EVIDENCES OF THE INHERITANCE OF TURPENTINE COMPOSITION IN SLASH PINE

by A. **E. Squillace and Gordon S. Fisher**[1]

Within-species variation in turpentine composition has promise of great utility in pine genetics. Most of the early work on turpentine composition dealt with species differences, and the utility of such variation in the taxonomy of pines is well known (Mirov 1961). With the development of gas chromatographic techniques, intensive study of individual tree differences has become possible Bannister *et al.* 1959; Williams and Bannister 1962); and this type of variation has been shown to occur in several pine species (Bannister *et al.* 1962; Blight and McDonald 1964; Smith, 1964a).

This paper shows that individual tree differences and racial variation occur in slash pine *(Pinus elliottii* Engelm.) and that differences in the content of some constituents are strongly inherited. Although further exploratory work is needed, the results reveal opportunities to breed for specific composition, to capitalize on geographic variation in commercial gum operations, and to develop a useful tool in pine genetics research.

Methods

Oleoresin samples were taken from 174 trees as outlined below.

1. Thirty-one 16-year-old rooted cuttings. There were 15 clones, each containing from 1 to 5 ramets. Most of the ortets had been selected for either high or average gum yielding ability.

2. Ten grafted trees. These were members of two of the clones noted above. The grafts had been made on plantation saplings 7 years prior to sampling. There were 5 grafted trees per clone.

3. Eleven selections. These were mature trees growing in natural stands. Most had been selected for high gum yielding ability.

4. Eighty progeny trees produced from matings among 9 of the ortets noted in "1" above. They were included in 9 crosses, one self, and 3 wind-pollinations. There were from 4 to 14 trees per progeny. About half of the progenies were 16 years old, while the remainder, in a separate plantation, were 11 years old.

5. Forty-two trees in 2 seed source tests. There were 15 seed sources, with from 1 to 5 trees sampled per source. Most trees were 7 years old at time of sampling, while a few were 9 years old.

[1] *Principal Plant Geneticist, Southeastern Forest Experiment Station, Forest Service, USDA, Olustee, Florida, and Research Chemist, Naval Stores Laboratory, Agricultural Research Service, USDA, Olustee, Florida, respectively.*

All trees sampled were growing within 15 miles of Olustee, Florida. Most oleoresin samples were taken in the fall of 1961, but some were taken at later dates.

Two kinds of oleoresin (gum) samples were taken, "cortex" and "stem." Cortex samples were obtained by excising branch terminals at about ½ inch from the tip. Samples were usually taken from branches in the lower crown. Usually, within a few minutes after cutting, a drop of gum exuded at the cut surface of the excised bud. This was removed with a spatula and inserted into a small vial. Vials were then sealed and stored in a refrigerator until analyzed. Samples collected in this manner consisted mostly, if not entirely, of gum exuding from resin ducts in cortical tissue.

Most stem gum samples were obtained by microchipping (Ostrom and True 1946) of the stem at about 2 feet above ground level. Storage was handled in the same manner as was done for cortex gum. Samples collected in this manner consisted mostly of gum exuding from resin ducts in secondary xylem tissues.

Chemical analyses were made by gas-liquid chromatography, and this was usually accomplished within 2 or 3 weeks after collection. Typical operating conditions were as follows: column, 10' x $^{1}/_{8}$", support, Chromosorb-W 60-80 mesh; substrate, carbowax-20M; loading, 30 percent; column temperature, 120°C.; helium inlet pressure, 45 p.s.i.; sample size, 0.1 mg. whole gum dissolved in 3 volumes of acetone; injection temperature, 275°C. With this substrate, the column temperature is critical for optimum separation of myrcene from other components. Peak areas were determined with a Disc integrator and the composition was calculated on the basis of the total area through ß-phellandrene. Only trace amounts of terpenes emerging after ß-phellandrene were encountered. In standardization runs all the major terpenes gave approximately the same peak area per mg.

Since the data encompassed various field plots, trees of different ages, and several sampling dates, highly efficient statistical techniques were not possible. Simple analyses of variance and regression techniques were employed to the extent possible, but the results of these must necessarily be considered as approximations. Wherever required, estimates of ortet composition were obtained by averaging the clonal data.

In presenting the results, percentages are shown for major constituents only (α-pinene, ß-pinene, myrcene, and ß-phellandrene). Percentages of minor constituents, including camphene, limonene, and unidentified compounds, are lumped and shown as "other."

Instrumental Reproducibility

Instrumental reproducibility was assessed by making 2 or 4 repeated injections of the same sample of cortex gum from each of 6 trees. Average composition and the average (pooled) standard deviation among determinations for the 6 trees are shown below.

Constituent	*Average composition (percent)*	*Average standard deviation (percent)*
α-pinene	18.8	.51
β-pinene	40.3	1.00
Myrcene	15.8	.70
β-phellandrene	22.0	.64
Other	3.1	—

The standard deviations were slightly higher than the average, 0.34 percent, reported by Bannister *et al. (* 1962) for major constituents in Monterey pine *(P. radiata D.* Don). But reproducibility was considered sufficiently accurate to discontinue replicate determinations.

Within-Tree Variation

Moderate correlations were found between the percentages of α-pinene, ß-pinene, and ß-phellandrenein cortex and stem gum (table 1). However, turpentines in gum from these two sources differed markedly in several respects.

1. Cortex gum usually contained less α-pinene and more ß-pinene than stem gum.

2. Myrcene (and rarely, limonene) was present in appreciable quantities in the cortex gum of some trees but was very low in the stem gum of all trees sampled.

3. Trees with appreciable quantities of ß-phellandrene in their stem gum invariably contained appreciable quantities in their cortex gum. However, the reverse was not always true. Some trees had high amounts of ß-phellandrene in their cortex gum and low amounts in their stem gum. Usually the percentage of this constituent was higher in cortex gum than in stem gum.

Table 1.--The relation between cortex and stem gum extracted from 69 trees

Constituent	Mean composition		Correlation coefficient
	Cortex gum	Stem gum	
	Percent	Percent	
α-pinene	29.1	57.3	.40**
ß-pinene	48.1	30.6	.47**
Myrcene	6.4	1.6	(1/)
ß-phellandrene	14.1	9.0	.56**
Other	2.3	2.2	(1/)

1/ Not computed because of the low amounts of these constituents in one or both sources.
** Significant at the 1-percent level.

Gum in plant parts other than stem wood and branch terminals was not studied. In respect to differences in gum composition within tissues, Blight and McDonald (1964) and Smith (1964a) found only minor differences in samples of stem gum taken at various circumferential and vertical positions.

Repeated samples were taken on some trees on different dates, spanning a period of 3 years. Pooled standard deviations, representing differences between sampling dates, varied from about 2 to 9 percent for various constituents (table 2). ß-pinene and ß-phellandrene, both in cortex gum, showed the greatest variation. No clear seasonal pattern could be shown.

The causes of the differences in composition of gum collected at various dates are unknown. Blight and MacDonald (1964) and Smith (1964a) reported low seasonal variation for several pine species. In another publication, Smith (1964b) suggested that age effects on stemwood gum in ponderosa pine *(P. ponderosa* Laws.) are minor. Further study of seasonal and age effects is needed for slash pine.

Although the differences noted above seem large, they were relatively small in comparison to differences between trees.

Individual Tree Variation

Large differences were found among trees in the composition of their turpentine in both cortex and stem gum, and evidence was obtained that much of the variation is genetic. Space prevents showing all of the data, but the results for 4 parent trees and 2 families sampled are given in table 3.

Note that parent trees G-1 and G-2 are unique in that they have a relatively low amount of α-pinene in comparison to ß-pinene and relatively large amounts of myrcene and ß-phellandrene in their cortex gum. Six out of 8 of the progenies of the mating between these two parents closely resembled their parents. Two other parent trees, and G-3 and G-4, are unique in having relatively high α-pinene in comparison to ß-pinene and containing only very small amounts of myrcene and ß-phellandrene. Eight of the 9 progenies of G-3 x G-4 resembled their parents. Although the possibility contamination cannot be excluded, the "maverick" individuals in the two families are probably due to control by few genes and heterozygosity in the parents. Mode of inheritance will be discussed later.

Table 2.--Variation in turpentine composition in samples taken from the same tree on different dates

(Percent)

Item	α-pinene	β-pinene	Myrcene	β-phellandrene	Other
		CORTEX GUM[1]			
Means	25.8	41.5	8.1	22.7	1.9
Standard deviations[2]	3.4	6.3	3.9	9.4	--
		STEM GUM[3]			
Means	62.8	26.2	2.7	6.6	1.7
Standard deviations[2]	4.4	2.2	2.8	3.7	--

1/ Based upon two samplings from each of 18 trees.

2/ Pooled "within-tree." The values are measures of the differences between percentages obtained from samples on the same tree but on different dates.

3/ Based upon two samplings from each of 9 trees.

Table 3.--A portion of the basic data, typifying the variation and inheritance of turpentine composition

(Percent)

Mating	Tree No.	Cortex gum α-pinene	Cortex gum β-pinene	Cortex gum Myrcene	Cortex gum β-phellandrene	Cortex gum Other	Stem gum[1] α-pinene	Stem gum[1] β-pinene	Stem gum[1] Myrcene	Stem gum[1] β-phellandrene	Stem gum[1] Other
					PARENTS						
	G-1	12	36	17	32	3	44	30	1	23	2
	G-2	16	52	17	13	2	44	40	1	12	3
	G-3	40	56	1	2	1	80	17	1	1	1
	G-4	38	59	1	1	1	62	35	1	1	1
					PROGENIES						
G-1 x G-2	1-5-5	13	48	19	18	2	60	25	1	10	4
	1-7-5	23	51	2	24	0	47	39	1	10	3
	1-10-6	10	27	32	28	3	47	29	1	20	3
	2-6-2	36	38	2	20	4	68	17	1	12	2
	3-6-5	13	32	20	31	4	70	16	1	11	2
	5-3-4	8	20	41	29	2	57	19	1	19	4
	7-10-7	12	29	20	36	3	58	15	1	22	4
	8-9-2	21	36	22	18	3	57	20	2	19	2
G-3 x G-4	5-10-16	49	49	1	1	0	51	49	0	0	0
	10-1-5	11	45	22	20	2	54	24	1	18	3
	10-2-5	42	54	0	1	3	56	41	1	1	1
	10-3-5	49	48	1	1	1	84	15	0	0	1
	10-5-5	40	56	1	1	2	64	34	1	1	0
	10-6-5	51	40	2	1	6					
	10-7-5	38	58	1	1	2					
	10-8-5	53	40	2	1	4					
	10-9-5	42	50	1	2	5	65	28	1	1	5

1/ Trees 10-6-5, 10-7-5, and 10-8-5 were not sampled for stem gum.

Similar variation and inheritance can be seen for β-phellandrene in stem gum. In stem gum, however, myrcene is very low in all trees; also, inheritance is obscure for α-pinene and ß-pinene in these data.

Analyses of variance were run on the progeny data, excluding the selfed progeny. Intraclass correlations, which show the percentage of the total variance that is associated with families, are shown in table 4. Heritability estimates were not made because of the nature of the data, but most constituents seem to be strongly inherited.

A part of the clonal data is shown in table 5. Note the similarity of ramets within clones. The

Table 4.-- Intraclass correlations obtained from analyses of variance of progeny and clonal data

Source of gum	α-pinene	β-pinene	Myrcene	β-phellandrene
		PROGENIES		
Cortex	.37**	.32**	.37**	.53**
Stem	.19*	.16*	--	.58**
		CLONES		
Cortex	.95**	.96**	.95**	.95**
Stem	.88**	.79**	--	.95*

* Significant at the 5-percent level.
** Significant at the 1-percent level.

consistency within clones is especially remarkable in view of the fact that some of the ramets were rooted cuttings growing at one location while others were grafted trees growing in another location about 10 miles distant. In the grafted trees, the cortex gum samples were taken from branches above the graft unions. Neither the differences in location nor type of propagation had any appreciable effect upon turpentine composition.

Intraclass correlations for clonal data are shown in table 4. Once again, strong genetic variation is indicated. Note that intraclass correlations for α-pinene and ß-pinene were stronger in cortex gum than in stem gum. This was also true for progeny data.

Table 5.-- A portion of the clonal data, typifying the variation and inheritance of turpentine composition

(Percent)

Clone	Ramet 3/	Cortex gum 1/					Stem gum 2/				
		α-pinene	β-pinene	Myrcene	β-phellandrene	Other	α-pinene	β-pinene	Myrcene	β-phellandrene	Other
G-1	1-3	16	36	18	28	2					
	2-3	13	34	22	28	3					
	4-3						46	29	1	21	3
	5-3						41	31	1	25	2
	6-3	13	32	23	29	3					
	a	10	39	15	33	3					
	b	10	40	14	33	3					
	c	11	37	14	36	2					
	d	11	37	15	36	1					
	e	10	37	15	36	2					
G-4	2-7	38	57	2	2	1	60	37	1	1	1
	3-7	32	63	1	2	2	64	33	1	1	1
	6-7	33	62	2	2	1					
	a	43	55	1	1	0					
	b	39	58	1	1	1					
	c	37	61	1	1	0					
	d	39	59	1	1	0					
	e	39	57	1	1	2					

1/ Ramets 4-3 and 5-3 of clone G-1 were not sampled.
2/ Only the 4 trees indicated were sampled.
3/ Ramets labelled "a" through "e" were grafted trees in a plantation near Lulu, Florida. All other trees were rooted cuttings growing near Olustee, Florida.

Table 6,-- Results of parent-progeny regression analyses

Constituent	Cortex gum (Basis, 12 families)		Stem gum (Basis, 10 families)	
	Regression coefficient	Coefficient of determination	Regression coefficient	Coefficient of determination
α-pinene	1.04**	0.83**	0.23	0.11
β-pinene	.35	.12	-.40	.10
Myrcene	.92**	.68**	--	--
β-phellandrene	.63*	.34*	.46	.35

* Significant at the 5-percent level.
** Significant at the 1-percent level.

Parent-progeny regressions were run as a further check on inheritance of turpentine composition. Progeny means were used as the dependent variable. For crosses, the average of the female and male parent values was used as the independent variable. For wind-pollinated progenies the value for the female parent was used as the independent variable.

Regression coefficients for all constituents of cortex gum, excepting ß-pinene, were strong (table 6). Those for ß-pinene in cortex gum and all constituents of stem gum were weak (negative in the case of β-pinene).

Mode of Inheritance

In order to obtain clues to the mode of inheritance, frequency distributions were compiled for each major constituent of turpentine. In compiling these, we omitted the seed source test data for Citrus, Volusia, and Polk Counties because many trees of these southern sources differed appreciably from the bulk of the data in the northern portion of the species range, a possible influence of south Florida slash pine (P. *elliottii* var. *densa* Little & Dorman)

The distributions for α-pinene in both cortex and stem gum and for ß-pinene in stem gum are quite normal, suggesting multigenic control for these compounds (figs. 1 and 2). The distribution for β-pinene in cortex gum is skewed to the left but is continuous and has a single mode. It is possible that the quantity of ß-pinene in cortex gum is controlled by few genes but that non-genetic effects caused the continuous pattern.

The distributions for myrcene in cortex gum and β-phellandrene in both cortex and stem gum are definitely abnormal, being skewed and having a tendency toward bimodality. Approximately 3/4 of the trees contained low amounts (0-4 percent) of myrcene, while about 1/4contained high amounts (10 percent or more) of this constituent. Control by few genes is suggested. The bimodality for β-phellandrene in both cortex and stem gum is not as clear as for myrcene, but nevertheless the distributions suggest control by few genes.

On the basis of these preliminary results, hypothetical models for the inheritance of myrcene in cortex gum and ß-phellandrene in cortex and stem gum were postulated. However, the limited data available were not adequate for reliable test of the models. More progenies, especially sells and other inbreds, are being sampled to test the hypotheses on mode of inheritance suggested for all major constituents. It is of interest to note that Forde (1964) postulated that the major difference between turpentines of knobcone pine (*P. attenuata* Lemm.) and Monterey pine is controlled by a single gene.

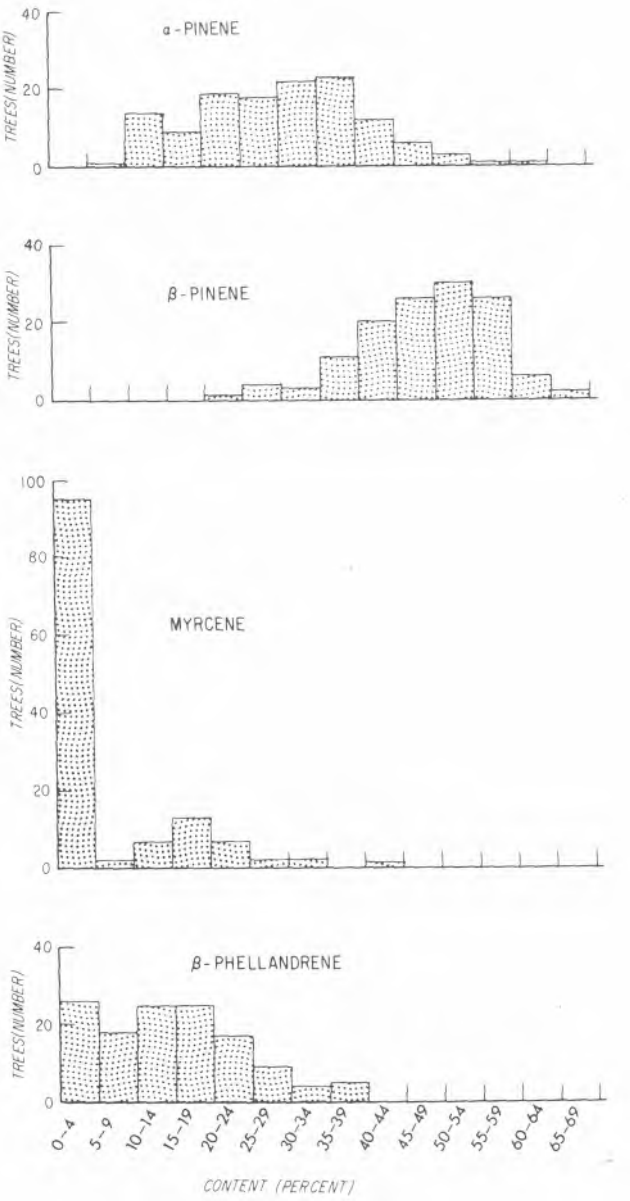

FIGURE 1. — Frequency distributions for the major constituents of turpentine in cortex gum. Basis, 129 trees.

Evidences of Racial Variation

In order to study racial variation, all trees of known geographic origin other than progenies were utilized (the latter could not be used because most had parents of different origins). For cortex gum, 60 trees in 12 sources, with from 3 to 16 trees per source, were available for analysis. For stem gum, there were 26 trees in 5 sources, with from 5 to 6 trees per source. Analyses of variance were run, using a simple "between- and within source" model.

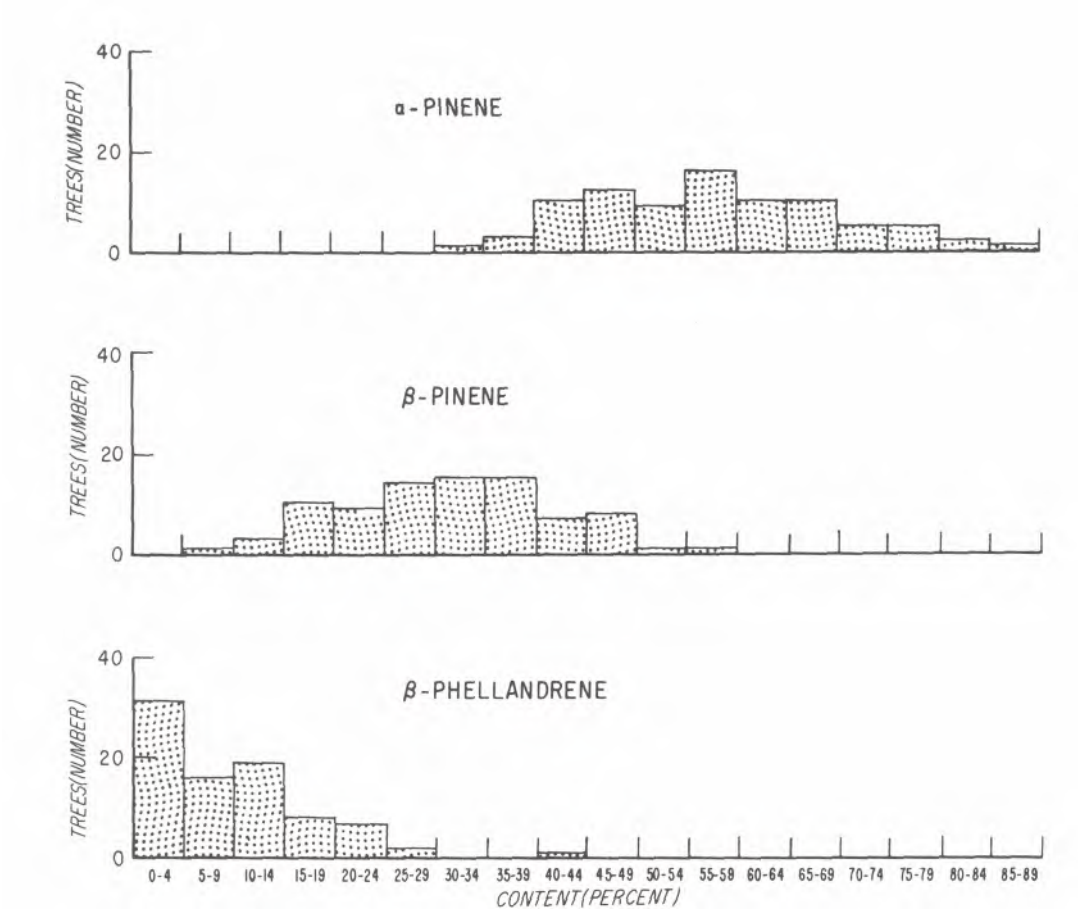

FIGURE 2. — Frequency distributions for the major constituents of turpentine in stem gum. Basis, 84 trees.

Seed source effects were significant or highly significant for all constituents except α-pinene in both cortex and stem gum and for ß-phellandrene in stem gum. ß-pinene in cortex gum showed a clear pattern — content of this constituent was low in south Florida and increased northward, reaching a high in south Georgia, and then decreased near the northern limits of the species range (fig. 3). This pattern is similar to the total height pattern reported by Squillace and Kraus (1959) and also to patterns for several traits reported by Squillace (1964). ß-phellandrene in cortex gum also showed a pattern, this constituent being low in the north and high in the south (fig. 4). Patterns for other constituents were either random or not clear. Because of the high variation among trees within sources and the few trees per source, these results are considered mainly as suggestive of further study.

Racial differences in turpentine composition have previously been reported for ponderosa pine (Mirov 1961), Monterey pine (Bannister et al. 1962) and bishop pine *(P. muricata D.* Don) (Forde and Blight 1964).

Discussion and Conclusions

This exploratory study has given us an insight into the variation and inheritance of turpentine composition in cortex and stem gum of slash pine. The data showed that (1) composition of turpentine, in both sources of oleoresin, varies greatly among trees within stands and also among trees of different geographic sources; (2) the content of most constituents is strongly inherited; and (3) some constituents seem to be controlled mainly by a few genes, while others show polygenic heritance.

The results suggest that we could easily breed for high or low amounts of some constituents, such as myrcene, α-pinene, and ß-pinene in cortex gum and ß-phellandrene in both cortex and stem gum. Unfortunately, the constituent presently considered to be most valuable, ß-pinene in stem gum, seems to have the weakest genetic control. The racial differences suggest that we can control turpentine composition on a practical basis by concentration of gum extraction in certain areas or by keeping gum separate by geographic areas.

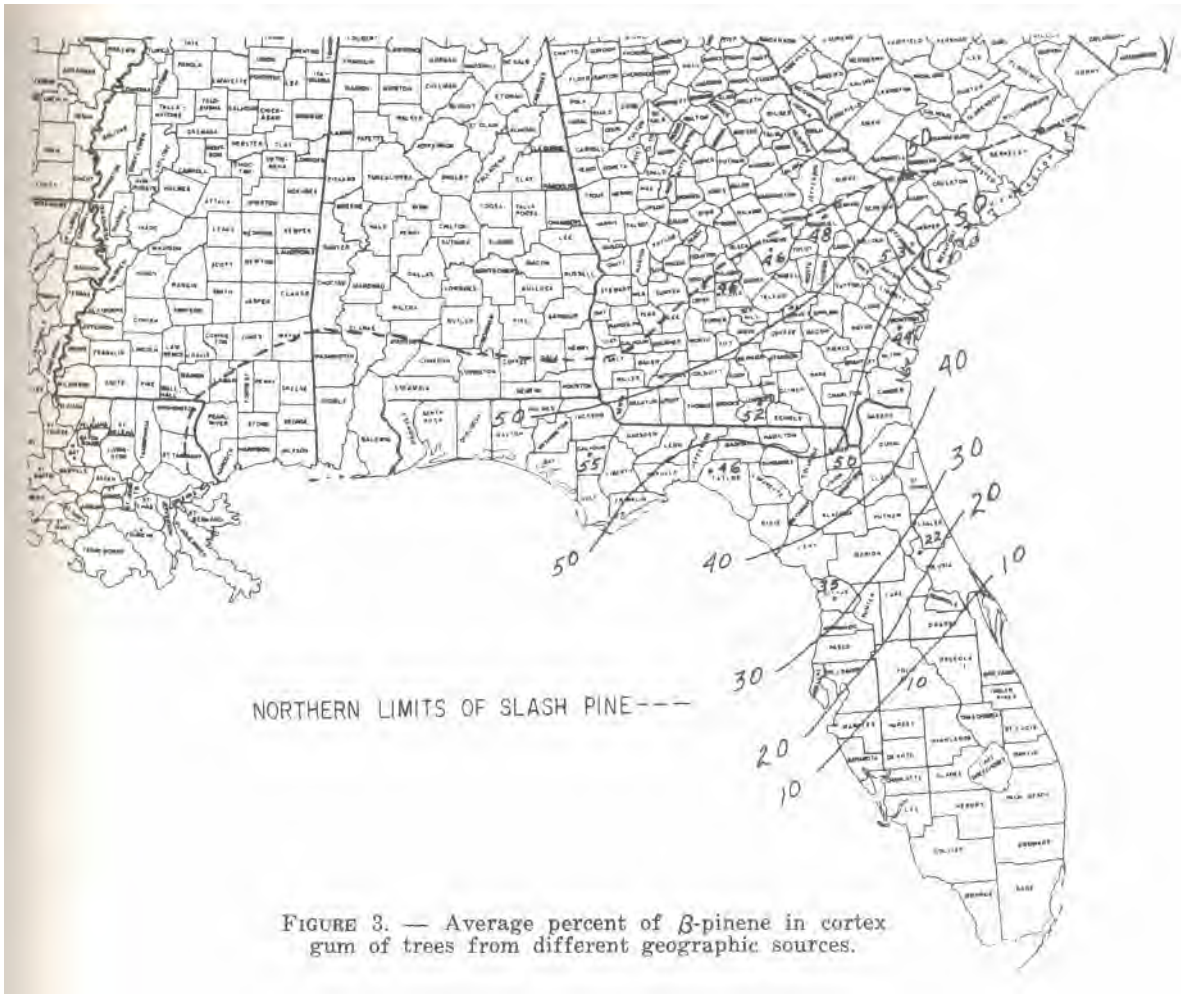

FIGURE 3. — Average percent of β-pinene in cortex gum of trees from different geographic sources.

Further study is needed, however, to verify the variation patterns and to determine more accurately the mode of inheritance of each constituent. Such knowledge will give us a better basis for judging the breeding potentials for each constituent. In addition, with precise knowledge of the mode of inheritance we may have a very useful tool for identifying relatives, hybrids, etc. The possibility of associations of differences in composition of both cortex gum and stem gum with insect resistance should be investigated.

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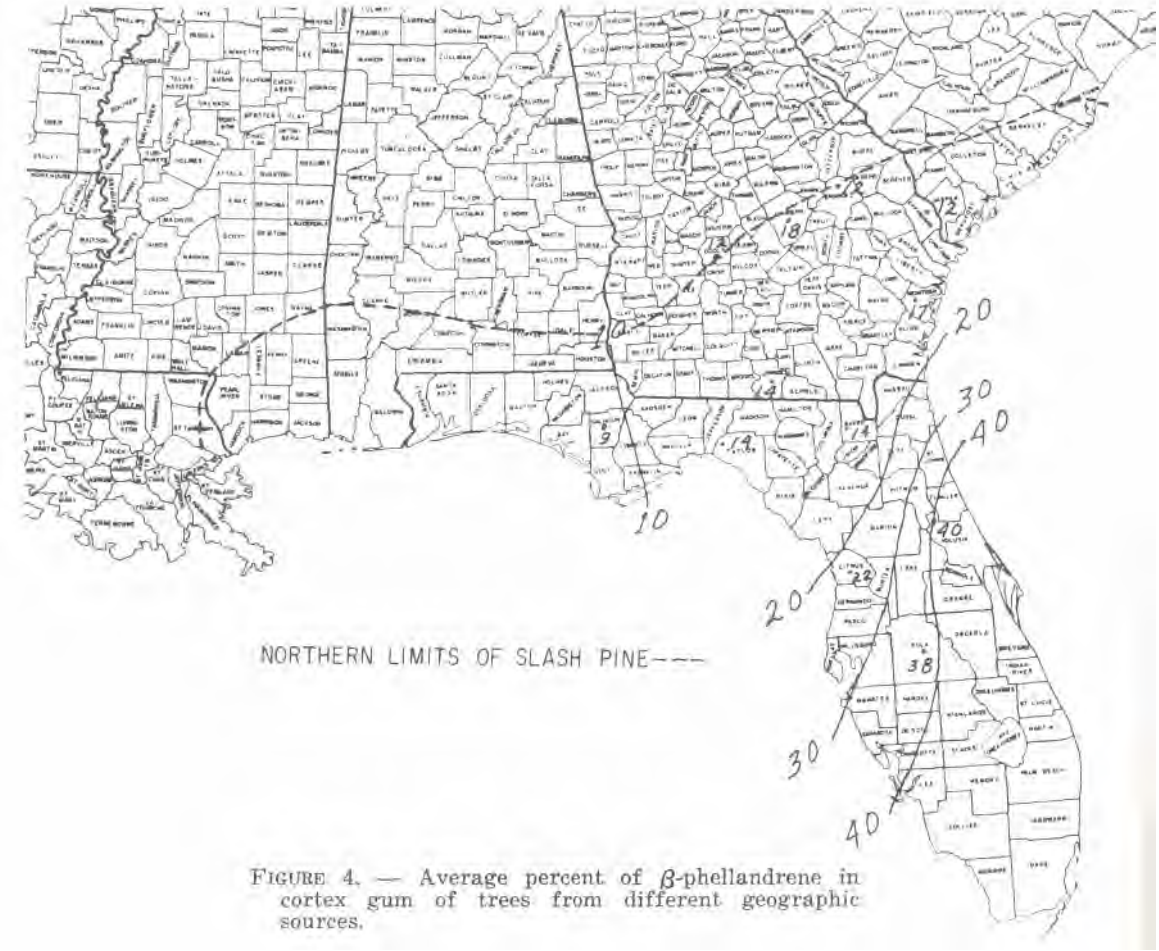

FIGURE 4. — Average percent of β-phellandrene in cortex gum of trees from different geographic sources.