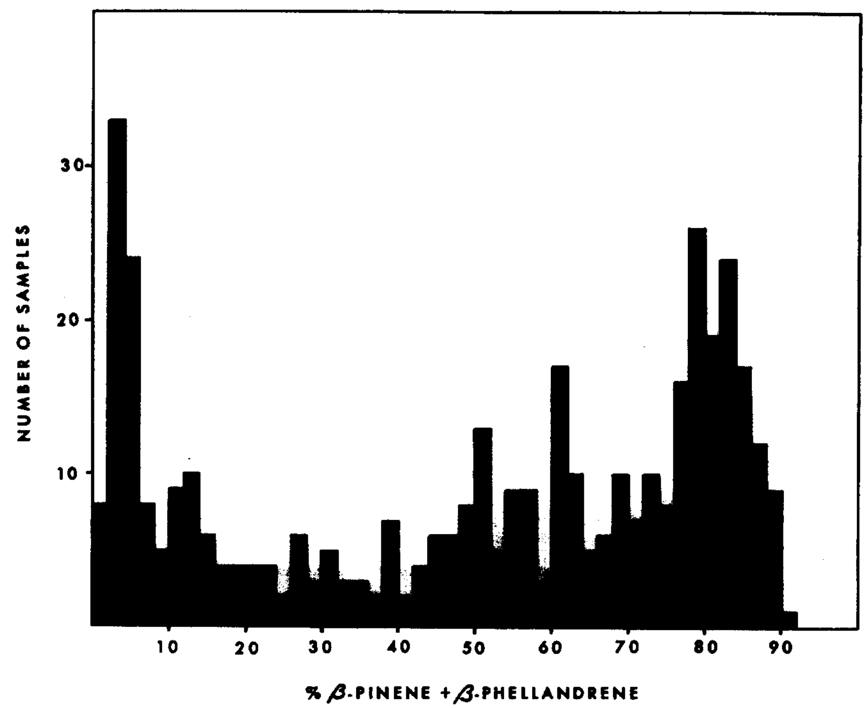

Fig. 7. Percentile distribution of β-pinene plus β-phellandrene.

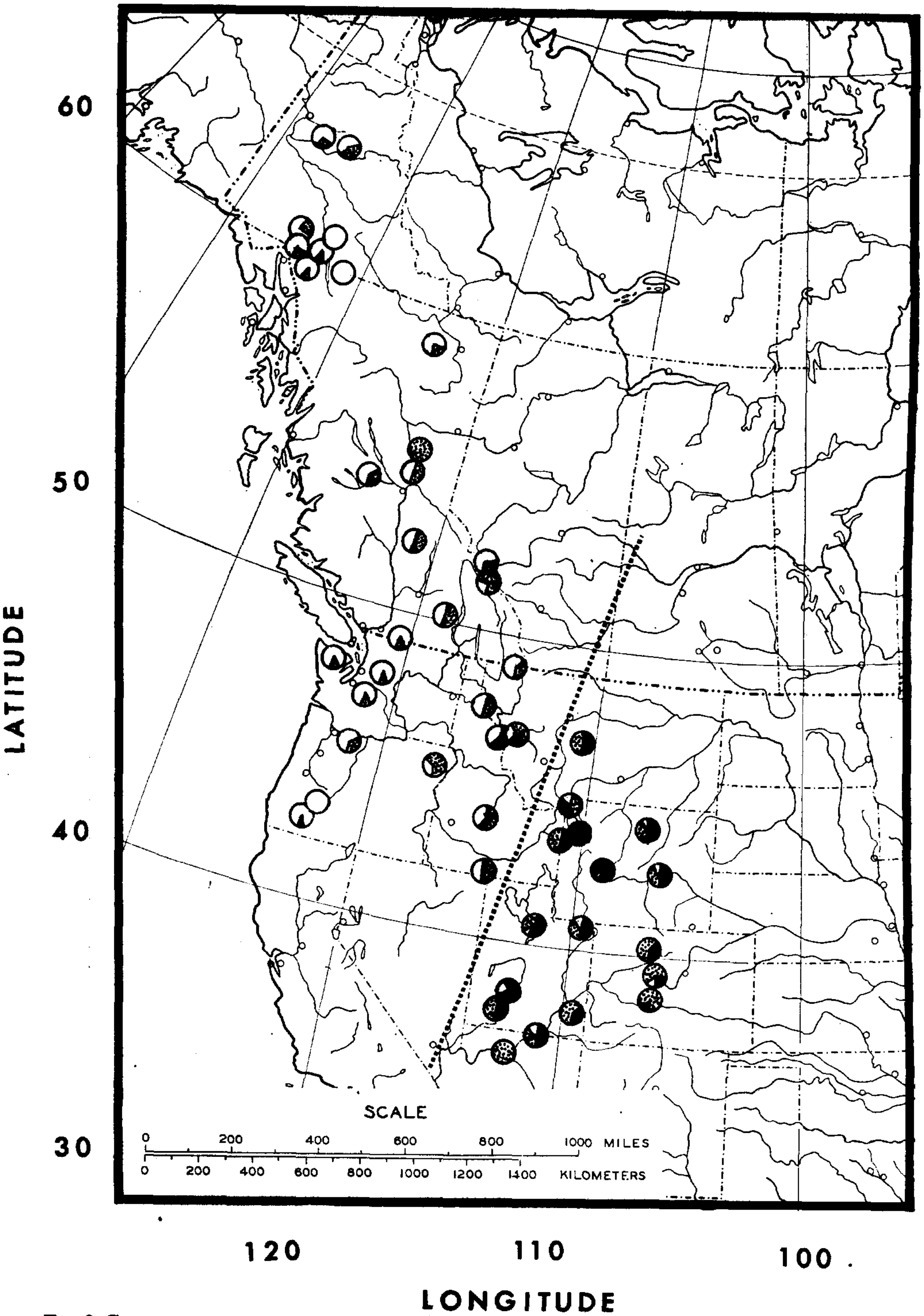

FIG. 8. GEOGRAPHIC DISTRIBUTION OF THE LP (DOTTED), LP (BLACK), AND lP (WHITE) TREE TYPES OF *Abies lasiocarpa*.

lation sampled, the number of trees rich in both limonene and β-pinene, β-phellandrene (LP), rich in limonene only (Lp), rich in β-pinene, β-phellandrene only (lP), and poor in either compound (lp) were calculated separately for each population from the number of trees belonging to each category and laws of probability. The results are shown in Table 3, where the Lolo Hot Springs population (fifty trees) has been tabulated separately while results for other populations were bulked. The results calculated agree fairly well with the experimentally obtained numbers, except for the absence of trees low in either of the three terpenes (lp). Several speculative explanations can be advanced for this. It is possible that *A. lasiocarpa* does not have a biosynthetic system allowing it to supplement the decrease in limonene, β-pinene, and β-phellandrene by an increased production of other terpenes—namely α-pinene, and 3-carene. This is supported in part by the considerations given later (see Fig. 10). As a consequence an exceptionally viscous oleoresin would be produced, affecting the health of the tree and leading to its possibly ultimate elimination from the stand. Another possibility is that a concurrent decrease would take place in the production of the nonvolatile part of the oleoresin, resulting in material of about the same viscosity but of less volume; in this case, bark blistering could be expected to be less and the tree would be overlooked in sampling. Finally, it must be stressed that the above test points only to the fact that limonene and β-pinene plus β-phellandrene are likely to be independently inherited; however, the presence of several peaks in the distribution diagrams (Figs. 6 and 7) suggests that the genetic situation is more complicated than that of two single genes acting independently—and that a model involving several genes acting in one locus seems more realistic.

Figure 8 shows the geographic distribution of the LP, lP and Lp individuals. The major feature of the variation pattern is a discontinuity in Montana and Idaho, which extends through western Montana and central Idaho, separating the Kings Hill, Montana samples and the Wyoming, Colorado, and Utah samples from the others. The north-western strain consists almost entirely of lP and LP trees. The frequency of LP trees increases gradually from west to east; in the west from the Yukon south to Oregon, LP trees are in the minority or absent, but in the eastern part of this region they make up about half of the population. The third type (Lp) is rare throughout the range of this strain, and absent in the western part. In the south-eastern strain, lP trees are uncommon or absent (compared to their high frequency in the north-western strain), Lp trees are common and in few samples the only type present (compared to their rarity in the north-western strain), and LP trees are common in most samples. So far, no morphological differences have been reported within populations of *A. lasiocarpa* investigated in this work. Still, as no quantitative studies have been performed on this species, it is possible that more detailed botanical work could uncover non-chemical distinguishing characteristics paralleling this chemical differentiation.

Experimental data were further analyzed by calculation of correlation and regression coefficients and constants for all possible pairs of variables, including α-pinene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene, terpinolene, and total terpene content of the oleoresin. The values were expressed in per cent on a total terpene and a total oleoresin basis. The data are reported numerically in Table 2 and in form of X/Y plots (Fig. 9 to 13).

One of the difficulties in working with data normalized to 100 per cent involves a possible introduction of a spurious part in the natural correlations existing between amounts of individual terpenoids, which can affect the values of correlation coefficients (r), constants (a), slopes (b), and standard deviations of the regression lines. In two component systems this spurious part is at a maximum, as any variability existing must result in this case in $r = -1{\cdot}0$, $a = 100$, and $b = -1{\cdot}0$ with standard deviations of zero. Thus, it is impossible to tell in this case whether an increase in the first compound, A, is being naturally made up by a corresponding decrease in the second compound B, or whether the rate of production of B

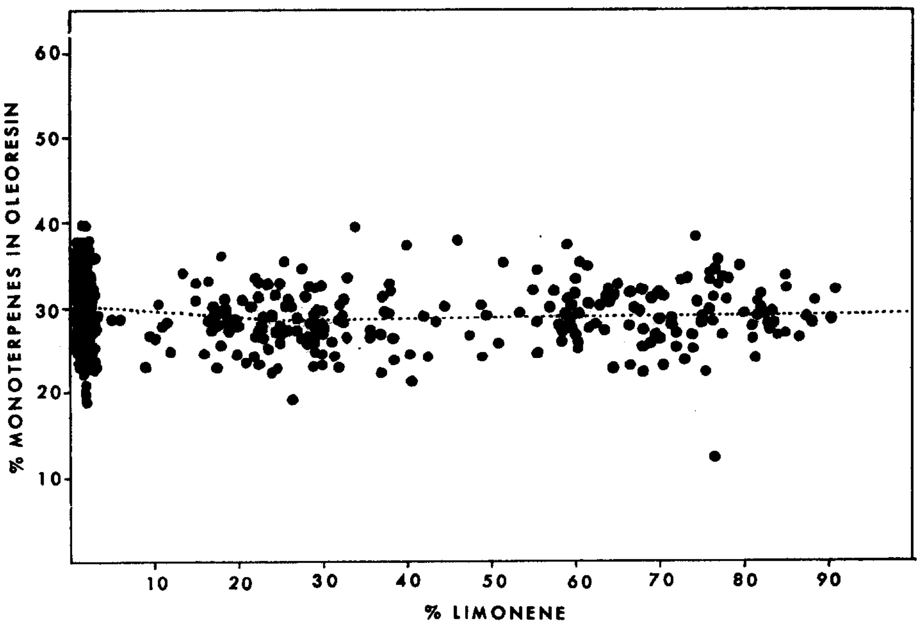

FIG. 9. PLOT OF PER CENT LIMONENE VS. PER CENT MONOTERPENES IN OLEORESIN.

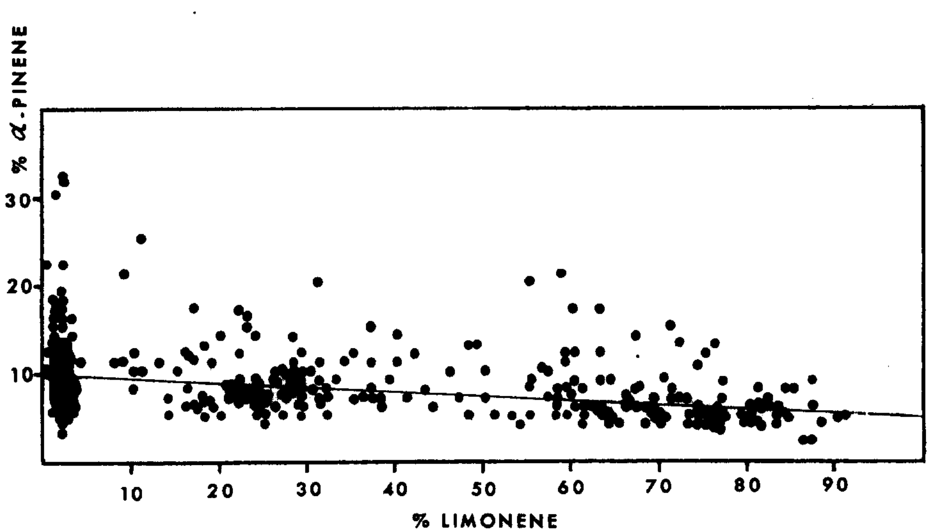

FIG. 10. PLOT OF PER CENT LIMONENE VS. PER CENT OF α-PINENE.

is unaffected by the increase in A—i.e. whether A is increasing independently of B. In multicomponent systems, with decrease in relative amounts of A and B the spurious part decreases also in that proportion as any change in A is made up to 100 per cent by all other components of the system thus affecting B to a proportionally lesser degree. This is why it seems

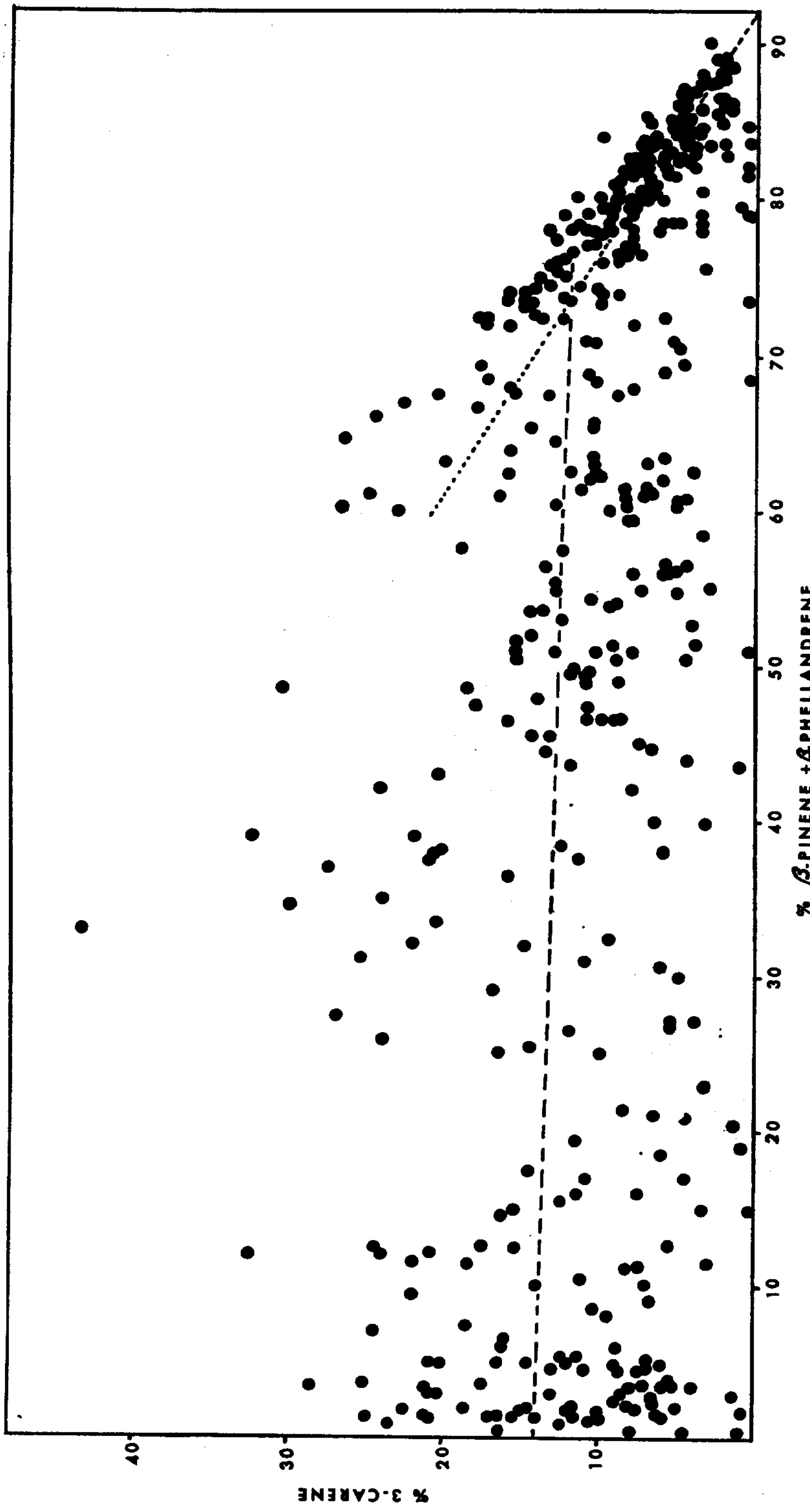

FIG. 11. PLOT OF THE SUM OF β-PINENE AND β-PHELLANDRENE VS. PER CENT OF 3-CARENE.

advantageous to express the terpene data not on total monoterpene but on total oleoresin per cent basis—more components are included in the system. Other workers have followed this practice.[11-13]

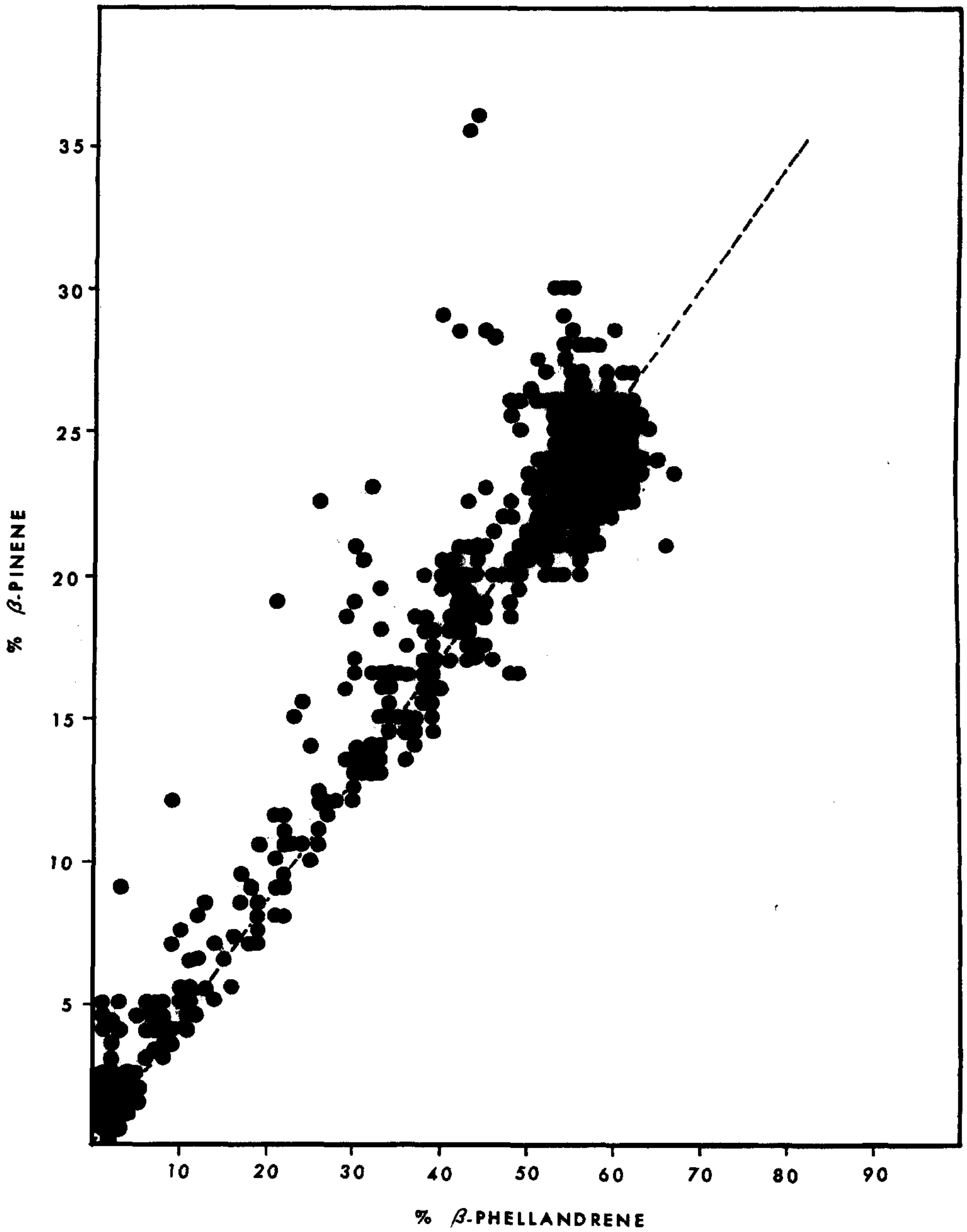

FIG. 12. PLOT OF PER CENT OF β-PINENE VS. PER CENT OF β-PHELLANDRENE.

[12] J. W. HANOVER, *Heredity* 21, 73 (1966).

[13] J. W. HANOVER and M. M. FURNISS, Joint Proc. 2nd Genetics Workshop of the Soc. of Amer. Foresters and the 7th Lake States Forest Tree Improv. Conf. (1965). U.S. Forest Serv. Res. Paper NC-6, p. 3 (1966).

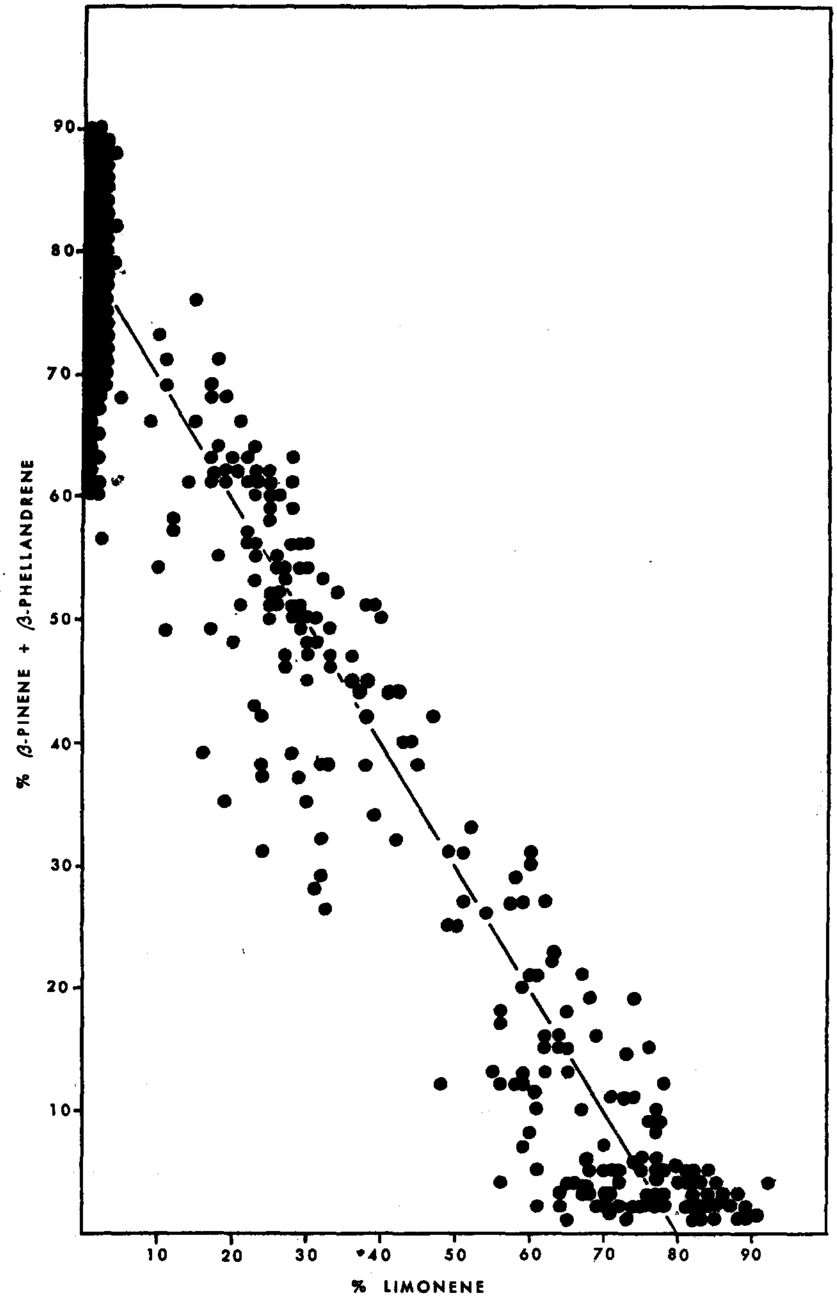

FIG. 13. PLOT OF PER CENT LIMONENE VS. PER CENT OF THE SUM OF β-PINENE AND β-PHELLANDRENE.

We checked the two methods by comparing the statistics obtained using data computed on either per cent basis. The results (Table 1) indicated that little difference existed in the values of correlation coefficients or the regression slopes, particularly in cases of better correlated terpenes. Apparently, spuriousness plays only a minor role in the correlations obtained, i.e., quantitative changes in one compound are made up to a large extent naturally by opposite changes in one or several other compounds included in the terpene set analyzed. This is reasonable from the biosynthetic point of view, as the terpene hydrocarbons represent a group of materials closely related through the same primary intermediate (geranyl-pyrophosphate) so that a change in the amount of one monoterpene is biogenetically intimately bound with changes of other monoterpenes.

Although the turpentine content of the oleoresin correlated occasionally with major monoterpenes, the values of the correlation coefficients were very small, accounting for less than 4 per cent of the total variability present—about 2 per cent on the average. It is possible

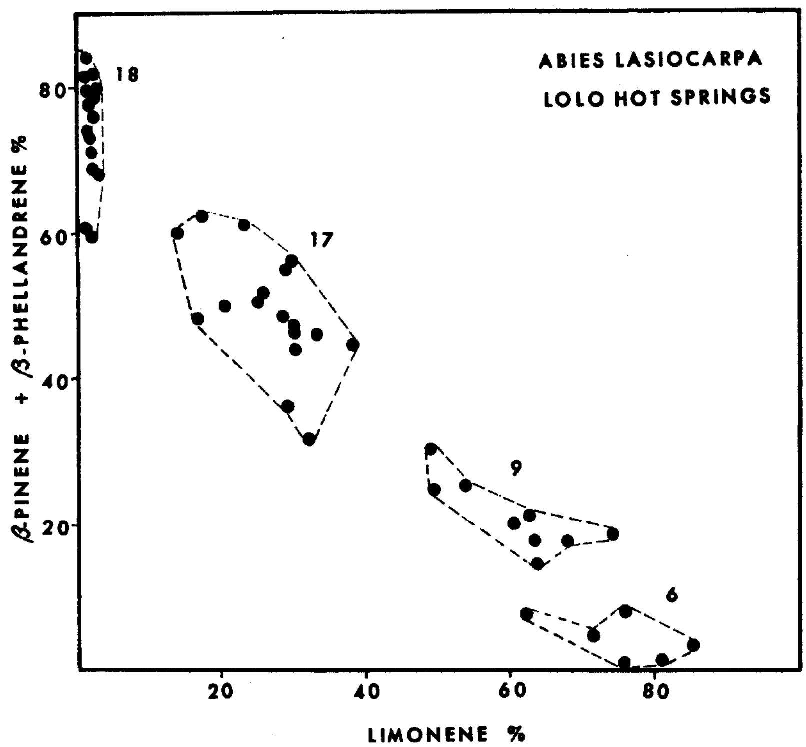

FIG. 14. PLOT OF PER CENT LIMONENE VS. PER CENT OF β-PINENE AND β-PHELLANDRENE FOR LOLO HOT SPRINGS (MONTANA) POPULATION ONLY.

that these correlations were introduced by some systematic error inherent in the experimental procedures used, as analysis and sample collection lasted over 5 years and involved a number of GLC instruments and operators as well as extensive sub-zero storage periods. The slopes of the regression lines, with individual terpenes as independent and oleoresin turpentine content as dependent variables, were also very small compared to regression constants (Table 1 and Fig. 9). Thus, a practical independence of the variations in the turpentine content of the oleoresin from fluctuations in limonene and other terpenoids can be assumed. Within monoterpenes, several strong quantitative correlations were encountered (Figs. 10 to 13).

An exceptionally high positive correlation was found to exist between β-pinene and β-phellandrene (Fig. 12), with r = +0·95, b = +0·41, and a = 1·68. Both of these compounds correlated negatively with limonene, with r = –0·95 for β-pinene, r = –0·95 and β-phellandrene, and r = –0·96 for the sum of both (Fig. 13). The amounts of all three compounds were furthermore connected through the equation:

$$\text{per cent } \beta\text{-Pinene} + \text{per cent } \beta\text{-Phellandrene} + \text{per cent Limonene} = 78 \text{ per cent}$$

In other words, any decrease (or increase) in limonene is compensated for by a concurrent identical increase (or decrease) in β-pinene plus β-phellandrene.

The substitution of limonene by β-pinene plus β-phellandrene mixture was generally accompanied by an increase in α-pinene and a decrease in 3-carene. The effect was a minor one, however, as long as both limonene and β-pinene, β-phellandrene were present in substantial amounts. However, if limonene or β-pinene, β-phellandrene were absent, or present in small amounts only, the regressions changed. With 3-carene the slopes increased to a value of roughly b = –0·7 in either case (from b = –0·070 for β-pinene, β-phellandrene as independent variable and from b = +0·063 for limonene as independent variable respectively) indicating that the contribution of 3-carene toward production of limonene or β-pinene, β-phellandrene mixture, becomes more important under those extreme conditions (Fig. 11). In the case of α-pinene and with β-pinene plus β-phellandrene as independent variable similar behavior was noted at the low limonene values, with the slope changing from about b = +0·021 to b = –0·3. However, at the high limonene values, and with limonene as independent variable the slope remained about the same throughout b = –0·044—i.e. the unavailability of β-pinene, β-phellandrene mixture did not result in diversion of increased amounts of material away from α-pinene and towards limonene (Fig. 10). This could suggest a larger than usual gap between biosynthesis of limonene and α-pinene.

The myrcene content ranged between 0·0 and 2·5 per cent in 97·5 per cent of the samples, and in the majority of cases it either did not or correlated only weakly with amounts of other terpenes present. The above seems reasonable, as myrcene is biosynthetically more distantly related to these terpenes. Terpinolene was found occasionally and in low amounts only—surprisingly, and in spite of the greater experimental error involved in its determination, its percentage correlated positively with the percentage of 3-carene. The cases of positive correlation between 3-carene and terpinolene have been noted before in case of *P. ponderosa* oleoresin.[14] Strong positive correlations between 3-carene and an "unknown", which according to retention data is probably identical with terpinolene, have been noted also by Hanover in *P. monticola* cortical oleoresin[12] and *Pseudotsuga menziesii* xylem oleoresin.[13]

EXPERIMENTAL

Collection Procedure

Oleoresin samples were collected in fifty localities spread as randomly as possible throughout the whole *Abies lasiocarpa* range, but not in areas of possible intergradation with *A. balsamea* or *A. lasiocarpa* var. *arizonica*. Each population was collected in an area covering several acres, although at times this was increased to a square mile or so chiefly in British Columbia and Yukon. To reduce the possibility of close parentage, trees standing within 100 ft or so of one another were not sampled. In most cases eight to ten trees were sampled in one locality. Table 4 gives the pertinent geographic and other collection data. Concurrently with oleoresin collection a small branch was secured from each tree sampled and preserved dry between newspapers for later reference. Collection and preservation procedure for the oleoresin has been described in detail earlier.[6]

TABLE 4. COLLECTION DATA FOR THE *A. lasiocarpa* SAMPLES

Population	Location (empirical designation)*	Latitude	Longitude	Elevation (ft)	Number of trees in each category†			Collector
Yukon		COASTAL BELT			lP	LP	Lp	
30	Quite Lake	61°07·5′	133°00′	4,600	8	0	0	Snajberk
31a	White Horse }	60 42	134 50	6,000 }	6	2	0	,,
31b	White Horse }	60 39	135 15	4,500 }				
32	Keno Mine	63 55	135 10	6,700	5	3	0	,,
34	Carcross	60 04	134 40	4,000	6	2	0	,,
36	Canol Rd.	60 35	133 05	4,000	7	1	0	,,
40	Clear Creek	63 47	137 22	2,500	6	2	0	,,
British Columbia								
16	Monashee Pass	50 02	118 30	3,900	4	4	0	Snajberk
17	Steamboat Mt.	58 40	123 42·5	2,600	3	1	0	,,
18	Horsefly Lake	52 22	121 19·5	2,600	4	3	1	,,
19	Pine Pass	55 24	122 37·5	2,700	0	4	0	,,
29	Francois Lake	54 00	125 00	2,600	4	3	0	,,
35	Manning Pass	49 03	120 46·5	4,200	7	1	0	,,
37	Atlin Lake	59 44	133 30	3,500	7	1	0	,,
38	Partridge Creek	59 59	131 13·5	2,900	7	0	0	,,
39	Prince George	54 29	122 41	2,370	3	3	0	,,
Washington								
44	Dog Lake	46 55	121 30	4,000	5	1	0	Duffield
74	Wenachee Lake	47 48	121 00	3,700	7	1	0	Zavarin
75	Olympic Hot Spr. }	47 57	123 42·5	3,800	8	1	0	,,
76	Hurricane Ridge }	47 58	123 31	5,000	3	1	0	,,
Oregon								
24	Union Creek	43 03	122 19·5	4,000	7	1	0	Zavarin
28	Crater Lake	42 26 to 42 52	122 15 to 122 43	5,700 to 7,400	8	0	0	Parker
60	Wallowa Mt.	45 12	117 18	6,100	3	7	0	Snajberk
65	Mt. Hood }	45 19	121 42·5	5,000	6	2	0	Critchfield
42	Mt. Hood }	45 20	121 42	5,100 to 6,000	2	2	0	Parker
Alberta		INTERMEDIATE BELT						
26	Lake Louise	51 31	116 50	5,600	1	3	0	Roller
41	Mt. Stewart	52 10	117 04	6,100	7	3	0	Snajberk
Idaho								
1	South Hills	42 11·5	144 17	6,800	3	3	0	Critchfield
59	Galena Summit	42 52	114 38·5	7,200	4	5	1	Zavarin
Utah								
2	Panguitch Lake	37 39·5	112 42·5	9,450	0	3	3	Critchfield
7	Navajo Mt.	37 02	110 49	10,100	1	2	3	,,
8	Abajo Blue Mt.	37 51	109 30	9,300	0	4	2	,,
43	Vernal	40 44·5	109 29·5	8,500	2	3	5	Zavarin
55	Salt Lake City	40 42·5	111 36·5	7,900	1	4	5	,,
113	Big Flat	38 16·2	112 22·1	10,200	2	1	5	Critchfield
Arizona								
6	Grand Canyon	36 18·5	112 06	8,800	0	2	0	Critchfield

TABLE 4—*continued*

Population	Location (empirical designation)	Latitude	Longitude	Elevation (ft)	Number of trees in each category†			Collector
Montana		U.S. ROCKY MOUNTAIN BELT			lP	LP	Lp	
33	Missoula	46 49·5	113 56·5	3,900	3	6	1	Zavarin and Weaver
54	Lolo Hot Spr.	46 38·5	114 33	4,400	18	26	6	Zavarin
58	King's Hill	46 50	110 41·5	7,393	0	5	5	,,
61	Kootenai Nat. For.	48 58	114 27	6,200	6	4	0	Snajberk
90	Lookout Pass	47 27·3	115 41·7	4,727	4	4	0	Critchfield
Wyoming								
3	Casper Mt.	42 44	106 20·5	7,800	1	4	5	Zavarin
4	Bighorn Nat. For.	44 09	107 00	8,600	1	4	5	,,
5	Lander	42 38·5	108 53	9,600	0	1	9	,,
15	Togwotee Pass	43 45·5	100 04	9,650	0	0	5	Weaver
23	Teton Pass	43 30	110 57	8,500	0	3	3	,,
57	Yellowstone Park	44 47	110 45	7,600	2	5	3	Zavarin
Colorado								
9	Monarch Pass	38 30	106 19·5	11,300	0	5	1	Critchfield
56	Rabbit's Ear Pass	40 23·5	106 35	8,800	0	6	4	Zavarin
111	Fremont Pass	39 24·7	106 11·5	10,800	1	5	2	Critchfield

* Bracketed populations considered together in data given in Fig. 9.
† P, p, Trees having more (P) or less (p) than 9% β-pinene and phellandrene. L, l, Trees having more or less than 7% limonene.

Analysis

Each sample was analyzed for monoterpenes present, as well as for total monoterpene content of the oleoresin, using an Aerograph Hy-Fi, model 6000 C, GLC instrument and a procedure described previously;[15] as before, an approximately 15% solution of isopropylbenzene in white oil was used as the internal standard. For integration of the peak area, an Aerograph model 470 digital integrator in combination with a Sargent Recorder, model SR, was used; the recorder was provided with an event marker which, when connected with the appropriate outlet of the integrator, marked under each peak the beginning and end of the integration. The curves and the integration values obtained were inspected and corrected for tailing if deemed necessary, and the per cent turpentine composition (both turpentine and oleoresin basis), turpentine content of the oleoresin, together with corresponding mean values and mean deviations for each population, were calculated using an IBM Model 1620 Computer. Statistical analysis was performed with an IBM Model 70/94 and CDC Model 6400 Computer partly in conjunction with the National Bureau of Standards Omnitab Program.[16]

Acknowledgements—We wish to thank Dr. William B. Critchfield of the Pacific Southwest Forest and Range Experiment Station for his help in evaluating evolutionary and genetic aspects of this study as well as for collection of some oleoresin samples, and Mr. Andre Kuzubov of the Lawrence Radiation Lab. and Mr. Duane Anderson of the University of California Computer Center for their help in setting up the computer programs. We also owe thanks to Professor John W. Duffield of the School of Forestry, North Carolina State College, to Mr. Eugene Parker, Medford, Oregon, to Mr. Tad Weaver, formerly of Montana State University, to Dr. K. J. Roller, Dept. of Forestry, Winnipeg, Manitoba, for collection of additional oleoresin samples, and to Mrs. Joanne Chin, Mrs. Margaret Henley and Miss Kathleen Costello for their participation in the oleoresin analyses. The work was financed in part by the National Science Foundation, Division of Biological and Medical Sciences Grant No. GB-3954, which is thankfully acknowledged.

[15] E. ZAVARIN, W. HATHWAY, T. REICHERT and Y. LINHART, *Phytochem.* **6**, 1019 (1967).
[16] *Omnitab a Computer Program for Statistical and Numerical Analysis.* NBS Handbook 101. U.S. Government Printing Office, Washington, D.C. (1966).