

Inheritance of Monoterpene Composition in Cortical Oleoresin of Loblolly Pine

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Abstract

Monoterpenes in samples of cortical oleoresin from 62 parent trees and 516 progenies were analyzed by gas-liquid chromatography. Major constituents were α -pinene, β -pinene, myrcene, limonene, and β -phellandrene. Among trees, there was considerable variation in the relative content of these constituents, and much of it was due to genetic factors. Variation in content of all of the major constituents except α -pinene is believed to be largely controlled by two alleles at a single locus, with high being dominant over low in all cases. The degree of dominance was estimated to be almost complete in β -pinene and limonene, low in myrcene, and essentially absent in β -phellandrene. Myrcene and limonene loci seemed to be closely linked. Knowledge of the inheritance patterns will enhance the use of monoterpenes as gene markers in studies of population structure and breeding mechanisms and in identifying relatives and seed origin.

Key words: *Pinus taeda*, terpenes, turpentine, essential oils, genetic variation.

Zusammenfassung

Vererbung der Monoterpenzusammensetzung im Harz der Rinde von *Pinus taeda*.

Monoterpene im Harz der Rinde von 62 Elternbäumen und 516 Nachkommenschaften wurden gaschromatographisch untersucht. Hauptbestandteile waren α -Pinen, β -Pinen, Myrcen, Limonen und β -Phellandren. Zwischen Bäumen ergab sich eine beträchtliche Variation im Gehalt dieser Bestandteile, ein großer Teil der Variation war auf genetische Faktoren zurückzuführen. Es wird angenommen, daß die Variation im Gehalt der Hauptbestandteile außer α -Pinen hauptsächlich von zwei Allelen eines Locus kontrolliert werden, in allen Fällen dominiert hoher Gehalt über niedrigen Gehalt. Der Dominanzgrad wurde als fast vollständig bei β -Pinen und Limonen, gering bei Myrcen und weitgehend fehlend bei β -Phellandren geschätzt. Myrcen und Limonen Loci scheinen eng gekoppelt zu sein. Die Kenntnis der Vererbungsmuster wird die Verwendungsmöglichkeit der Monoterpenen als Genmarker in Untersuchungen über Populationsstrukturen und Züchtungsverfahren sowie bei der Identifizierung von Verwandten und Saatgutherkünften erweitern.

Introduction

Monoterpenes are useful as genetic markers in forest genetics experiments, as criteria for taxonomic studies, and as a means of identifying geographic origin of seed (SQUILLACE, 1976). Such uses, however, are greatly facilitated if the mode of inheritance of the major constituents is known. Here we investigate the mode of inheritance of

four major monoterpenes found in the cortical oleoresin of loblolly pine (*Pinus taeda* L.).

ROCKWOOD (1973) previously reported on the inheritance of two monoterpenes in loblolly pine, based only on progeny data. In the present study we sampled the parents of those progeny and also a number of other parents and progeny. Both Rockwood's data and the newly obtained data are used here to examine inheritance patterns.

Materials and methods

Samples of cortical oleoresin were obtained from loblolly pines in three separate experiments:

- 1) Selfing experiment, established by the Southern Forest Experiment Station, Harrison Experimental Forest, Harrison, Mississippi, 1970–71. Sampled in December 1977.
- 2) Selfing experiment, established by the Southeastern Forest Experiment Station and North Carolina State University, Olustee, Florida, November 1967. Sampled in February 1976.
- 3) Heritability study, established by the International Paper Company and North Carolina State University on the Southlands Experiment Forest, Bainbridge, Georgia, 1960–61. Mostly sampled in September 1969, with supplementary samples taken in December 1978.

Further details of the trees sampled are shown in Table 1. Oleoresin samples were obtained by excising lower crown-branch terminals at about 1 cm from the tips. Exuded oleoresin was placed in screw-cap vials and stored in a refrigerator until analyzed. Monoterpene composition was determined by gas-liquid chromatography with a carbowax-20 M column. Relative amount of each monoterpene was expressed as a percent of total monoterpenes.

Several individuals in selfed families were believed to be contaminants. The occurrence of contaminants in self-pollinations is not surprising, as recently pointed out by RUDIN and LINDGREN (1977). Suspected contaminants were omitted only when there was evidence based on at least two characters such as monoterpenes, isozymes, tree size, and tree appearance. Exclusions were: nine individuals in family A3PT-31 X self which was believed to be a mix of selfs and outcrosses, and one individual from each of the selfed families of parents A6PT-31, A3PT-40, A6PT-35, F182, and F255. Family 26 X 26A is believed to have misidentified parents or to contain contaminants, but no reliable bases were available for checking it and we retained the data.

Results and Discussion

Variation and classification

Monoterpenes that appeared in relatively large amounts in at least some trees were α -pinene, β -pinene, myrcene, limonene, and β -phellandrene. Minor constituents were camphene and α -phellandrene. Variation among the major constituents was great, especially between families but also often within families. Four of the constituents (β -pinene, myrcene, limonene, and β -phellandrene) showed evidence of bimodality, which suggested Mendelian inheritance involving few genes. The fifth major constituent, α -pinene, did not show bimodality. These findings agree with

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those found for slash pine (*P. elliottii* ENGELM.) (GANSEL and SQUILLACE, 1976).

To investigate qualitative inheritance, we first developed classification schemes for each constituent. That is, we determined criteria for assigning each tree as having relatively high or low amounts of each constituent. In doing so, we considered the fact that the phenotypic expression of a gene can be affected by the presence or absence of other genes (SQUILLACE, 1976). Since all constituents in a sample must add to 100 percent, there tend to be negative correlations between constituents. Also, biological correlations may be present. Hence, in addition, to examining frequency distributions for each constituent, we prepared scatter diagrams between pairs of individual constituents (or groups of them) to look for aids in classification, as suggested by SQUILLACE (1976).

β-pinene. The frequency distribution for *β*-pinene (Figure 1) indicates that trees with about 0 to 2 percent of this constituent could be considered low *β*-pinene trees while those with 4 percent and above as high. However, a scatter diagram of *β*-pinene over the sum of limonene plus *β*-phellandrene showed a tendency for the low *β*-pinene trees to be slightly higher at low values of limonene + *β*-phellandrene than at higher values of the latter (Figure 2). Hence, the line with a slight negative slope arbitrarily drawn in the figure was used for separation. Trees above the line were considered "high" and those below the line "low."

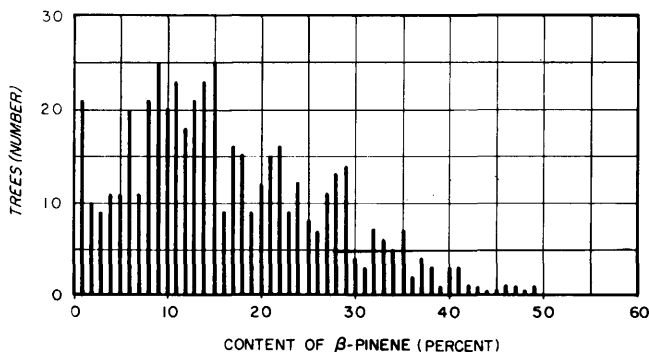


Figure 1. — Frequency distribution for *β*-pinene (578 trees).

Myrcene. The frequency distribution for myrcene showed rather clear bimodality, with the separation between low and high being at about 7 percent (Figure 3). However, within the group of low myrcene trees, myrcene tended to be positively correlated with limonene, as indicated in the scatter diagram of Figure 4. That is, myrcene tended to be lower in trees having low limonene than in trees having high limonene. This relationship was also found in slash pine (GANSEL and SQUILLACE, 1976). Accordingly, the line with a slight positive slope was arbitrarily drawn (Figure 4). Trees to the left of the line were considered to have low myrcene, while those to the right were considered to have high myrcene.

Limonene. The frequency distribution for limonene showed rather clear bimodality, with the break between low and high occurring at about 7 percent (Figure 5). Relationships of this constituent with other constituents showed no clear trends that would be beneficial for

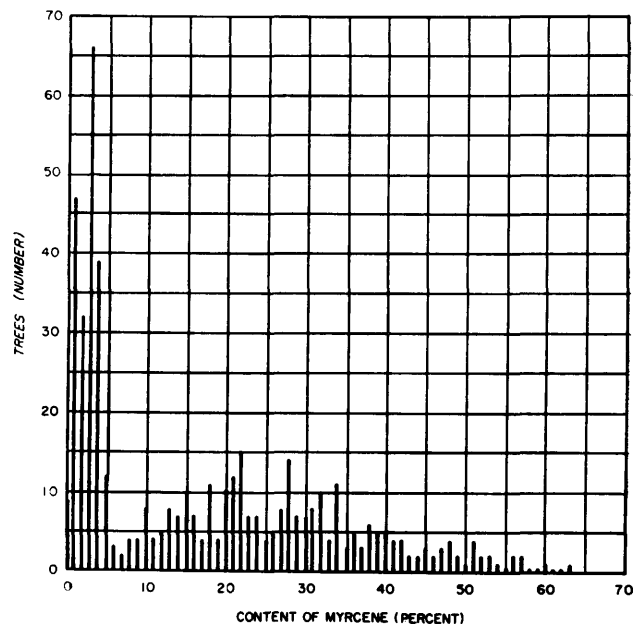


Figure 3. — Frequency distribution for myrcene (578 trees).

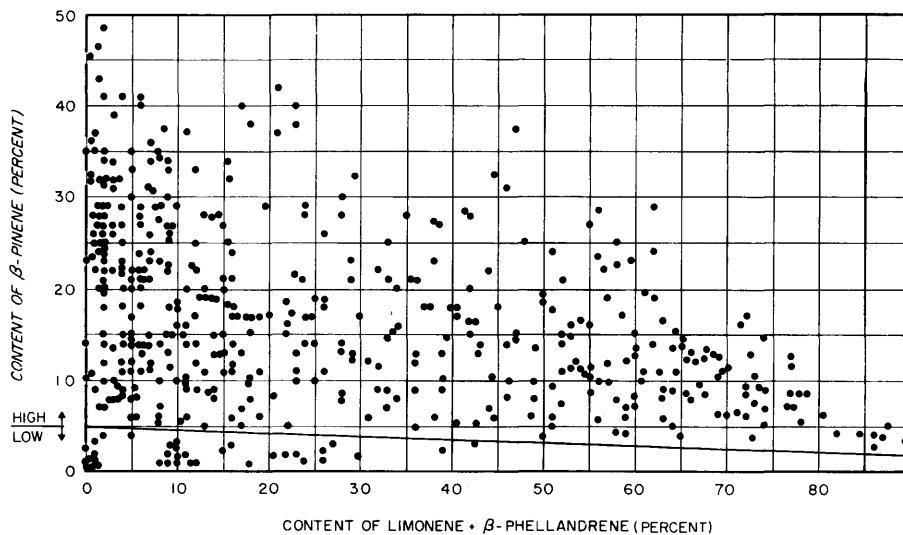


Figure 2. — Scatter diagram of *β*-pinene over limonene + *β*-phellandrene.

classification. Hence, trees with 0 to 6 percent limonene were considered low, while those with 8 or more percent were considered high.

β-phellandrene. The frequency distribution for *β-phellandrene* was the least clear of the major constituents in respect to modality (Figure 6). However, it suggested a low mode at 0 to 2 percent. The remaining trees seemed to be divisible into two modes, one centering at about 6 percent and the other at about 30 percent. The two relatively high modes could perhaps be due to incomplete

dominance, with the central mode consisting largely of heterozygotes. (Degree of dominance will be discussed later). In any event, relationships with other constituents failed to suggest any refinements for classification, so we classified trees with 0 to 1 percent *β-phellandrene* as low and those with 3 percent or more as high. Trees with 2 percent *β-phellandrene* were not used in the study of mode of inheritance that follows. This is similar to the scheme used for slash pine (GANSEL and SQUILLACE 1976).

Composition types. Classification of the four major monoterpenes into high and low classes results in 16 possible phenotypic combinations. In the trees sampled, 13 of the possible types occurred (Table 2). Although a large

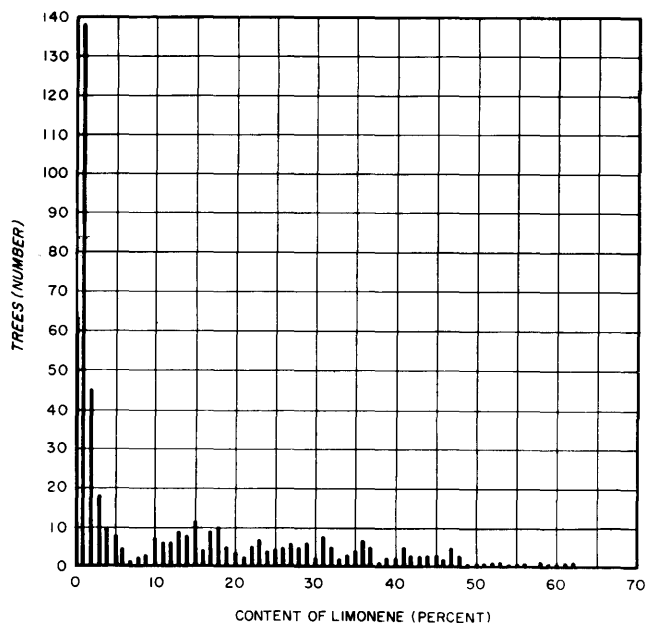


Figure 5. — Frequency distribution for limonene (578 trees).

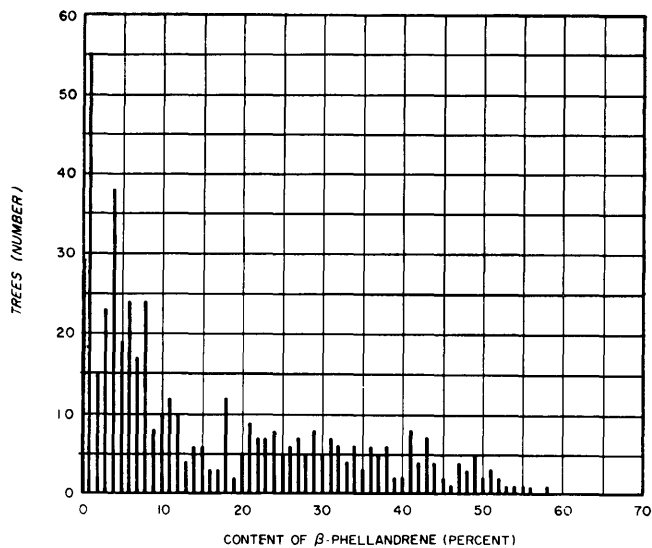


Figure 6. — Frequency distribution for *β-phellandrene* (578 trees).

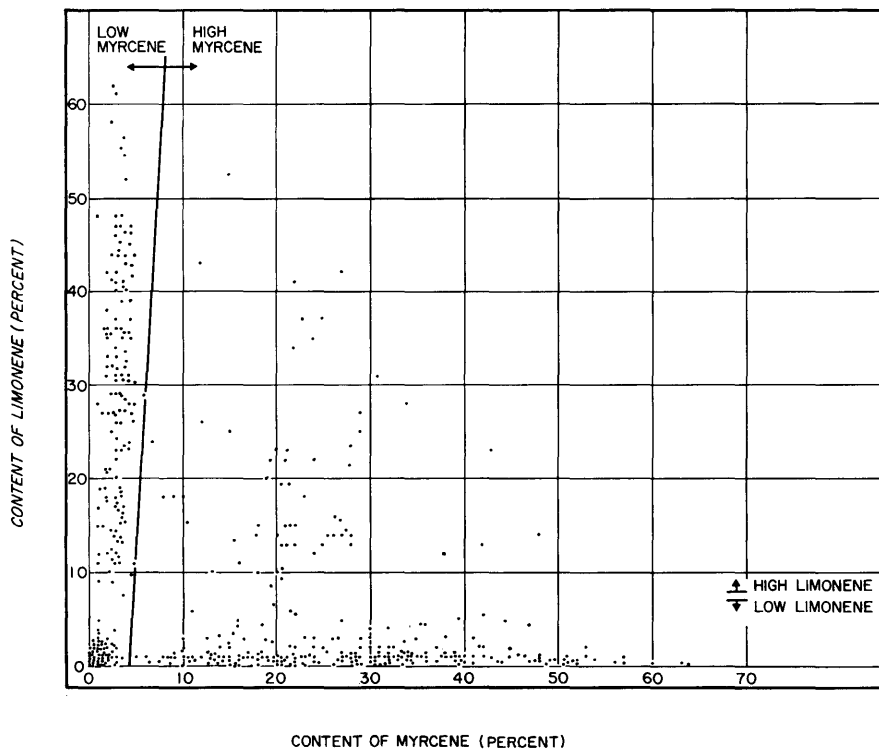


Figure 4. — Scatter diagram of limonene over myrcene.

Table 1. — Number, location, and type of loblolly pines sampled for cortical monoterpene composition

Location	Parents ^{1/}	Families	Individual progeny
Harrison Exp. For.	9	8 ^{2/}	126
Olustee Exp. For.	24	15 ^{3/}	81
Southlands Exp. For.	29	25 ^{4/}	309

^{1/} The parents sampled were vegetative propagules. The geographic origin of the ortets were Texas, North Carolina, and Georgia for Harrison Exp. For., Olustee Exp. For., and Southlands Exp. For. trees, respectively.

^{2/} All were self-full-sib families.

^{3/} Eleven were self-full-sib families while four were half-sib polycross families. In the latter, each of the four mother trees were sired by a mix of pollen from five trees.

^{4/} Twenty-four were full-sib families previously sampled and reported by Rockwood (1973), while one was a wind-pollinated family.

Table 2. — Average monoterpene composition in branch cortical oleoresin of 13 loblolly pine phenotypes.

Phenotype ^{1/}	Basis, trees ^{2/}	Monoterpene				
		α -pinene	β -pinene	Myrcene	Limonene	β -phellandrene
	number	percent				
BMLP	60	25.1	12.9	20.6	19.4	20.9
BMLp	9	52.3	13.3	20.7	12.4	.7
BMlP	171	38.3	19.0	27.6	1.3	12.7
BMlp	62	49.2	24.3	24.1	.8	.7
BmLP	140	25.3	14.8	3.2	29.1	26.4
BmLp	4	66.2	11.6	2.2	18.1	.8
BmlP	60	61.5	21.2	1.3	1.4	13.3
Bmlp	12	71.1	24.6	1.3	1.2	.7
bMLP	1	48.0	3.0	20.0	23.0	4.0
bMLp	0	--	--	--	--	--
bMlP	17	39.2	2.1	37.6	1.3	18.9
bMlp	18	52.4	1.3	45.0	.4	.4
bmLP	1	52.2	1.7	2.5	17.9	24.3
bmLp	0	--	--	--	--	--
bmlP	7	86.1	1.5	1.0	9	9.9
bmlp	0	--	--	--	--	--
Total	562					

^{1/} B, M, L, and P represent high amounts of β -pinene, myrcene, limonene, and β -phellandrene, respectively, while lower-case letters represent low amounts. Small amounts of camphene and α -phellandrene occurred frequently.

^{2/} Fifteen trees could not be classified for β -phellandrene content and 1 for limonene content; these are omitted.

Ann.: In photoprinted Tables 2, 7, 8, 9, 11, 12 the lower case of L is another type than in 1, 3, 4, 5, 6, 10 and 13 and also in text.

proportion of trees fall into five of the phenotypes (BMLP, BMlp, BmLP, and Bmlp), appreciable numbers fall into several other types indicating that monoterpene composition in loblolly pine is highly variable. Note that considerable variation also occurs among high phenotypes of individual constituents. For example, the average content of β -pinene in BMLP trees was 12.9 percent, whereas its average content in Bmlp trees was 24.6 percent. Usually the average content of a given high phenotype tends to increase with decreasing numbers of other high phenotypes present, and this is largely due to the constraint factor mentioned earlier. β -phellandrene, however, seems to be an exception to this rule.

The composition types seem to be rather similar to those found in slash pine (SQUILLACE 1977a, 1977b). Notable differences in phenotypic expressions seem to occur, however. For example, the content of β -pinene and limonene in loblolly pine trees that are phenotypically high for these constituents is less than in slash pine.

Mode of inheritance

β -pinene. In 35 of the families sampled, both parents had high β -pinene. Among these, seven showed segregation (Table 3). Three families were progeny of "low \times low" parents and none of these segregated—all progeny were of the low type. Therefore, assuming single-gene inheritance,

Table 3. — Segregation for β -pinene content in cortical oleoresin of 39 loblolly pine families.

Family or group of families	Observed		Expected		Individual family and pooled tests	
	High	Low	High	Low	χ^2	Significance
-----number of trees-----						
NONSEGREGATING FAMILIES with 1 or 2 HIGH PARENTS (BB x --)						
29 families ^{1/}	350	1	351	0	--	--
SEGREGATING FAMILIES with 2 HIGH PARENTS (Bb x Bb)						
F120 x self	3	1	3.00	1.00	0.33	NS
F189 x self	3	4	5.25	1.75	2.33	NS
F216 x self	5	1	4.50	1.50	.00	NS
F255 x self	7	4	8.25	2.75	.27	NS
2C x 2	7	3	7.50	2.50	.00	NS
18A x 18	9	1	7.50	2.50	.53	NS
58D x 58	9	1	7.50	2.50	.53	NS
All families	43	15	43.50	14.50	.00	NS
Heterogeneity test ^{2/}					4.00	NS
FAMILIES with LOW PARENTS (bb x bb)						
3 families	0	19		19	--	--

^{1/} In 28 of these families both parents were known to be high while 1 was high \times low. Parental phenotypes four other families were not known.

^{2/} Chi-square test of the degree of dissimilarity among families, with 6 d.f. (SNEDECOR 1956, p. 214).

NS = Non significant.

ce, high must be dominant over low. Segregation patterns in the seven segregating high \times high families were not significantly different from expected 3:1 ratios for single-gene inheritance. There were no high \times low segregating families. Thus, we conclude single-gene inheritance with high being dominant over low.

Myrcene. Among 22 families in which both parents had high myrcene, 10 segregated while segregation was absent in 9 low \times low families (Table 4). The 10 segregating high \times high families all closely approximated the expected 3:1 ratio for single-gene inheritance. In the nine high \times low families which segregated, the ratios were not greatly different from the expected 1:1. So here again we conclude single-gene inheritance with high being dominant over low, which agrees with Rockwood's (1973) findings.

Limonene. Two families were problematic in respect to inheritance of limonene (Table 5). Out of 10 high \times high families, 6 segregated. Among the 22 low \times low families, one (26A \times 26) segregated 21:17, which is unlikely under any hypothesis. The remaining 21 low \times low families did not segregate.

Nineteen wind-pollinated progeny of 26A were sampled to get more information of this parent. None contained high limonene. Thus it appears likely that the female parent of 26A \times 26 was not actually 26A, or that the family was a mixture of two or more families. In any event, the

evidence is highly in favor of high limonene being dominant over low, which agrees with the results for slash pine (SQUILLACE 1977a, 1977b), but does not agree with Rockwood's (1973) analyses of the loblolly data from the Southlands Experiment Forest. However, Rockwood did not have the benefit of parental data, which were available here. In four of the five L1 \times L1 families and all of the 11 L1 \times l1 families, segregation patterns were not significantly different from expected ratios. In A6PT-30 \times self, the 6:11 ratio was highly significantly different from the expected 3:1 ratio. The results as a whole favor the single-gene hypothesis, with high being dominant over low, but it cannot be considered conclusive.

β -phellandrene. Out of 31 high \times high families, 9 segregated, while out of 5 low \times low families, none segregated (Table 6). Thus, we hypothesized a single gene with high being dominant over low for β -phellandrene, as in all other major constituents. Some of the segregation ratios were unusual, such as that in family 58C \times 58; this was perhaps partially due to the difficulty of classification. Also, β -phellandrene seems to be the most unstable of the monoterpenes studied because it can apparently polymerize on long storage (SQUILLACE, 1971) and it seems to be the least consistent in repeated samplings. Some families, such as 24E \times 24 segregated cleanly, the 10 progeny having 23, 14

Table 4. — Segregation for myrcene content in cortical oleoresin of 40 loblolly pine families

Family or group of families	Observed		Expected		Individual families and pooled tests	
	High	Low	High	Low	χ^2	Significance
----- number of trees -----						
NONSEGREGATING FAMILIES with 1 or 2 HIGH PARENTS (MM x --)						
12 families ^{1/}	108	0	108	0	--	--
SEGREGATING FAMILIES with 2 HIGH PARENTS (Mm x Mm)						
A6PT-30 x self	12	5	12.75	4.25	0.01	NS
F135 x self	3	1	3.00	1.00	.33	NS
F147 x self	3	2	3.75	1.25	.07	NS
F162 x self ^{2/}	4	0	3.00	1.00	.33	NS
2B x 2	8	2	7.50	2.50	.00	NS
2C x 2	8	2	7.50	2.50	.00	NS
5A x 5	8	2	7.50	2.50	.00	NS
5C x 5	18	4	16.50	5.50	.24	NS
24B x 24	6	4	7.50	2.50	.53	NS
45A x 45	7	3	7.50	2.50	.00	NS
All families	77	25	76.50	25.50	.00	NS
Heterogeneity test ^{3/}					1.51	NS
SEGREGATING FAMILIES with HIGH AND LOW PARENTS (Mm x mm)						
5E x 5	4	6	5	5	0.10	NS
18A x 18	7	3	5	5	.90	NS
18B x 18	5	5	5	5	.10	NS
18D x 18	2	8	5	5	2.50	NS
26A x 26	16	22	19	19	.66	NS
24A x 24	3	7	5	5	.90	NS
45E x 45	4	6	5	5	.10	NS
58C x 58	6	2	4	4	1.12	NS
58D x 58	5	5	5	5	.10	NS
All families	52	64	58	58	1.04	NS
Heterogeneity test ^{3/}					5.44	NS
FAMILIES WITH LOW PARENTS (mm x mm)						
9 families	1	111	0	112	--	--

¹⁾ In all of these, both parents were known to be high. Parental phenotypes of three other families were not known.

²⁾ F162 was taken to be a heterozygote rather than homozygote because its polycross family segregated four high and one low.

³⁾ Chi-square test of the degree of dissimilarity among families (f) with f-1 d.f. (SNEDECOR 1956, p. 214).

NS = Nonsignificant.

1, 27, 1, 12, 31, 1, 14, and 21 percent β -phellandrene. In others, segregation was not so distinct.

Linkages

Data were insufficient to study linkage between most pairs to the four simply inherited monoterpenes. However, six families permitted a test for linkage between myrcene and limonene. To make the test we grouped families having similar genotypes:

Group I (Mm ll × Mm Ll):
2B × 2, 2C × 2, and 45A × 45.

Group II (mm Ll × MmLl): 18A × 18.

Group III (mm ll × Mm Ll): 18B × 18 and 18D × 18.

A preliminary check of data for the families suggested that the two loci are closely linked and that in the particular parents involved, the linkage is in the repulsion phase.

For example, the progeny of Group III can be of four types: ML, Ml, mL, ml. Observed numbers were 0, 13, 7, and 0, respectively. Under free recombination, the expected numbers would be 5, 5, 5, and 5, respectively. Under complete linkage in coupling, expected numbers would be 10, 0, 0, and 10, while in repulsion they would be 0, 10, 10, and 0, respectively. Therefore, we pursued the hypothesis that the two loci are linked in repulsion.

To compute the rate of recombination (closeness of linkage) we followed procedures used by BARADAT *et al.* (1975). The first step was to compute expected phenotypic frequencies in terms of p, the rate of recombination, from gametic frequencies. The computation for Family Group I is shown in Table 7 as an example. Results for all groups are given in Table 8.

The next step was to compute the numbers of the various phenotypes expected under complete linkage and

Table 5. — Segregation for limonene content in cortical oleoresin in 43 lobloly pine families

Family or group of families	Observed		Expected		Individual family and pooled tests	
	High	Low	High	Low	X ²	Significance
----- number of trees -----						
NONSEGREGATING FAMILIES with 1 or 2 HIGH PARENTS (LL × --)						
4 families	68	0	68	0	--	--
SEGREGATING FAMILIES with 2 HIGH PARENTS (Ll × Ll)						
A3PT—35 × self	5	1	4.50	1.50	0.00	NS
A3PT—40 × self	17	4	15.75	5.25	.14	NS
A6PT—26 × self	11	2	9.75	3.25	.23	NS
A6PT—30 × self	6	11	12.75	4.25	12.25	**
F135 × self ¹⁾	4	0	3.00	1.00	.33	NS
18A × 18	8	2	7.50	2.50	.00	NS
All families	51	20	53.25	17.75	.23	NS
Heterogeneity test ²⁾					12.73	*
SEGREGATING FAMILIES with HIGH AND LOW PARENTS (Ll × ll)						
2A × 2	3	6	4.5	4.5	.44	NS
2B × 2	6	4	5.0	5.0	.10	NS
2C × 2	3	7	5.0	5.0	.90	NS
5E × 5	6	4	5.0	5.0	.10	NS
18B × 18	5	5	5.0	5.0	.10	NS
18D × 18	8	2	5.0	5.0	2.50	NS
23A × 23	6	4	5.0	5.0	.10	NS
24A × 24	5	5	5.0	5.0	.10	NS
26C × 26	7	3	5.0	5.0	.90	NS
45A × 45	7	3	5.0	5.0	.90	NS
58C × 58	2	6	4.0	4.0	1.12	NS
All families	58	49	53.5	53.5	.59	NS
Heterogeneity test ²⁾						
FAMILIES with 2 LOW PARENTS (ll × ll)						
26A × 26	21	17	0	38	--	--
21 other families	0	197	0	197	--	--

¹⁾ F135 was taken to be heterozygous rather than homozygous because its polycross progeny segregated two high and three low.

²⁾ Chi-square test of the degree of dissimilarity among families (f) with f-1 d.f. (SNEDECOR 1956, p. 214)

* Significant at 0.05 level.

** Significant at 0.01 level.

NS = Nonsignificant.

Table 7. — Computation of expected phenotypic frequencies under linkage in repulsion and under no linkage for Family Group I¹⁾

Female, frequency and gamete	Male, frequency and gamete			
	p/2, ML	p/2, mL	(1-p)/2, Ml	(1-p)/2, mL
1/2, Ml	p/4, ML	p/4, Ml	(1-p)/4, Ml	(1-p)/4, ML
1/2, mL	p/4, ML	p/4, mL	(1-p)/4, Ml	(1-p)/4, mL

Sums of phenotypic frequencies in terms of p:

(1+p)/4 ML, (2-p)/4 Ml, (1-p)/4 mL, and p/4 ml.

Sums of phenotypic frequencies if p = 0, completely linked:

1/4 ML, 1/2 Ml, 1/4 mL, and 0 ml.

Sums of phenotypic frequencies if p = 1/2, no linkage:

3/8 ML, 3/8 Ml, 1/8 mL, and 1/8 ml.

¹⁾ The genotypes of the parents of these families are Ml/ml and Ml/ml, if the two loci are linked in repulsion.

Table 6. — Segregation for β -phellandrene content in cortical oleoresin of 43 loblolly pine families.

Family or group of families	Observed		Expected		Individual family and pooled tests	
	High	Low	High	Low	χ^2	Significance
-----number of trees-----						
NONSEGREGATING FAMILIES with 1 or 2 HIGH PARENTS (PP x --)						
26 families ^{1/}	307	2	309	0	--	--
SEGREGATING FAMILIES with 2 HIGH PARENTS (Pp x Pp)						
F-135 x self	3	1	3.00	1.00	0.33	NS
F-216 x self	5	1	4.50	1.50	.00	NS
23A x 23	4	5	6.75	2.25	1.75	NS
23C x 23	9	1	7.50	2.50	.53	NS
24A x 24	5	5	7.50	2.50	2.13	NS
24B x 24	6	3	6.75	2.25	.03	NS
24E x 24	7	3	7.50	2.50	.00	NS
58A x 58	9	2	8.25	2.75	.03	NS
58C x 58	2	5	5.25	1.75	5.76	*
All families	50	26	57.00	19.00	2.96	NS
Heterogeneity test ^{2/}					7.60	NS
SEGREGATING FAMILIES with HIGH AND LOW PARENTS (Pp x pp)						
2A x 2	7	1	4.0	4.0	3.12	NS
23E x 23	11	10	10.5	10.5	.00	NS
45E x 45	3	5	4.0	4.0	.12	NS
All families	21	16	18.5	18.5	.43	NS
Heterogeneity test ^{2/}					2.81	NS
FAMILIES with LOW PARENTS (pp x pp)						
5 families	0	43	0	43	--	--

^{1/} In 22 of these, both parents were known to be high, while four were high x low.
^{2/} Chi-square test of the degree of dissimilarity among families (f), with f-1 d.f. (SNEDECOR 1956, p. 214).
 NS = Non significant.
 * Significant at 0.05 level.

Table 8. — Expected phenotypic frequencies for linkage in repulsion or no linkage, in terms of p (the proportion of recombination) for all family groups.^{1/}

Family group	Genotypes ^{2/}		Phenotypes			
	Female	Male	ML	Ml	mL	ml
I	Ml/mL	Ml/mL	(1+p)/4	(2-p)/4	(1-p)/4	p/4
II	mL/mL	Ml/mL	(1+p)/4	(1-p)/4	(2-p)/4	p/4
III	mL/mL	Ml/mL	p/2	(1-p)/2	(1-p)/2	p/2

^{1/} For linkage in repulsion p = 0 and for no linkage p = 1/2.
^{2/} Alleles before the slash (/) are on one of a pair of homologous chromosomes, while those after it are on the other.

under no linkage (Table 9). As an example, note in Table 8 that the expected proportion of ML types in Family Group I was (1 + p)/4. For complete linkage p = 0, and therefore, we would expect $\frac{1+0}{4}(30) = 7.50$ trees of this type. For no linkage, p = 1/2 and the proportion of expected ML types is $\frac{(1+1/2)}{4}(30) = 11.25$.

The final step was to compute estimated rates of recombination (\hat{p}). For Family Group III, \hat{p} is simply the observed number of recombinant types (ML and ml) divided by the total number observed, or $\frac{0}{20} = 0$ suggesting complete linkage in repulsion. For the other two groups of families, we combined the data for phenotypes having the same expected frequencies (Table 10) and computed an estimate of p using the cubic equation given in BARADAT et al. (1975).

The result, $\hat{p} = 0.175$, suggests relatively close linkage. More data would be desirable, but the results suggest that myrcene and limonene are closely linked, with a recombination rate of about 0.10 (a rough average of the two estimates obtained.) BARADAT *et al.* (1975) showed a similar close linkage between myrcene and 3-carene in *P. pinaster* AIR.

Genotypes

Genotypes of the various parents involved were deduced from the above analyses to the extent possible, with the assumption of linkage between myrcene and limonene (Tables 11 and 12). Genotypes of some of the parents are incomplete, usually because of insufficient data. More complete genotypes could be readily obtained with ad-

Table 9. — Observed number of phenotypes and expected number under complete linkage in repulsion and under no linkage.

Item	Phenotype			
	ML	Ml	mL	m ℓ
FAMILY GROUP I (M ℓ /m ℓ x M ℓ /mL)				
Observed	10	13	6	1
Expected under complete linkage	7.50	15.00	7.50	0.00
Expected under no linkage	11.25	11.25	3.75	3.75
FAMILY GROUP II (mL/m ℓ x M ℓ /mL)				
Observed	5	2	3	0
Expected under complete linkage	2.50	2.50	5.00	0.00
Expected under no linkage	3.75	1.25	3.75	1.25
FAMILY GROUP III (m ℓ /m ℓ x M ℓ /mL)				
Observed	0	13	7	0
Expected under complete linkage	0	10	10	0
Expected under no linkage	5	5	5	5

X^2 values for observed numbers vs. numbers expected under no linkage were 3.8, 2.3, and 23.6 for the three groups respectively, the latter being significant at the 1 percent level.

Table 10. — Computation of rate of recombination for Family Groups I and II combined.¹⁾

Phenotypes of expected frequency	Expected number of progeny under:		Observed number
	Complete linkage (p = 0)	No linkage (p = 1/2)	
(1 + p)/4	10	15	15 = n ₁
(2 - p)/4	20	15	16 = n ₂
(1 - p)/4	10	5	8 = n ₃
p/4	0	5	1 = n ₄

¹⁾ Data obtained by summing values from Table 9 that are based on the same phenotypic frequencies. Thus, data in

- row 1 = ML of Group I + ML of Group II,
- row 2 = Ml of Group I + mL of Group II,
- row 3 = mL of Group I + Ml of Group II, and
- row 4 = m ℓ of Group I + m ℓ of Group II.

Computation of p (BARADAT *et al.* 1975):

$$p^3 (n_1 + n_2 + n_3 + n_4) - p^2 (3n_1 + n_3 + 2n_4) + p (2n_1 - n_2 - 2n_3 - 2n_4) + 2n_4 = 0$$

Substituting observed values of n we have,

$$40 p^3 - 55 p^2 - 3p + 2 = 0$$

Under the condition that $0 < \hat{p} < 1/2$, we obtain $\hat{p} = 0.175$, the estimated rate of recombination.

Table 11. — Phenotypes and genotypes of Texas and North Carolina parents for relatively high or low content of β -pinene (B), myrcene (M), limonene (L), and β -phellandrene (P) in their cortical oleoresin.¹⁾

Parent	Phenotype	Genotype
TEXAS PARENTS		
A3PT-31	BmLP	BB mL/mL PP
A3PT-34	BmLP	BB mL/mL PP
A3PT-35	BmLP	B- mL/mL P-
A3PT-37	BmLP	BB mL/mL PP
A3PT-40	BMLP	BB ML/Ml PP
A6PT-25	BmLP	BB mL/mL PP
A6PT-26	BmLP	BB mL/mL PP
A6PT-30	BMLP	BB M ^l /mL PP
NORTH CAROLINA PARENTS		
F114	bMlP	bb Ml/-l P-
F120	BMLP	Bb Ml/Ml PP
F135	BMLP	BB Ml/mL Pp
F147	BMLp	B- Ml/mL pp
F162	BMLP	BB Ml/mL PP
F182	bMlP	bb Ml/Ml pp
F189	BMLP	Bb Ml/-l P-
F216	BMLP	Bb Ml/-l Pp
F255	BmlP	Bb Ml/Ml pp
F1009	bmlP	bb ml/ml P-
F1024	BMLP	B- Ml/-l P-

¹⁾ The incomplete genotypes, such as B-, are most likely homozygous dominants, but could be heterozygotes; the bases for these determinations were weak, there being only four to seven trees in the families involved.

ditional sampling. Some, however, would require additional matings. For example, the genotype of parent No. 5 (Table 12) could be completed by mating with parent 2C and sampling their progeny. Both the complete and incomplete genotypes, however, would be very useful as gene markers for basic studies.

Degree of dominance

Average content of each monoterpene in parents and progenies of known genotype were used to estimate degrees of dominance. Computations were made separately for groups of trees having the same number of high phenotypes in order to reduce effects of variation in phenotypic expression caused by constraint (Table 13). That is, for example, the content of β -pinene in a tree of the phenotype BMLP will tend to have less β -pinene than a tree of the phenotype Bmlp. Results for β -pinene were very erratic, partly due to the few number of trees in some phenotypic groups. But the weighted average degree of dominance for it was very high, 0.83. The degree of dominance estimated for limonene was likewise high (0.90), while it was low for myrcene (0.31) and essentially absent for β -phellandrene (-0.22). The results are rather similar to those found for slash pine (SQUILLACE 1977a, 1977b).

Conclusions

Composition of the monoterpenes in cortical oleoresin of loblolly pine varies greatly among trees. Trees can be classified as having high or low amounts of four of the major constituents (β -pinene, myrcene, limonene, and β -phellandrene), and such differences seem to be controlled by single genes with high being dominant over low in all cases. Further research on inheritance of limonene however, would be desirable because of some inconsistencies in the data. The degree of dominance seems to be almost complete in β -pinene and limonene, low in myrcene, and essentially absent in β -phellandrene. Further sampling would be desirable to determine the degrees of dominance

Table 12. — Phenotypes and genotypes of Georgia trees for relatively high or low content of β -pinene (B), myrcene (M), limonene (L), and β -phellandrene (P) in their cortical oleoresin.¹⁾

Parent	Phenotype	Genotype	Parent	Phenotype	Genotype
2	BMLp	Bb Ml/mL pp	24	BMLP	B- Ml/mL Pp
2A	BMLP	BB Ml/Ml Pp	24A	BmLP	B- mL/mL Pp
2B	BMLP	BB Ml/mL PP	24B	BMLP	B- Ml/mL Pp
2C	BMLp	Bb Ml/mL pp	24E	BMLP	B- Ml/Ml Pp
5	BMLP	B- Ml/mL P-	26	FmLP	BB ml/ml PP
5A	BMLP	B- Ml/mL P-	26A	FMlP	Bb Ml/mL pp
5C	BMLP	B- Ml/mL P-	26B	FmLP	B- ml/ml P-
5E	-mLP	-- mL/mL P-	26C	bmLP	bb mL/mL P-
18	BMLP	Bb Ml/mL PP	45	BMLp	B- Ml/mL pp
18A	BmLP	Bb mL/mL pp	45A	BMLP	B- Ml/mL PP
18B	BmlP	BB ml/mL P-	45D	BMLp	B ⁵ Ml/Ml pp
18D	BmlP	BB ml/mL P-	45E	BmlP	B- ml/mL Pp
23	--lP	-- -l/-l Pp	58	BMLP	Bb Ml/mL Pp
23A ^{2/}	BMLP	B- M- Ll Pp	58A	BMLP	BB Ml/Ml Pp
23C	--lP	-- -l/-l Pp	58C	BmLP	BB mL/mL Pp
23E	BMLp	B- Ml/-l pp	58D	BmlP	Bb ml/mL PP

¹⁾ The incomplete genotypes were due to 1) lack of knowledge of parental phenotypes (5E, 23, and 23C, which were dead) or to 2) the impossibility of determining genotypes when two high parents are crossed and no segregation occurs.

²⁾ Impossible to assign the proper linkage in this case.

Table 13. — Estimates of degrees of dominance, based on average monoterpene content of parents and progenies of known genotype.

Phenotypic group or groups ^{1/}	Homozygous dominants (D)		Heterozygotes (H)		Homozygous recessives (R)		Degree of dominance ^{2/}
	Basis Average trees content		Basis Average trees content		Basis Average trees content		
	No.	%	No.	%	No.	%	
β-PINENE							
MLP	30	10.4	1	7.4	1	3.0	0.19
ML, MP, and LP	109	15.4	12	15.0	17	2.1	.94
M, L, and P	5	15.6	8	18.0	25	1.4	<u>1.34</u>
Weighted average ^{3/}							.83
MYRCENE							
BLP	22	24.6	21	19.9	140	3.2	.56
BL, BP, and LP	7	35.4	35	20.1	64	1.4	.10
B, L, and P	10	29.1	10	18.1	19	1.2	<u>.21</u>
Weighted average ^{3/}							.31
LIMONENE							
BM, BP, and MP	71	30.9	39	27.9	137	1.1	.80
β-PHELLANDRENE ^{4/}							
BML	3	25.7	19	7.7	9	.7	-.44
BM, BL, and ML	9	18.8	64	7.6	66	.7	-.24
B, M, and L	2	13.0	12	8.3	30	.5	<u>.25</u>
Weighted average ^{3/}							-.2

^{1/} B, M, L, and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lower case letters represent low amounts.

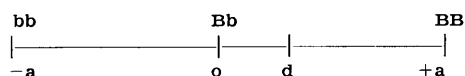
^{2/} Computed as in KEMPTHORNE (1957, p 373). For example, the degree of dominance for β-pinene group MLP was computed as:

$$D-R = 10.4 - 3.0 = 3.7$$

$$a = \frac{D-R}{2} = \frac{3.7}{2} = 1.85$$

$$b = a - (D-H) = 1.85 - (10.4 - 7.4) = 0.7$$

$$\text{Degree of dominance} = \frac{a}{d} = \frac{0.7}{3.7} = 0.19, \text{ depicted below}$$



^{3/} Weighted by number of D and H trees involved.

^{4/} Includes only the Olustee and Southland Experiment Forest trees. The Harrison Experimental Forest trees of known genotype were all homozygous dominant and averaged 36.2 percent.

more precisely, which may then permit distinguishing heterozygotes from homozygous dominants, for some constituents at least. The composition and inheritance patterns seem to be similar to those found in slash pine, although differences occur in mean phenotypic expression of the gene involved.

Loci controlling variation in myrcene and limonene seem to occur in close proximity on the same chromosome, having a low rate of recombination.

Although more research is required on some aspects, the results show that monoterpenes can be very useful as gene markers for study of population structure, breeding mechanisms, and geographic variation.

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