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Geographic Variation of Monoterpenes in Cortical Oleoresin of Loblolly Pine

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Summary

Monoterpene composition of cortical oleoresin was determined for 2,724 planted trees originating from areas throughout the range of loblolly pine. Major constituents were α-pinene, β-pinene myrcene, limonene, and β-phellandrene. Content of α-pinene, myrcene, and limonene showed strong clinal trends, although with some interruptions, in an east to west direction. β-phellandrene showed a definite disjunction — all trees west of the Mississippi River had high β-phellandrene. β-pinene showed the least variation, with no distinct geographic pattern. Some indications of a relationship between monoterpene composition and fusiform rust infection were found, but these were inconclusive and only suggest further study. Content of limonene was found to be related to some seed traits studied by others. The geographic variation in monoterpenes, together with supplemental data on seed traits reproduced here, allows judgment of the geographic origin of seed used in plantations, and could perhaps also be used to certify seed from seed orchards.

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

Zusammenfassung

An 2.724 Bäumen aus dem Verbreitungsgebiet von *Pinus taeda* wurde die Zusammensetzung des Rindenharzes untersucht. Hauptkomponenten waren α-Pinen, β-Pinen, Myrcen, Limonen und β-Phellandren. Der Gehalt an α-Pinen, Myrcen und Limonen zeigte, abgesehen von einigen Abweichungen, einen ausgeprägten klinalen Trend in Ost-West-Richtung. β-Phellandren zeigte eine klare Disjunktion — alle Bäume westlich des Mississippi hatten einen hohen Gehalt an β-Phellandren. β-Pinen wies die geringste Variation auf, es ergab sich kein deutliches geographisches Muster. Es ergaben sich einige Anzeichen für eine Beziehung zwischen Monoterpenzusammensetzung und der Infektion durch *Cronartium fusiforme*, die jedoch noch weiterer Untersuchungen bedürfen. Der Gehalt an Limonen zeigte einige Beziehungen zu Saatgutmerkmalen aus Untersuchungen anderer Autoren. Die geographische Variation der Monoterpene gestattet zusammen mit den ergänzenden Saatgutmerkmalen eine Beurteilung der geographischen Herkunft des für die Kulturen verwendeten Saatgutes und könnte ferner zur Zertifikation des Saatgutes aus Samenplantagen dienen.

Introduction

Loblolly pine (*Pinus taeda* L.) is the most important softwood species in the southern pine region, and forest geneticists need to learn as much as possible about patterns of genetic variation over the species range. Such knowledge is useful to delineate breeding zones and to determine seed origin of plantations. Composition of monoterpenes in cortical oleoresin is ideally suited for study of geographic variation because it is strongly inherited and not greatly affected by environment (SQUILLACE 1976). Geographic patterns of variation in monoterpene composition for loblolly pine are presented, and the relationship between monoterpene composition to fusiform rust infection and some other traits are examined.

Materials and Methods

Oleoresin samples were taken from loblolly pine trees growing in nine plantations and one natural stand (*Table 1*). A total of 2,724 trees were sampled from 113 prove-

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Table 1. — Summary of provenances and trees sampled.

Location of plantation	Owner	Provenances sampled [2]	Trees sampled
		Number	
Olustee, Fl.	Owens-Illinois Corp.	9	219
Callahan, Fl.	Container Corp.	6	149
Bainbridge, Ga. [1]	International Paper Co.	1	29
Colquitt, Ga.	Southeastern For. Exp. Stn.	13	257
Cordele, Ga.	Southeastern For. Exp. Stn.	9	216
Dothan, Al.	Auburn University	8	192
Baton Rouge, La.	Crown Zellerbach Corp.	26	654
Fordyce, Ar.	Georgia-Pacific Co.	26	627
Millard, Ms.	Crown Zellerbach Corp.	4	110
Homer, La.	Louisiana State University	11	271
Total		113	2724

[1]) A natural stand.
[2]) Locations of provenances sampled are indicated in subsequent maps showing patterns of variation.

nances scattered over the species range. The number of trees per source varied from 18 to 33 and averaged 24. In view of findings by GANSEL and SQUILLACE (1976) and of others (as reviewed by SQUILLACE 1976), we assumed that plantation, age, and seasonal effects on monoterpene composition were negligible. Sampling was begun November 1976 and completed in February 1978. Trees varied from about 9 to 40 years of age.

Cortical rather than xylem oleoresin was sampled because preliminary investigation showed that the former contained more major constituents and was less affected by location in the tree. Samples were obtained from cortical tissue by excising branches at about 10 mm or less from the tips. Exuded oleoresin was placed in screw-cap vials, sealed, and stored in a refrigerator until analyzed. Samples were analyzed within 8 weeks after collection. The monoterpene composition of each sample was determined by gas-liquid chromatography, using a 20-foot (6.1 m), 3/16-inch (4.76 mm), 60/80-mesh chromosorb W column packed with 20% carbowax 20 M. The relative amount of each monoterpene was expressed as a percentage of total monoterpenes.

To examine the relationship between monoterpene composition and fusiform rust *Cronartium fusiforme* HEDGC. and HUNT ex CUMM.) infection, sample trees that contained a rust gall were noted. A total of 2,409 trees were examined for this trait.

Results

Individual Tree Variation and Classification

Major monoterpene found were similar to those in slash pine (*P. elliottii* ENGELM.) (GANSEL and SQUILLACE 1976): α-pinene, β-pinene, mycrene, limonene, and β-phellandrene. Minor constituents were camphene and α-phellandrene, with small amounts (up to about 2%) found in most trees. Three trees contained rather large amounts of $\triangle$-3-carene (8, 16, and 28%), two of them originating from Calvert Co., Maryland, and one from Clay Co., Alabama. COYNE and KEITH (1972) found this constituent in the xylem oleoresin of two of the loblolly pines they sampled.

Frequency distributions for the five major constituents were similar to those reported by SQUILLACE *et al.* (1980 *b*). Four of them (β-pinene, myrcene, limonene, and β-phellandrene) showed bimodality, while the fifth (α-pinene) did not. The authors showed that the bimodal constituents were monogenically inherited, with high amounts dominant over low in each case. Therefore, we used their classification scheme, which took into account relationships between constituents, to assign each tree as being "high" or "low,"as follows:

β-pinene: Considered to be high when its content was greater than Y, where Y = 4.8 - 0.035 X, and X is the sum of limonene + β-phellandrene contents, otherwise low.

Myrcene: Considered to be high when its content was greater than Y, where Y = 4.5 + 0.058 X, and X is content of limonene, otherwise low.

Limonene: Considered to be high when its content was 8% or more, and low when 6% or less.

β-phellandrene: Considered to be high when its content was 3% or more, and low when 1% or less.

Classifying each tree as high or low for each of the four monoterpenes resulted in 16 possible phenotypes. The frequencies and mean composition for each of these are shown in *Table 2.* Although 96% of the trees fell into six of the classes (BMLP, BMlP, BMlp, BmLP, BmlP, and bMlP), some trees occurred in all other classes. The composition for one tree, which lacked genes for high amounts of the four simply inherited constituents, was dominated by α-pinene. Curiously, trees with the phenotypes mL and Ml occurred much more frequently than expected from random pairing of these alleles, while ML and ml types occurred less frequently than expected. Implications of this are not clear, although it suggests that natural selection has occurred for mL and Ml types. SQUILLACE *et al.* (1980 b) showed that the two genes are closely linked.

Geographic variation

The percentage of trees with high amounts of each of the four simply inherited major constituents was plotted on maps and isograms were drawn to depict patterns of variation. Although α-pinene was not shown to be simply

Table 2. — Average content of major monoterpenes in branch cortical oleoresin of 16 loblolly pine phenotypes.

Phenotype [1]	Basis, trees	Monoterpene [2]				
		α-pinene	β-pinene	Myrcene	Limonene	β-phellandrene
	Number	percent				
BMLP	373	20.4	14.1	20.5	20.3	23.5
BMLp	20	39.1	17.5	17.4	24.4	.7
BMlP	1132	30.4	18.6	30.2	1.6	17.8
BMlp	112	48.9	17.0	31.4	.9	.8
BmLP	526	22.0	14.3	3.4	29.9	29.0
BmLp	19	53.6	18.0	1.5	25.3	.4
BmlP	321	45.8	24.4	2.1	1.8	24.2
Bmlp	31	70.3	25.2	1.4	1.2	.6
bMLP	14	20.3	1.8	36.7	20.0	20.0
bMLp	2	53.5	1.5	24.7	18.2	1.1
bMlP	92	37.1	2.4	38.3	1.3	19.5
bMlp	7	67.1	2.0	28.2	.7	.6
bmLP	9	35.7	1.8	3.0	32.5	25.4
bmLp	1	73.7	1.3	2.2	21.8	.0
bmlP	8	73.0	2.6	1.9	1.4	19.5
bmlp	1	94.8	1.8	1.8	.0	.0
Total trees & weighted averages	2668 [3]	31.1	17.0	19.7	10.5	20.4

[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]) Small amounts of camphene and α-phellandrene were also found in most trees, and appreciable amounts of Δ-3-carene in three trees.
[3]) Fifty-six additional trees could not be classified because the content of one or more of their constituents fell between the "high" and "low" criteria noted in the text.

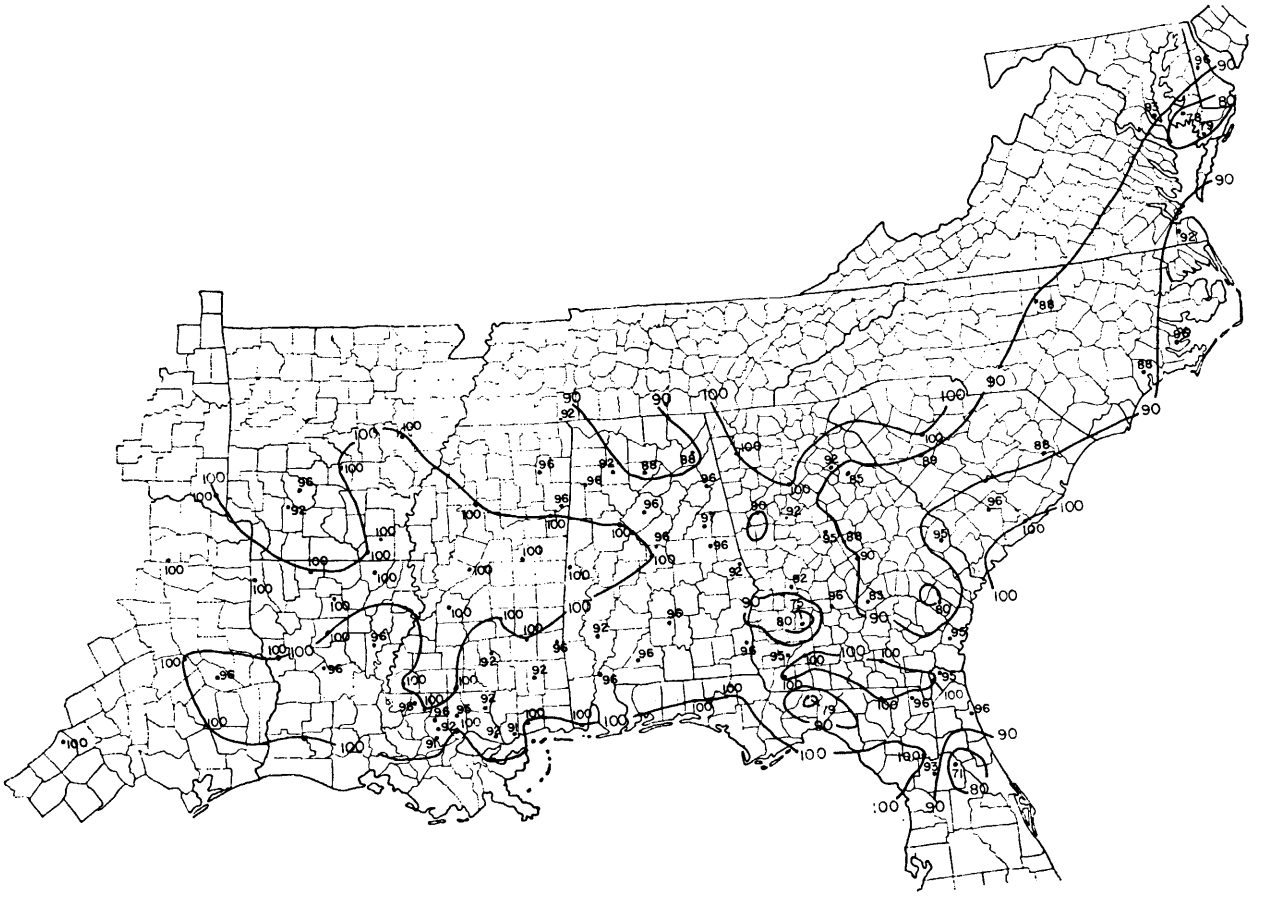

Figure 1. — Percentage of trees with high β-pinene.

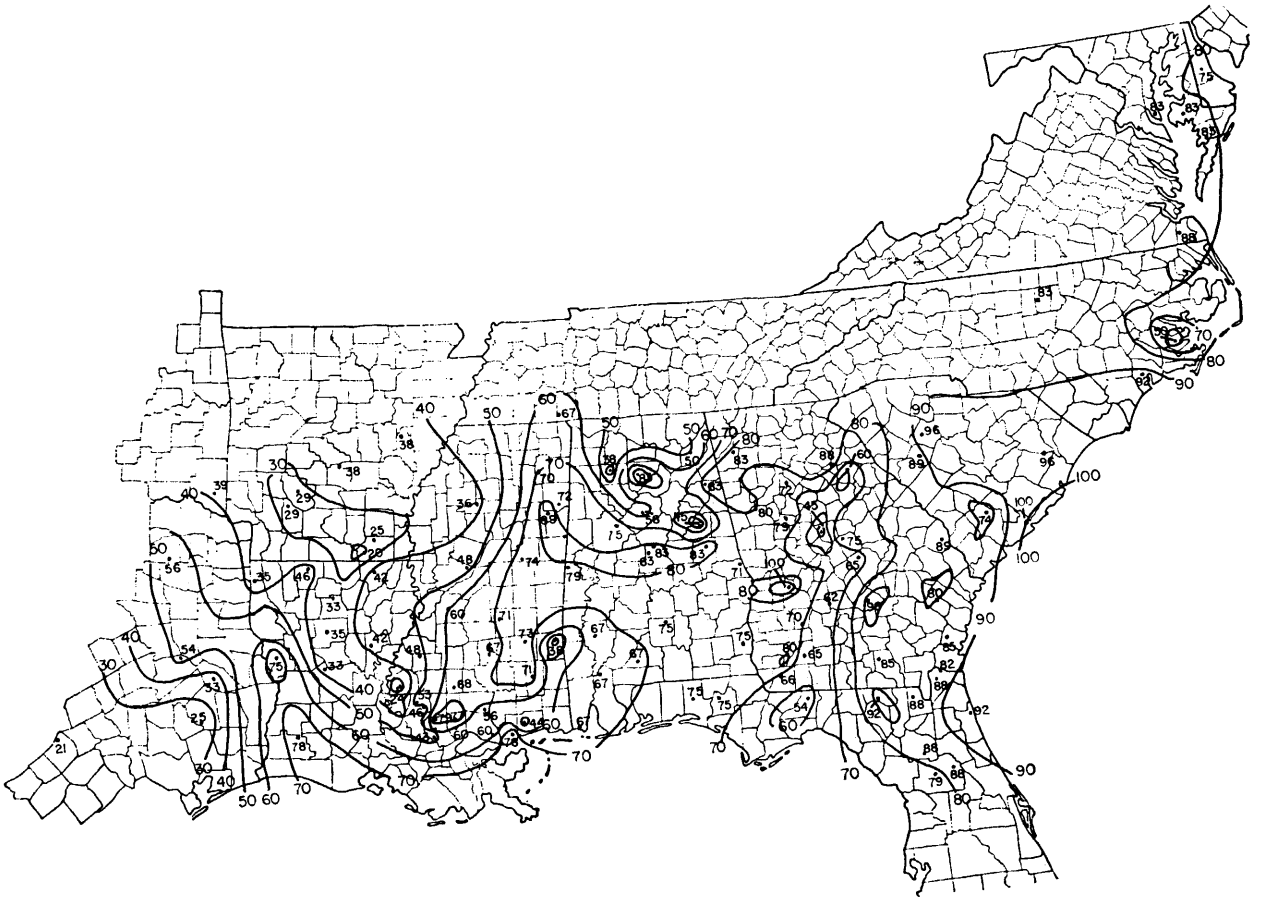

Figure 2. — Percentage of trees with high myrcene.

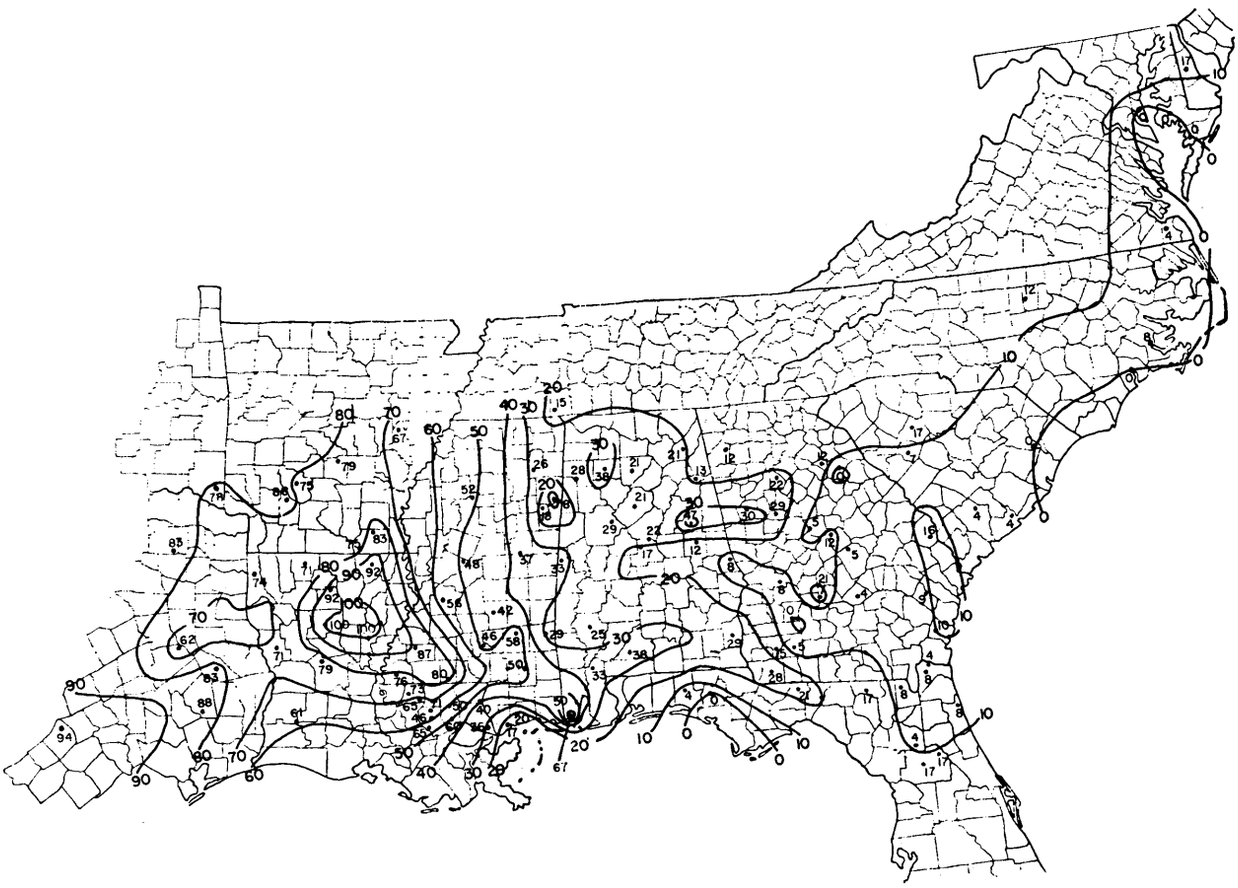

Figure 3. — Percentage of trees with high limonene.

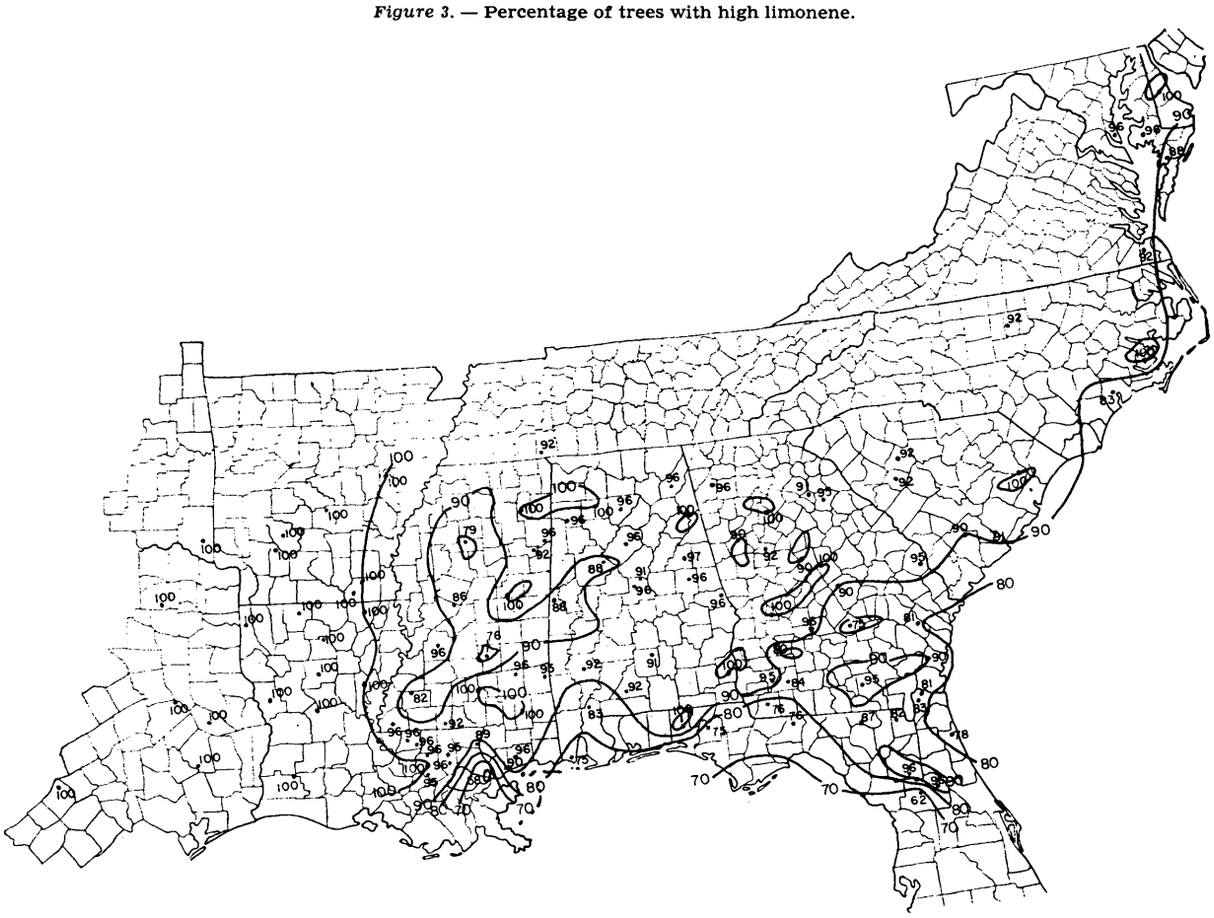

Figure 4. — Percentage of trees with high β-phellandrene.

inherited, the average content of this constituent was also studied.

β-pinene. This constituent showed relatively little variation among seed sources *(Fig. 1).* Although there were some definite high and low patches, no clear geographic trends were apparent. The percentage of trees with high amounts varied from 75 to 100, but was mostly 90+ throughout the range.

Myrcene. Percentages of trees with high myrcene varied from 21 to 100 *(Fig. 2).* More trees in the eastern portion of the range had high myrcene than those in the west. This overall west to east gradient was interrupted by "valleys" and "ridges". For example, the percentage of trees with high myrcene was very low in Texas, increased to a high in western Louisiana, then decreased to a low in north-central Louisiana and southern Arkansas. A very distinct valley also occurred, running south to north, through central Georgia. Some high or low patches occurred at various points, which in some cases may be random variation.

Limonene. The pattern for limonene showed a definite east to west gradient, with most sources in the east having 0 to 10% of trees with high amounts and those in the west having 70 to 100% *(Fig. 3).* There is, however, a high ridge beginning in northeast Louisiana, and extending southeasterly into southeast Mississippi and continuing easterly through southern Alabama and southwest Georgia. Sources from the Gulf and Atlantic coastal areas tended to have fewer trees with high limonene content than those inland. The overall pattern tends to be opposite to that for myrcene.

β-phellandrene. The percentage of trees with high β-phellandrene varied from 58 to 100 *(Fig. 4).* The striking feature, however, was that every tree sampled west of the Mississippi River had high β-phellandrene, while east of the river the percentages varied from 58 to 100 and averaged 91. As with limonene, there was also a general tendency for coastal sources to have fewer trees with high β-phellandrene than inland trees. There were also numerous small random patches of moderate highs and lows.

α-pinene. Average content of α-pinene was lowest (about 15%) in the western portion of the species range *(Fig. 5).* It then increased toward the east, reaching a high of about 40% in eastern Mississippi. In the remainder of the range, content was relatively high in coastal areas (40 to 50%), with a decrease to the north and northwest, to about 25 to 35%. The pattern was generally similar to that found by COYNE and KEITH (1972) and GILMORE (1971) for loblolly pine, but the content of α-pinene in stem xylem oleoresin they found was considerably higher (50 to 85%), which was likely due to the fact that xylem oleoresin lacks high amounts of myrcene and limonene.

Relationship to Fusiform Rust Infection

To determine the relationship between monoterpene composition and fusiform rust infection, the numbers of infected and uninfected trees within high and low monoterpene content classes were subjected to chi-square tests of independence. Separate tests were run for each plantation because of high variation in the degree of infection among plantations. Some of the plantations included seed

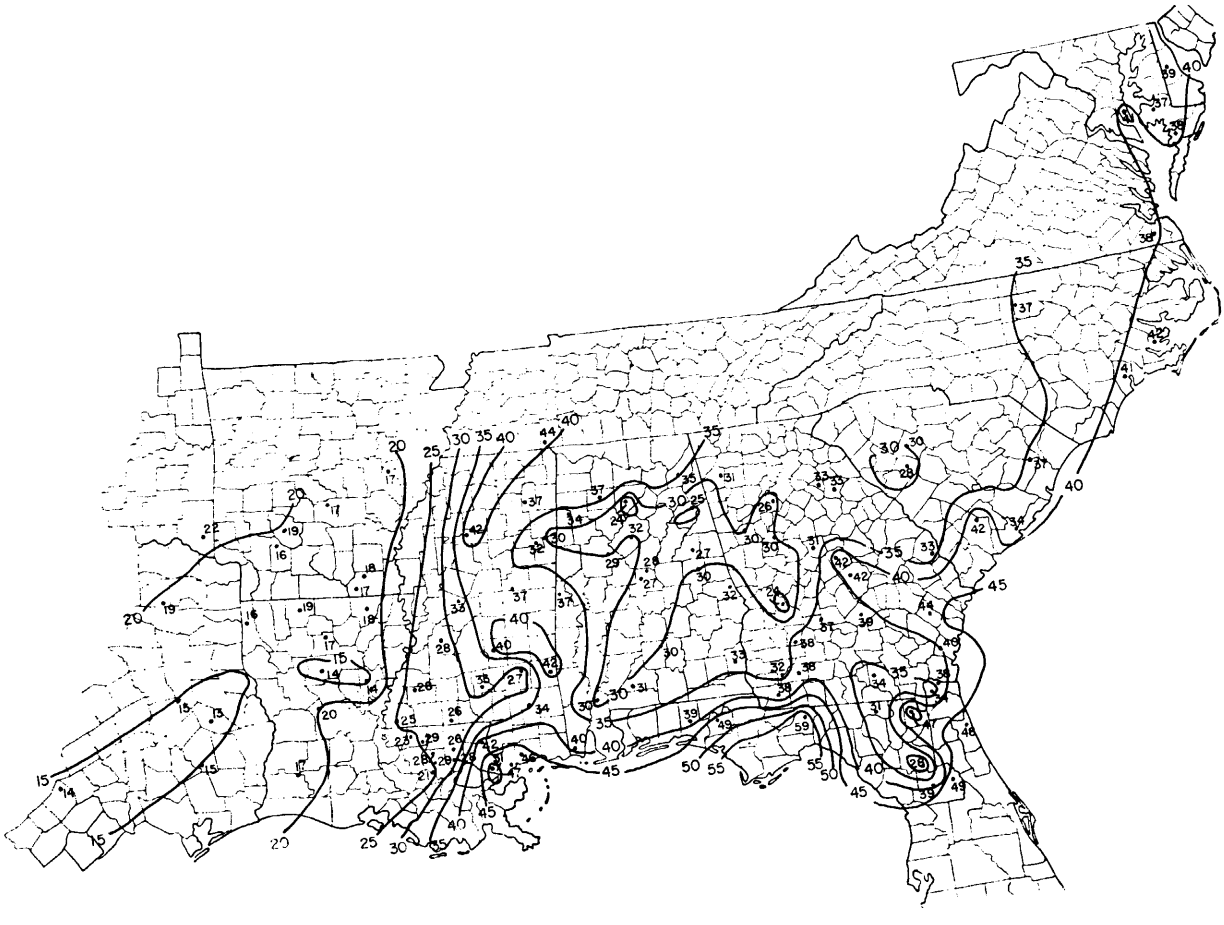

Figure 5. — Mean percentage content of α-pinene.

Table 3. — Percent of trees infected with fusiform rust in high and low monoterpene content classes.

Plantation	Region of seed source[1]	Trees sampled	β-pinene		Myrcene		Limonene		β-phellendrene	
			High	Low	High	Low	High	Low	High	Low
		Number	Percent							
Olustee, Fl.	Central	192	16	17	17	13	23	15	16	20
Colquitt, Ga.	West	20	15	--[2]	25	12	7	40	15	--[2]
	Central	237	78	68	78	72	72	77	78	70
	All sources	257	72	68	77	60**	48	76**	72	70
Cordele, Ga.	West	48	0	0	0	0	0	0	0	0
	Central	144	23	9	23	18	14	23	22	14
	Northeast	24	5	0	5	0[2]	--[2]	4	5	0
	All sources	216	16	6	18	10	6	19*	15	10
Dothan, Al.	Central	192	41	40	40	43	37	43	41	50
Baton Rouge, La.	Central	654	60	71	60	61	59	62	58	74*
Fordyce, Ar.	West	271	6	0	10	4	5	10	6	--[2]
	Central	261	18	0	16	21	18	17	17	24
	Northeast	95	5	0	5	0	0	4	4	0
	All sources	627	11	0	12	7*	7	13*	10	20

[1]) West = seed sources from west of the Mississippi River.
Northeast = seed sources from Virginia, Maryland, and Delaware.
Central = seed sources from areas between west and northeast regions.
[2]) No trees occurred in the category.
* Significant at 0.05 level.
** Significant at 0.01 level.

Table 4. — Fusiform rust infection in mLP[1]) monoterpene types vs. all other types.

Plantation	Region of seed source[2]	mLP trees		Other trees		χ^2	Percent infection	
		Infected	Not infected	Infected	Not infected		mLP trees	Other trees
		Number					Percent	
Olustee, Fl.	Central	1	9	30	152	0.29	10	16
Colquitt, Ga.	West	0	13	3	4	6.55*	0	43
	Central	5	4	177	51	2.37	56	78
	All sources	5	17	180	55	28.95**	23	77
Cordele, Ga.	West	0	28	0	20	--	0	0
	Central	2	13	29	100	1.25	13	22
	Northeast	0	0	1	23	--	--	--
	All sources	2	41	30	143	4.39*	5	17
Dothan, Al.	Central	8	15	71	98	.44	35	42
Baton Rouge, La.	Central	76	56	312	200	.49	58	61
Fordyce, Ar.	West	7	154	10	100	.61	4	9
	Central	2	3	43	213	1.85	40	17
	Northeast	0	2	4	89	.09	0	4
	All sources	9	159	57	402	6.51*	5	12

[1]) Trees having the combination low myrcene-high limonene-high β-phellandrene.
[2]) West = seed sources from west of the Mississippi River.
Northeast = seed sources from Virginia, Maryland, and Delaware.
Central = seed sources from areas between west and northeast regions.
* Significant at the 0.05 level.
** Significant at the 0.01 level.

sources from widely scattered portions of the species range. For these, the seed sources were grouped into three broad geographic regions based on the fact that trees from western sources (west of the Mississippi River) and from northwestern sources (Virginia, Maryland, and Delaware) tend to be relatively resistant to fusiform rust, while trees from sources between these regions (central region) tend to be relatively susceptible (Wells and Wakeley, 1966; Grigsby 1973). From this grouping it could be determined whether relationships occurring over the species range would within regions. Any relationships found within seed source regions would be more meaningful, from a practical standpoint, than relationships over the species range.

Within seed source regions, only one statistically significant difference was found *(Table 3).* In the Baton Rouge plantation there was 58% infection among trees with high β-phellandrene, compared to 74% infection among trees with low β-phellandrene. Several significant differences were found in the combined data for plantations with widespread seed sources. In these, infection was lower among trees with low myrcene and high limonene than in trees with high myrcene and low limonene. No significant differences were found between trees with high and low β-pinene content.

We also compared rust infection in trees with the combination low myrcene-high limonene-high β-phellandrene (mLP trees) vs. all other monoterpene types. Results were similar to those obtained when monoterpenes were considered individually. Within common seed source regions, only one significant difference was found—mLP trees from a western source planted at Colquitt had no infection, while 43% of other types were infected *(Table 4).* In the combined data for plantations having widely scattered seed sources, significant differences were found. The weighted average of infection for these plantations was 9% in mLP types and 29% in other types. Data from the six central seed source groups are perhaps the most meaningful, since infection rates were relatively high in them. Although differences were statistically significant

in only one of these, the groups tended to show similar patterns, with infection averaging 40% in mLP types and 46% in other types. Thus, the data suggest that infection might be slightly less in mLP types than in other monoterpene types, regardless of seed source, which substantiates the fact that western trees are predominantly mLP and relatively rust resistant.

Although trees from both the western and northeastern regions tend to be relatively rust resistant, monoterpene composition of trees from these regions differs greatly. For example, about 60% of the western trees were of the mLP type, wheras only about 2% of the northeastern trees were of this type. Some investigators believe that the physiological basis for resistance of northeastern trees is different from that of western trees.

If the northeastern trees are ignored, our geographic patterns of monoterpene composition show some similarities to the patterns of fusiform rust infection reported for a statewide test in Mississippi (WELLS and SWITZER, 1971), and for a rangewide test (GRIGSBY 1973). The Baton Rouge and Fordyce plantations which we sampled were from these two studies, respectively. However, the cited authors determined rust infection on many more trees than we did. Hence, we used their data to further examine relationships with monoterpene composition. Correlation and multiple regression analysis were run using the percentage of trees with high myrcene, limonene, and β-phellandrene in each seed source as independent variables. The dependent variable was the number of cankers per tree for the WELLS and SWITZER data and the percentage of infected trees in the GRIGSBY's data. The western seed sources alone showed no meaningful relationships, but this was expected because they had uniformly low infection *(Table 5)*. In the central sources, infection tended to increase with increasing percentage of trees with high myrcene and to decrease with increasing percentage of trees with high limonene and high β-phellandrene. When western and central sources were combined, the correlations were stronger. Variance accounted for by the multiple regression was only 14% in the WELLS and SWITZER data but was 67% in GRIGBY's data, the difference likely

Table 5. — Results of correlation and multiple regression analysis of fusiform rust infection and monoterpene composition.

Region of seed source	Number of seed sources	Correlation of degree of infection[1] vs. percentage of trees with high: Myrcene	Limonene	β-phellandrene	R^2 [2]
DATA FROM WELLS AND SWITZER (1971)					
Western	5	-0.22	0.97**	0.00[3]	1.00[4]
Central	107	.19	-.15	-.16	.07*
Western & central	112	.26**	-.23*	-.24**	.14**
DATA FROM GRIGSBY (1973)					
Western	11	-.18	-.09	.00[3]	.10
Central	21	.08	-.33	-.58**	.40**
Western & central	32	.58**	-.71**	-.74**	.67**

[1]) Number of cankers per tree in the Wells-Switzer data and percentage of trees infected in Grigsby's data.
[2]) Proportion of variance accounted for by the multiple regression.
[3]) These values are 0 because all trees in western sources had high β-phellandrene.
[4]) This value has a single degree of freedom.

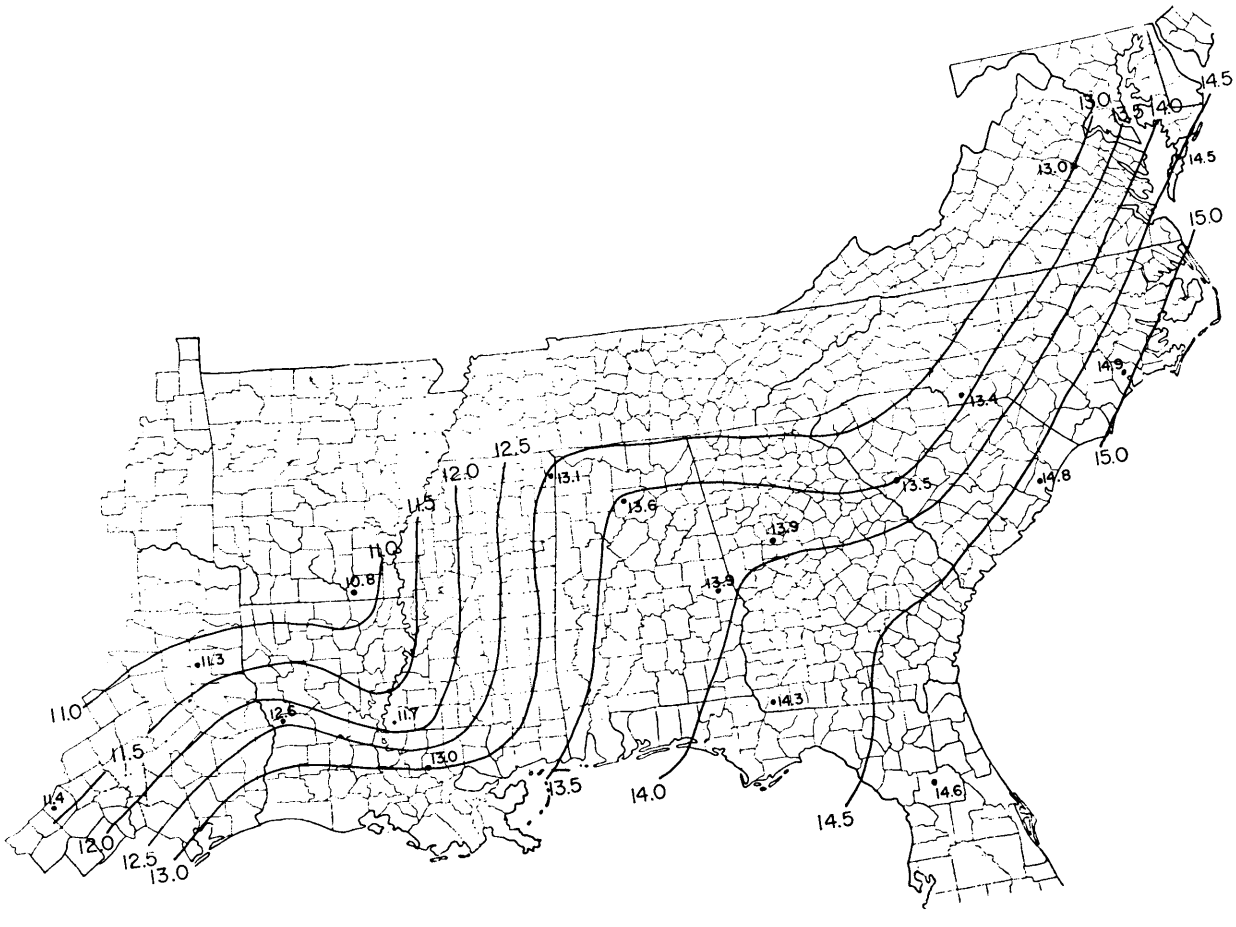

Figure 6. — Geographic variation in seed coat thickness (inches × 1000) in loblolly pine (Adapted from THORBJORNSEN 1961).

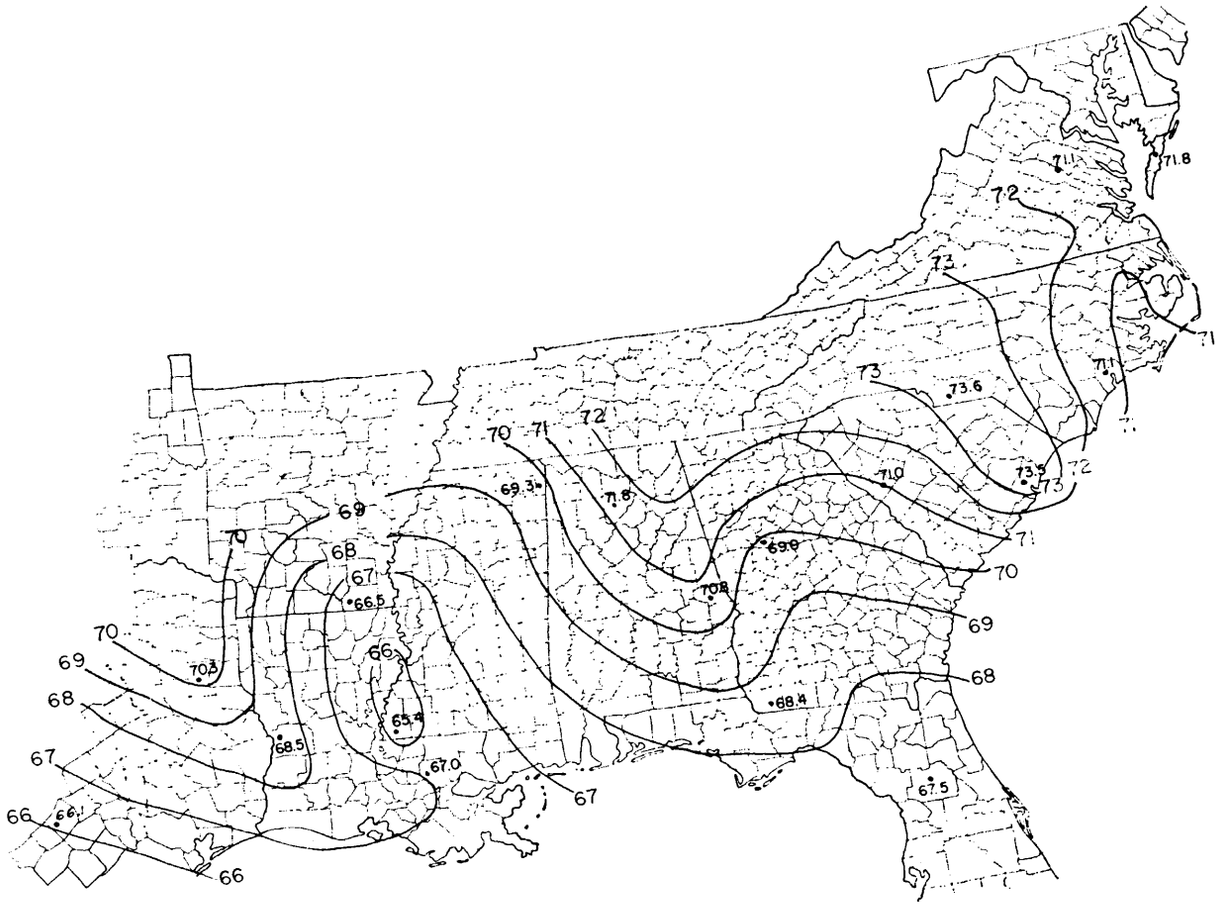

Figure 7. — Geographic variation in seed form (seed width as percentage of seed length) in loblolly pine (Adapted from THORBJORNSEN 1961).

being due to the more widespread sampling encompassed by the latter. The results as a whole were similar to those obtained with our rest-infection data presented earlier. From all of the analyses we conclude that there is a tendency, although weak, for less infection to occur among trees with low myrcene, high limonene, and high β-phellandrene.

HARE (1970) reported that loblolly pine trees from rust resistant geographic sources generally contained more β-pinene and limonene in stem xylem gum than did those from susceptible sources. ROCKWOOD (1973) found that low branch-cortex β-phellandrene was associated with rust resistance in a group of loblolly pine progeny in southwest Georgia. In slash pine an opposite trend was found, with high β-phellandrene associated with resistance (ROCKWOOD 1974), Obviously, more work is needed to clarify relationships of monoterpenes with fusiform rust resistance.

Relationship to Other Traits

Strong correlations were found between the percentage of trees with high limonene and some seed traits reported by THORBJORNSEN (1961). Limonene content data were interpolated from *Figure 3* for each of the 18 provenances in THORBJORNSEN's study. The correlation coefficient with seed coat thickness, for example, was -0.85, and with seed form (seed width as percentage of length), -0.77. The southeast-northwest gradients found for several monoterpenes here agree in general with THORBJORNSEN's findings on seed morphology.

Some of the patterns observed, especially that for β-phellandrene, also closely resemble some allozyme patterns found by FLORENCE and RINK (1979).

Discussion und Conclusions

Considerable variation in cortical monoterpene composition was found throughout the range of loblolly pine. Patterns of variation differed among the various constituents. The most prominent feature was a strong east to west clinal trend for limonene, myrcene, and α-pinene. Also, there was a definite disjunction for content of β-phellandrene, with all trees west of the Mississippi River high in β-phellandrene. A modest increasing trend from south to north in the eastern portion of the species range also occurred for β-phellandrene. Some patterns were patchy, probably random, especially for β-pinene and β-phellandrene in the east. Larger samples (say about 50 trees per source) would likely have made patterns clearer and are recommended for future work.

There was some indication that trees with a combination of low myrcene, high limonene, and high β-phellandrene are less frequently infected with fusiform rust than trees of other types. No cause and effect relationship is implied, and the relationship, if indeed real, is not strong enough for use in selecting rust-resistant trees. The results are merely suggestive of further study, which should be done preferably with trees of known genetic resistance.

Monoterpene composition patterns could be useful for identifying the seed origin of plantations and also for verifying origin of certified seed from seed orchards (SQUILLACE 1977; SQUILLACE *et al.*, 1980 a). Most portions of the species range are unique for monoterpene composition, but some areas, especially in the eastern portion of the range, are not readily distinguishable. Use of supplementary traits would be desirable. For such use, patterns for two seed traits, assuming that they are genetically controlled to an appreciable degree, are shown in *Figures 6* and *7* (adapted from THORBJORNSEN 1961).

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A complementary gene inheritance of a needle morphology of outward hooking in sugi, Crypto meria japonica D. Don

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Summary

It is concluded that outward hooking needles of a sugi variant found in a plantation is produced by an interaction of two dominant complementary genes. To elucidate the mode of this inheritance, crossing experiments were made. For crossing materials, F_1-normal individuals from outcrossed progenies (normal × variant) which segregated the variant and the normal in a ratio of 1:3, were used. Segregation ratios in F_2 families were examined after selfing, backcrossing and full-sib crossing.

From the effect of complementary genes that are heterozygous in the variant parent, no segregation of the variant was seen after the selfing of F_1-normal individuals. For back-crosses between F_1-normal individuals and variant parent, segregation of the variant was observed in all cross combinations. Segregation ratios of the variant and the normal were 1:3 and 3:5. After full-sib cross combinations of F_1-normal individuals that produced the variant in backcrossing, it was concluded that the genotype of one partner was Aabb and the other was aaBb. In the second of the full-sib crosses in 1978, individuals identified by their genotypes were used and the experimental results showed a good fit with expected segregation ratios. In this way, it was possible to determine the genotypes of the females (F_1-normal individuals) used as partners.

From these cross experiments, it was proved that two dominant genes, A and B, located on two different pairs of chromosomes, interact to produce the needle morphology with outward hooking and each of them alone result in normal needles.

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Zusammenfassung

Die nach außen gekrümmten Nadeln einer in einer Pflanzung gefundenen Variante von *Cryptomeria japonica* D. DON gehen auf 2 dominante Komplementärgene zurück. Um den Vererbungsmodus zu klären, wurden Kreuzungsversuche unternommen. Als Kreuzungsmaterial wurden normale F_1-Individuen benutzt, die aus Fremdungen (normal × Variante) hervorgegangen waren und im Verhältnis 1:3 Variante zu normalen Individuen aufspalteten. Die Aufspaltung in den F_2-Familien wurde nach Selbstung, Rückkreuzung und Vollgeschwisterkreuzung untersucht. Durch die komplementäre Wirkung der Gene, die in den Elternvarianten heterozygot waren, wurde keine Aufspaltung der Varianten nach dem Selbsten der normalen F_1-Individuen sichtbar. Bei den Rückkreuzungen zwischen normalen F_1-Individuen und den Elternvarianten spalteten die Varianten in allen Kreuzungskombinationen auf. Die Spaltungsverhältnisse von Varianten zu Normalen waren 1:3 und 3:5. Nach den Vollgeschwister-Kreuzungskombinationen normaler F_1-Individuen, die nach Rückkreuzung die Variante ergaben, wurde festgestellt, daß der Genotyp des einen Partners Aabb und der des anderen aaBb war. In der 2. Serie der Vollgeschwisterkreuzungen 1978 wurden durch ihren Genotyp identifizierte Individuen benutzt, und die Ergebnisse des Experimentes zeigten eine gute Übereinstimmung mit den erwarteten Spaltungsverhältnissen. Auf diese Weise war es möglich, die Genotypen der als Partner benutzten weiblichen Individuen (normale F_1-Individuen) zu bestimmen.

Aus diesen Kreuzungsexperimenten ging hervor, daß 2 dominante Gene A und B, die auf verschiedenen Chromo-