Geographic variation in shortleaf pine (*Pinus echinata* Mill.) - Cortical monoterpenes

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Abstract - Cortical monoterpenes were assayed in bud tissue from 16 Southwide Southern Pine Seed Source Study (SSPSS) sources and from 6 seed orchard sources from across the natural range of the species, to examine geographic variation in shortleaf pine. Spruce pine and pond pine were also sampled. The results show geographic differences in all of the major terpenes. There was no north-south trend in any of the terpenes, but there was clinal variation in alpha pinene from west to east. One source, from New Jersey (SSPSSS) had very low alpha pinene and did not fit the trend, possibly because of hybridization with pitch pine. Some of the western sources had high limonene content, probably as a result of hybridization with loblolly pine, which has high limonene in western populations. Spruce pine had terpene levels similar to shortleaf pine, while pond pine had low alpha pinene and much higher limonene compared to shortleaf pine.

Keywords: Shortleaf pine, *Pinus echinata* Mill., loblolly pine, *Pinus taeda* L., pond pine, *Pinus serotina* Michx., spruce pine, *Pinus glabra* Walt., terpenes, geographic variation, hybridization.

INTRODUCTION

Geographic variation in loblolly pine has been very well documented, but very little has been published on geographic variation in shortleaf pine. Shortleaf pine is not widely planted, because in many situations loblolly pine grows much faster. There is increased interest in shortleaf pine as restoration of native species is becoming more popular. Because shortleaf has the widest north-south distribution of any of the southern pines, it may have the greatest variation among provenances.

Judging from the 25-year analysis of the Southwide Southern Pine Seed Source Study (SSPSSS) (Schmidtling 1995), which may be the only published study on growth of geographic races, variation in shortleaf pine follows a pattern common to many forest tree species. Southern sources are generally less cold-hardy but faster growing than northern sources. This north-south variation appears to be clinal.

In loblolly, populations west of the Mississippi River valley generally are slower growing, survive better and are more resistant to fusiform rust then those east of the Mississippi. It has been hypothesized that the pineless expanse of the Mississippi River Valley serves as a barrier to gene flow among the two populations allowing them to evolve separately. East-west variation in growth of shortleaf pine does not appear to be as extensive as that found in loblolly pine, if it exists at all (Schmidtling 1995). In spite of this, in many tree improvement programs including that of the Southern Region (R-8), the Mississippi river is used as the dividing line to separate western from

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eastern populations in determining planting zones.

An extensive sampling of xylem monoterpenes of shortleaf and loblolly trees *in situ* by Coyne and Keith (1972) showed clinal variation in the concentration of alpha pinene from east to west for both species. They also found a tendency for higher limonene contents in the western sources. Terpenes are probably related to insect resistance, qualitatively as well as quantitatively.

Cortical monoterpenes are more useful than xylem monoterpenes for examining population structure because there is generally more genetic variation in the minor constituents and they are affected very little by environment. There is also evidence for a simple inheritance pattern for the cortical monoterpenes (Squillace *et al* 1980), and they have the additional advantage of being highly variable among populations (Squillace and Wells 1981), at least in loblolly pine. Cortical monoterpenes have not been previously examined in shortleaf pine.

The present study will utilize cortical monoterpenes to examine geographic variation in shortleaf pine, and to explore phylogenetic relationships with other species.

MATERIALS

The study primarily utilizes material from a demonstration planting of the SSPSSS shortleaf phase on the Harrison Experimental Forest (HEF) in south Mississippi. All sources used for the 3 different series of the SSPSSS shortleaf phase are included in this planting (Fig. 1). Material from the US Forest Services Southern Region (R-8) tree improvement program located in the HEF clone bank were included, as well as arboretum material (Table 1).

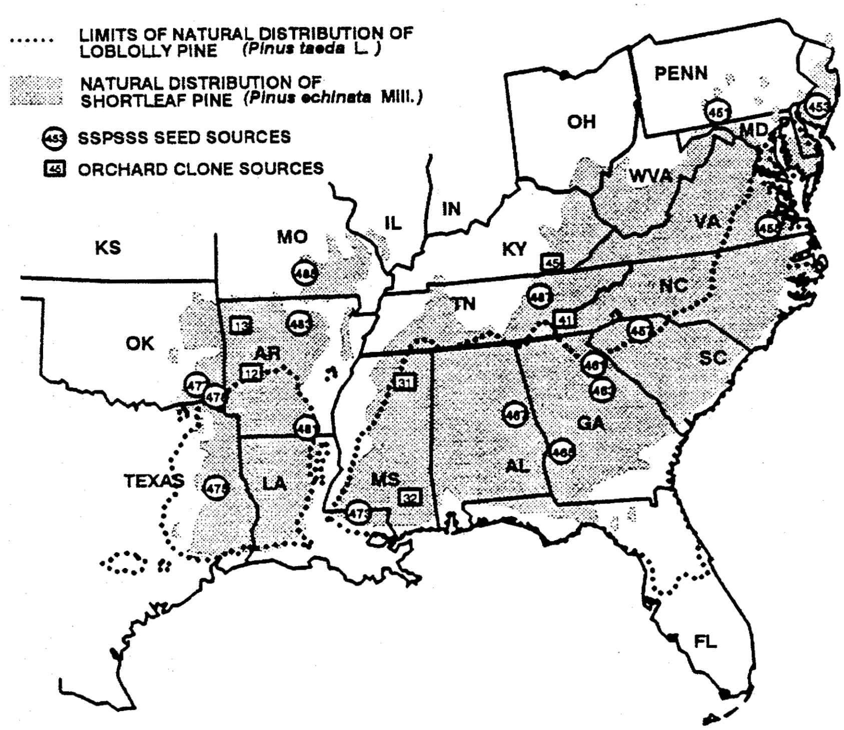

Figure 1. Map of the southeastern United States showing the natural distribution of shortleaf pine (*Pinus echinata* Mill.) and the location of the sampled populations. Also shown are the limits of

the natural distribution of loblolly pine *(Pinus taeda* L.).

Table 1. Original provenance of the shortleaf pine trees used in the study.

I.D	State	County	No. Trees	Longitude	Latitude
		Seed Orchard Clones			
12	AR	Scott	17	94.0	34.5
13	AR	Pope, Franklin, Johnson	11	94.1	35.8
31	MS	Chickasaw, Benton	17	88.8	34.4
32	MS	Stone, George, Perry	9	88.7	30.9
41	TN	Monroe	12	83.8	35.5
45	NC	Cherokee	10	83.9	36.9
	Southwide Southern Pine Seed Source Study				
451	PA	Franklin	9	77.7	39.8
453	NJ	Burlington	10	74.6	39.7
455	VA	Southampton	10	77.0	36.9
457	SC	Union	10	81.7	34.7
461	GA	Clarke	10	83.4	34.0
463	GA	Putnam	10	83.3	33.3
465	GA	Webster	10	84.6	32.1
467	AL	Talapoosa	10	85.8	32.9
473	LA	St Helena	12	90.8	30.8
475	TX	Cherokee	10	94.7	31.9
481	AR	Ashley	10	91.8	33.1
483	AR	Stone	10	92.2	35.9
485	MO	Dent	10	91.3	37.6
487	TN	Anderson	10	84.2	36.1
477a	OK	Pushmataha	10	95.6	34.2
477b	OK	McCurtain	10	94.8	33.9
		Other Species			
P. glabra		Harrison, MS	15	89.5	30.0
P. serotina		NC	7	80.0	35.1

METHODS

The distal 1/8" of 2 buds per sample tree were excised with a razor blade and placed in a vial containing ethel The razor blade was rinsed in ether between each sample tree. Vials were labeled, sealed and stored in a freezer until analysis.

In the laboratory, extracts from the bud tips were assayed for terpenes by gas-liquid chromatography, using a 6.1 m , 4.76 mm, 60/80 mesh chromosorb W column packed with 20%

carbowax 20 M.

RESULTS

The results show geographic differences in nearly all the terpenes (Appendix). The major constituents were alpha pinene (averaging 33 percent), and beta phellandrine (averaging 38 percent). The monoterpene composition of spruce pine was similar to shortleaf pine. The composition of pond pine was quite different. Pond pine had very high limonene content (48 percent), and very low beta phellandrine content (Appendix 2). The terpene composition of shortleaf pine cortical oleoresin is comparable to those found by Squillace and Wells (1981) for loblolly pine, except that loblolly pine has higher limonene.

There were east-west trends in the concentration of several constituents. The correlation of alpha pinene content with longitude was R = 0.85 (Fig. 2). Alpha pinene content decreased from east to west. The east-west variation appears to be linear, with no discontinuity at the Mississippi River. One source, New Jersey 452 did not fit the pattern of east-west variation. This source has long been suspect as it may be pitch pine or a hybrid.

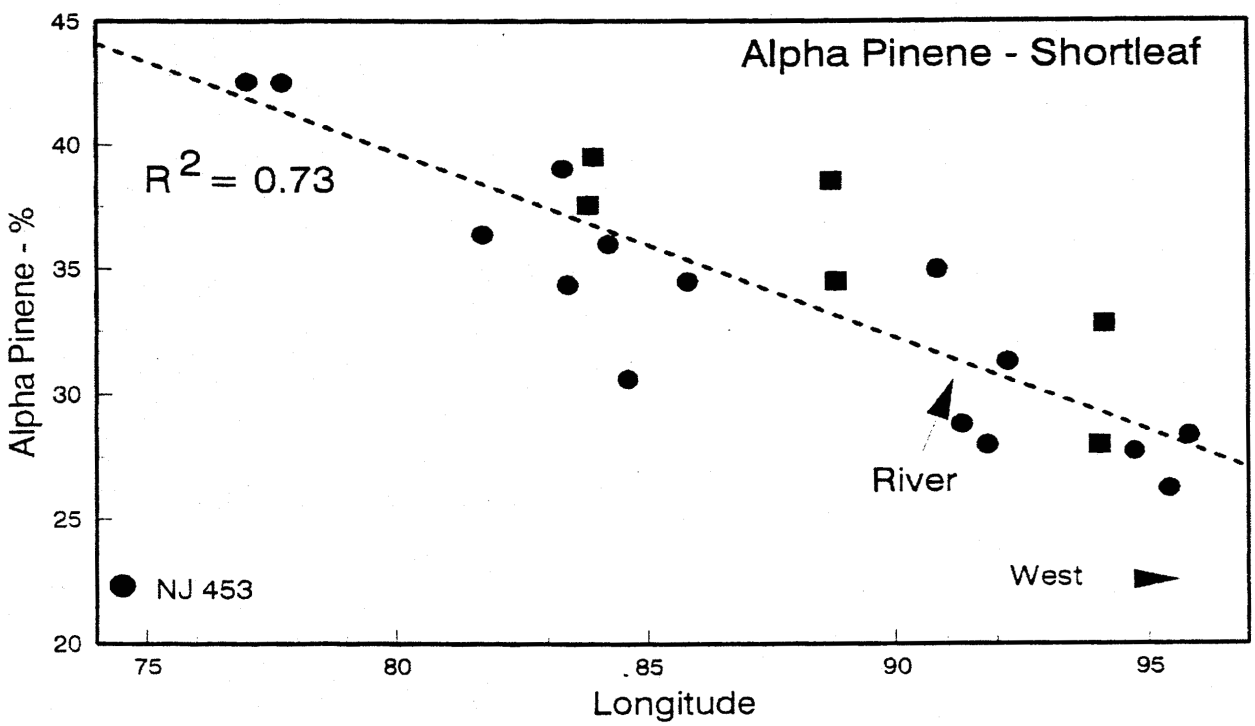

Figure 2. Alpha pinene concentration in cortical monoterpenes of buds of geographic races of shortleaf pine plotted by longitude.

Squillace and Wells (1981) intensively examined geographic variation in cortical monoterpenes in loblolly pine. They also found a decrease in alpha pinene from east to west. If their data are plotted similarly to the shortleaf data in Fig. 2, it is apparent that in loblolly pine there is a distinct discontinuity in alpha pinene concentration at the Mississippi River (Fig. 3). Within the eastern population or within the western population, there is no significant east-west trend. This supports the two-population refugium hypothesis for loblolly pine, versus one population for shortleaf pine

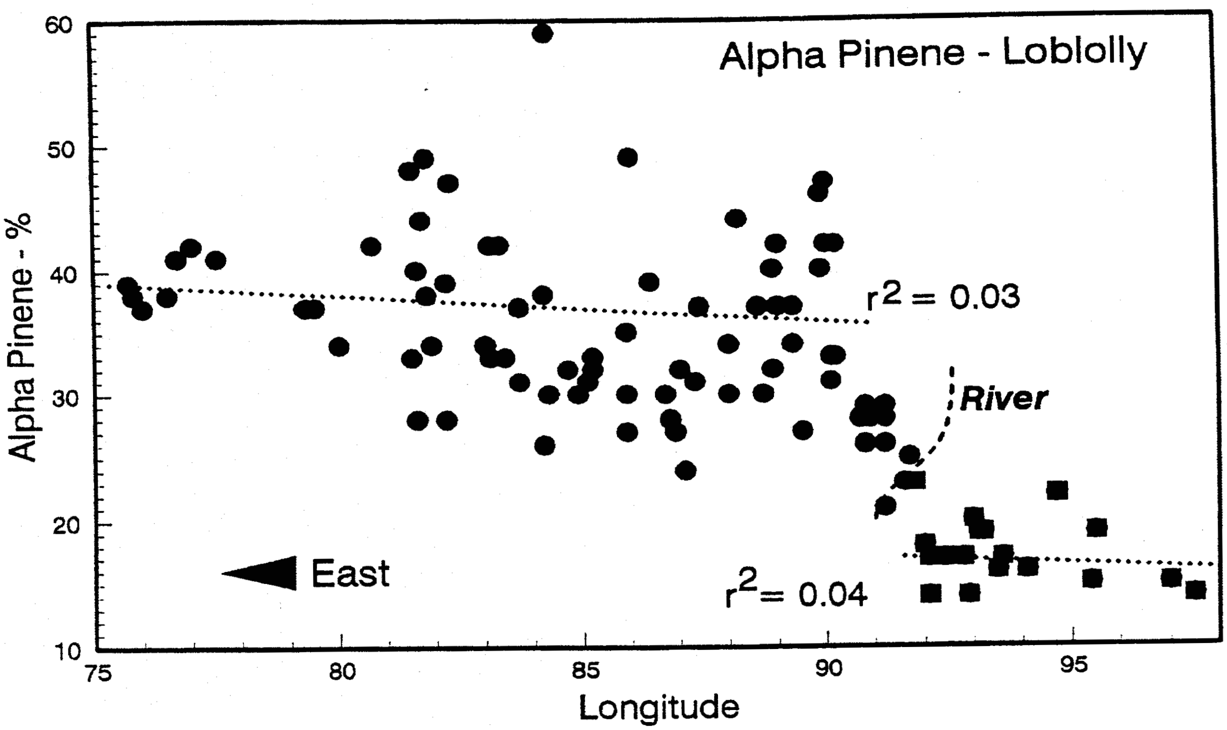

Figure 3. Alpha pinene concentration in cortical monoterpenes of buds of geographic races of loblolly pine plotted by longitude. Data from Squillace and Wells 1981.

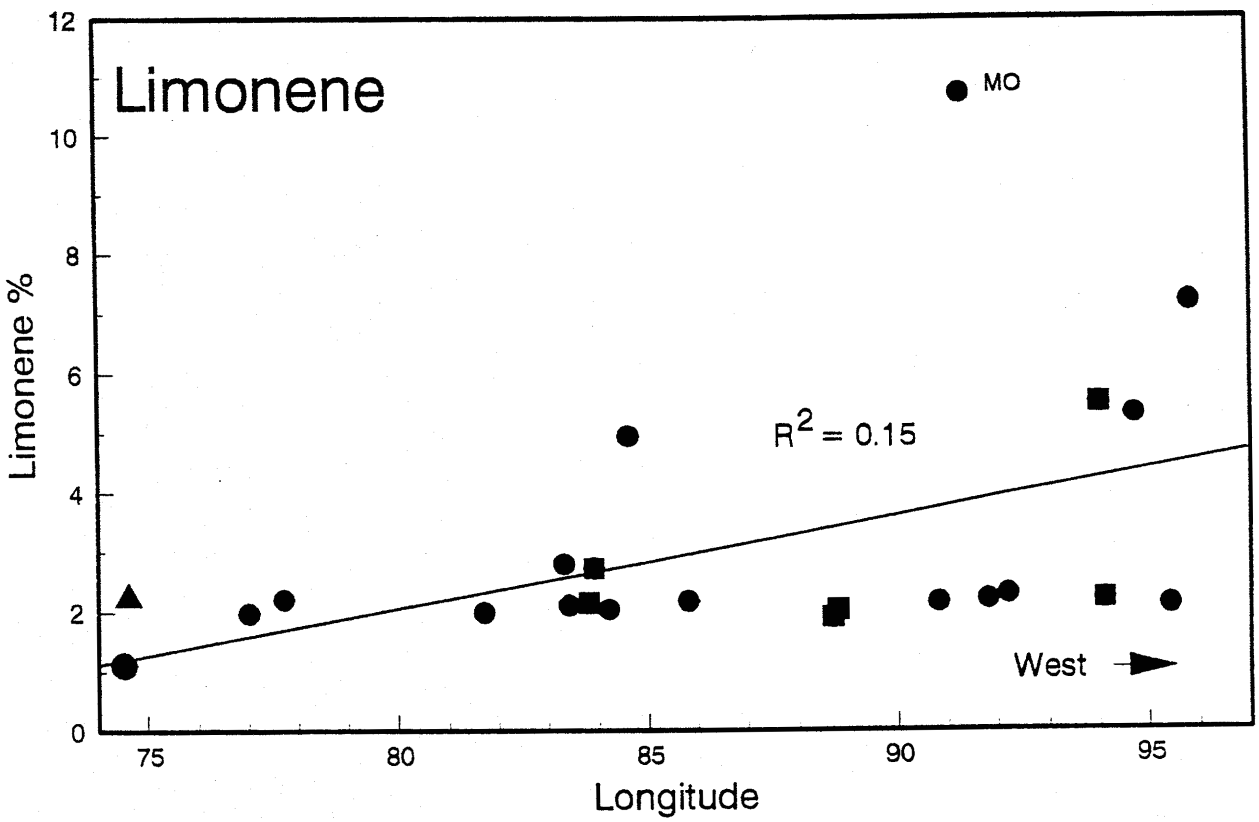

Figure 4. Limonene concentration in cortical monoterpenes of buds of geographic races of shortleaf

pine plotted by longitude.
A correlation coefficient of R = 0.39 between longitude and limonene content indicates a tendency for the western sources to have higher limonene contents, similar to loblolly pine (Squillace and Wells 1981). A plot of limonene content versus longitude, however, indicates that this correlation is due to a few of the western sources having high limonene contents (Fig. 4). This may be due to hybridization with loblolly pine. Western loblolly pine sources also have high limonene contents (Squillace and Wells 1981).

Hybridization of shortleaf and loblolly pines appears to be common in northwestern part of the natural range of shortleaf. Allozyme analysis of some of these same sources has also indicated hybridization with loblolly pine (Raja et al. 1997)

One problem with the hybridization theory is that many of the shortleaf pine sources with probable loblolly parentage are well north of the current loblolly pine distribution (Fig. 1). The Missouri source is perhaps 300 km distant from the nearest loblolly pine. Gene flow as well as long-distance pollen transport is a possibility. Also, the loblolly pine distribution probably extended farther north 5,000 to 7,000 years ago during the Hypsithermal geological period, when the climate was warmer that it is now. Stumps of Scots pine (*Pinus* sylvestris L.) dating from the end of the Hypsithermal have been found well north of the current distribution, indicating that the species expanded northward in the warmer climate, then retreated in the colder present-day climate (Gear and Huntley 1991)..

CONCLUSIONS

1.There is a moderate east-west trend in alpha pinene content in buds of shortleaf pine.

2.The variation is continuous across the Mississippi River, unlike loblolly pine, where the content varies discontinuously across the river.

3.There is Indication of hybridization between shortleaf and loblolly pine in the Limonene content of some shortleaf from the northwestern part of the natural range

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Appendix. Mean values for cortical monoterpenes of geographic sources of shortleaf pine and of spruce pine and pond pine samples. Also included is data on loblolly pine from Squillace and Wells (1981).

Source	α-pinene	camphene	β-pinene	Sabinene	myrcene	α-Phell	Limonene	β-Phell
12	27.994	1.0118	11.665	0.7824	9.735	1.6824	5.4941	38.229
13	32.818	1.3000	8.100	0.9364	7.736	1.0182	2.1909	42.127
31	34.524	1.1941	8.847	0.9765	8.488	0.7588	2.0235	38.188
32	38.533	1.0556	9.944	0.8556	6.900	0.7778	1.8889	36.144
41	37.550	1.1583	11.483	0.7417	9.400	0.6667	2.1417	32.692
45	39.520	1.2100	7.830	0.8200	4.830	1.5300	2.7300	36.530
451	42.500	1.3333	18.556	0.6111	5.200	0.6000	2.2000	26.078
453	22.110	1.1700	11.920	1.1400	8.494	1.0400	2.2300	46.510
455	42.530	1.3300	12.270	0.7000	6.820	0.7100	1.9700	30.790
457	36.360	1.2500	8.440	0.8300	8.030	0.8900	1.9800	39.130
461	34.380	1.2000	10.900	0.8600	8.730	0.8500	2.1000	37.380
463	39.030	1.3000	9.430	0.8100	6.830	0.8100	2.8200	35.850
465	30.630	1.2700	6.570	1.0000	7.630	0.9500	4.9500	43.630
467	34.510	1.3100	14.010	0.8100	9.530	0.8000	2.1700	33.860
473	34.992	1.2000	13.650	0.8583	7.483	0.9250	2.1417	34.750
475	27.740	1.1200	10.930	1.0000	6.020	0.9200	5.3000	42.300
477	26.260	1.2600	12.060	1.0800	8.510	1.1300	2.0900	45.030
478	28.370	1.1200	14.710	0.8400	7.360	0.8600	7.1900	36.710
481	28.000	1.2500	8.930	1.2700	6.500	1.0500	2.1900	47.980
483	31.320	1.2500	10.710	0.9000	7.890	0.9800	2.2800	42.090
485	28.820	1.1300	13.980	0.8100	6.910	0.8000	10.7100	34.550
487	35.989	1.3889	11.922	0.7333	10.511	0.7778	2.0333	33.611
Mean	33.234	1.2110	11.144	0.8809	7.804	0.9508	3.2436	37.956
Spruce pine								
	29.940	0.9800	4.113	0.8533	11.420	1.0400	5.353	43.601
Pond pine								
	10.386	0.4429	35.657	0.1000	1.214	0.0143	47.857	1.320

Loblolly pine (Squillace and Wells 1981)

31.1	17.0	19.7	10.5	20.4

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