

Racial Variation in Physiological Characteristics of Shortleaf Pine Roots

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Introduction

Genetic variations in physiological traits of forest tree roots have been reported. VOIGT (1953) found differences in root respiration among *Pinus banksiana* LAMB., *P. resinosa* AIT., and *Robinia pseudoacacia* L., and STEINBECK and McALPINE (1966) found clonal differences within as well as differences among species of hardwoods. There is, however, relatively little information available on racial variation in roots such as that in root form of *P. palustris* MILL. described by SNYDER (1961), or the ecotypic variation in root plasticity of *Thuja occidentalis* L. described by HABECK (1958). Because of the lack of information on geographic variation in roots of forest tree species, and because of an interest in the possible contribution of tree roots to variation in height growth, some physiological properties of roots of young seedlings from different geographic sources of seeds were measured in the study described here.

Experimental Material and Methods

Plant material. — Shortleaf pine (*P. echinata* MILL.) was selected as the source of geographic variation because seeds were available²⁾ from the same collections that had demonstrated significant racial variation in the field (WAKELEY, 1961). The seeds had been collected at various places in the species' range from New Jersey to Texas (Fig. 1). The mean annual temperature and physiography at seed origins are given in Table 1 because of the primary consideration given to them in determining the points of collection (Committee on Southern Forest Tree Improvement, 1952, 1956).

Three groups of seedlings were grown for the study. The seeds in groups 1 and 2 were from eight sources of a 1955 collection. Those in Group 3 were from six; none were available from the Georgia and Mississippi sources and shortages necessitated the substitution of seeds from a 1951 collection for the Texas, Arkansas, and South Carolina sources (Table 1 and Fig. 1).

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²⁾ Seeds were supplied by the Committee on Southern Forest Tree Improvement.

The seeds in Group 1 were germinated in flats of sand and vermiculite in a lathhouse in early March. They were watered weekly with a commercial water-soluble fertilizer. In June the entire root systems of several hundred seedlings of each source were cut from the tops, washed clean, and blotted dry. The roots of each source were divided into two groups. Each group was chopped into small pieces, and a 15 g sample was weighed out for biochemical studies.

Group 2 seeds were germinated in a sand and vermiculite mixture in the laboratory in late July, and the seedlings were grown out of doors until used in respiration and growth-inhibition experiments. When they were about 40 days old, the Virginia and Mississippi seedlings began dying from what appeared to be a damping-off fungus (isolations were not made), but there was no detectable sign of it on the seedlings of the other sources. The seeds from South Carolina started to germinate about 1 week later than other seeds in the group, and the average age of South Carolina seedlings appeared to be more than 1 week less than that of other seedlings. Only records of the start of germination were kept.

Group 3 seeds were germinated in pots of soil in June of the following year. They also were grown out of doors

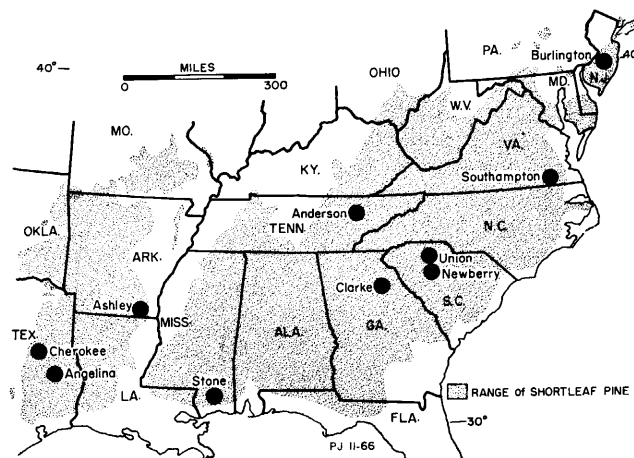


Figure 1. — Location of seed sources.

Table 1. — Temperature and physiography of the areas where seeds were collected.¹⁾

State	County	Seedling group	Mean annual temperature zone (°F)	Physiography
New Jersey	Burlington	1, 2, 3	53	Coastal Plain
Virginia	Southampton	1, 2, 3	58	Coastal Plain
Tennessee	Anderson	1, 2, 3	58	Cumberland Plateau
South Carolina	Union	1, 2	62—63	Piedmont
South Carolina	Newberry	3	63	Piedmont
Georgia	Clarke	1, 2	62—63	Piedmont
Arkansas	Ashley	1, 2	63	Mississippi Embayment
Arkansas	Ashley	3	63	Mississippi Embayment
Texas	Cherokee	1, 2	67	Coastal Plain
Texas	Angelina	3	67	Coastal Plain
Mississippi	Stone	1, 2	67	Coastal Plain

¹⁾ Data supplied by P. C. WAKELEY.

for respiration studies. No disease problems were encountered in the Virginia seedlings in this group, nor was the germination of this South Carolina collection behind that of the other sources.

Preparation of extracts. — Acetone powders were prepared from Group 1 root samples. Each 15-g sample was blended in 200 ml of -20° C acetone and filtered. Three-ml samples of the filtrate were examined for pigment content. The acetone was evaporated from the filter cake, and the cake screened. The resulting powder was weighed and divided in half.

Enzymes were extracted from one-half of the powder with cold phosphate buffer (pH 6.4). The proteins were purified by precipitating them twice from buffer with cold acetone. The final enzyme preparation was brought to a volume of 25 ml with buffer. Enzyme activity of one of the Arkansas samples was inadvertently destroyed by heating during blending; therefore, the Arkansas source was not included in the results of the enzyme experiments.

An indoleacetic acid (IAA) oxidase inhibitor was extracted from the other half of the acetone powder with 70 ml of 80° C water. The mixture was cooled and filtered, and the filtrate was brought to 100 ml with water.

Determination of characters. — The IAA oxidase activity of each enzyme sample was determined with 8 ml of reaction mixture which initially contained 4 ml of enzyme extract, IAA at 25 ppm, 2-4-dichlorophenol at 10^{-4} M, MnCl_2 at 10^{-4} M, and maleic hydrazide at 10^{-3} M. After 15, 30, and 60 minutes, 1-ml aliquots were pipetted into colorimeter tubes containing 2 ml of GORDON-WEBER (1951) reagent, and readings were taken. Sixty-minute reaction times proved suitable.

The IAA oxidase inhibitor activity was determined by measuring its inhibition of the IAA oxidase reaction. One-tenth ml of each inhibitor extract was mixed with 4 ml of a stock enzyme solution before the IAA and cofactors were added. Measurements were made after a 3-hour reaction time.

Peroxidase activity of the enzyme samples was measured by the methods of SHARPENSTEEN *et al.* (1956) with slight modifications in H_2O_2 and pyrogallol concentrations.

Pigment content of the filtrates obtained in preparing the acetone powders was determined with a spectrophotometer.

In earlier unreported tests, elongation of detached slash pine (*P. elliotii* ENGELM.) hypocotyl sections was inhibited

when the sections were incubated with root tips. In the present experiment, an 8-mm section of root tip was cut from each of 10 month-old shortleaf seedlings of each source in Group 2. Each tip was placed with four slash pine hypocotyl sections in a small stoppered bottle containing 1 ml of 0.01 ppm IAA. Twenty bottles containing hypocotyl sections but no roots were the controls. Hypocotyl elongation was measured after incubation for 22 hours. The root sections were not measured.

Root respiration of seedlings from Groups 2 and 3 was measured by conventional WARBURG manometric techniques (UMBREIT *et al.*, 1957). Each WARBURG flask contained the root systems of eight seedlings from one source. There were two flasks per source. Respiration was determined at 27° C because the temperature of the medium in which the seedlings were growing prior to lifting was 26.5° C. Seedlings were 45 days old when these determinations were made. At this age the primary roots were less than 7 cm. in length and no secondary roots were visible.

Another respiration experiment was performed with Group 3 seedlings from Angelina County, Texas; Newberry County, South Carolina; and Burlington County, New Jersey. Respiration was measured for 1-hour periods at 30° , 35° , 40° , and 30° C in the sequence given. The temperature was raised to 45° C for 15 minutes before it was reduced to the final 30° C, but no measurements were made at the high temperature. Temperature changes took about 5 minutes — measurements were started 10 minutes after the desired bath temperature was reached.

Results

Statistically significant (0.05 level used throughout this paper) differences by source were found in contents of pigment, IAA oxidase and peroxidase, IAA oxidase inhibitor, acetone powder, and elongation inhibitor, and in root respiration. The results are summarized in Tables 2 and 3, where geographic sources are arranged in north-to-south order. Differences were analyzed by DUNCAN'S multiple range test; means followed by the same letter do not differ significantly at the 0.05 level of probability.

Pigment. — The filtrates were yellow, and the major peak of absorbance was at $333 \text{ m}\mu$ for all sources. The chemical identity of the compound(s) was not determined. Pigment content was lowest in the Mississippi and Texas seedlings and highest in the Georgia and Tennessee.

Table 2. — Physiological characteristics measured in shortleaf roots.

Seed source — State	Character					
	Pigment absorbance ¹⁾	IAA oxidase transmittance ²⁾	Peroxidase absorbance ²⁾	IAA oxidase inhibitor absorbance ²⁾	Acetone powder ³⁾	Elongation in- hibition ⁴⁾
	Percent				Grams	Percent
New Jersey	74 ab ⁵⁾	45 cd	27 cd	52 ab	1.844 b	29 bc
Virginia	66 bc	55 ab	33 ab	30 c	1.862 b	40 a
Tennessee	82 a	48 bc	28 bcd	42 abc	2.147 a	42 a
South Carolina	66 bc	52 b	32 bc	26 c	1.887 b	21 c
Georgia	80 a	63 a	38 a	38 bc	2.150 a	32 ab
Arkansas	75 ab	—	—	52 ab	2.050 a	36 ab
Texas	60 cd	38 d	24 d	58 a	2.055 a	32 ab
Mississippi	54 d	42 cd	24 d	26 c	1.862 b	41 a

¹⁾ Absorbance at $333 \text{ m}\mu$. Group 1 seedlings.

²⁾ Transmittance or absorbance. The larger the value the higher the activity. Group 1 seedlings.

³⁾ Weight of acetone powder extracted from 15 g (fresh weight) of roots. Group 1 seedlings.

⁴⁾ The higher the value the greater the inhibition — controls had zero inhibition. Group 2 seedlings.

⁵⁾ Values followed by a given letter do not differ at the 0.05 level.

Table 3. — Respiration of shortleaf pine seedling roots.

Seed source — County and State	Respiration ¹⁾	
	Group 2	Group 3
Burlington, New Jersey	30.2 b ²⁾	27.2 c
Southampton, Virginia	—	41.4 b
Anderson, Tennessee	34.6 b	37.4 bc
Union, South Carolina	40.1 ab	—
Newberry, South Carolina	—	56.2 a
Clarke, Georgia	31.2 b	—
Ashley, Arkansas ³⁾	33.0 b	—
Ashley, Arkansas ⁴⁾	—	35.8 bc
Cherokee, Texas	45.7 a	—
Angelina, Texas	—	44.9 ab

¹⁾ Microliters O₂/10 mg dry wt/hr.²⁾ Values followed by a given letter do not differ at the 0.05 level.³⁾ 1955 collection.⁴⁾ 1951 collection.

Enzymes. — The activities of IAA oxidase and peroxidase were highest in the Georgia, Virginia, and South Carolina sources and lowest in the Texas and Mississippi sources. There was a strong correlation between the activities of the two enzymes. This is not surprising, since peroxidase is thought to be a component of the IAA oxidase system (GALSTON and HILLMAN, 1961).

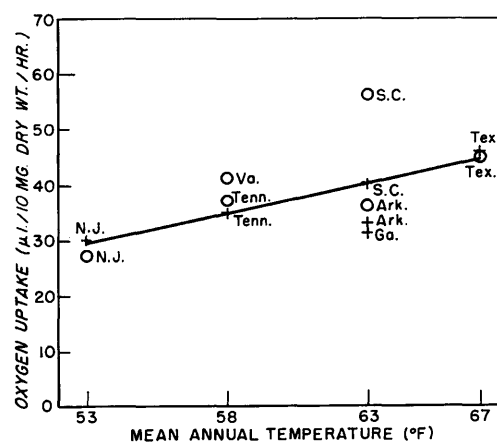
IAA oxidase inhibitor. — There were statistically significant differences in inhibitor content attributable to seed source, but the differences did not seem to fit a geographical pattern. It is not known if the inhibitor extracted plays any role in auxin metabolism of intact roots. Because the extract inhibited oxidation of IAA *in vitro* does not mean necessarily that it has a similar function in the plant.

Acetone powder. — Based on the weight of acetone powders extracted from the 15-g samples (fresh weight), the geographic sources were in two groups — the Georgia, Tennessee, Texas, and Arkansas sources comprised the heaviest group, and the South Carolina, Virginia, Mississippi, and New Jersey sources the lightest. Less than 15 percent of the variation in IAA oxidase, peroxidase, or IAA oxidase inhibitor activity could be attributed to variation in powder weight. About 30 percent of the variation in pigment concentration could be attributed to variation in powder weight, but even this relation was not statistically significant.

Elongation inhibitor. — Significant differences in activity of the elongation inhibitor were obtained among the sources but again the differences did not appear to have any geographical pattern. The low value obtained for the South Carolina seedlings in this group were younger than the other seedlings. The chemical nature and biological role of the root compound(s) that inhibited the elongation of hypocotyl sections are unknown.

Respiration. — Root respiration was positively correlated with mean annual temperature at seed origin (Fig. 2). The simple correlation coefficient of root respiration with mean annual temperature at seed origin was 0.58; with average January minimum temperature, 0.54; with average July maximum temperature, 0.51; with average length of frost free season, 0.35; with average June-August precipitation, 0.32; and with average annual rainfall, 0.32. The 5-percent level of significance is 0.40.

BOURDEAU (1963) measured respiration of *P. strobus* L. needles from various geographic sources and found no significant effect of seed origin. He did, however, observe that needles from southern sources tended to have higher

Figure 2. — Respiration of seedling roots by temperature at seed source (+ = mean of Group 2 seedlings; 0 = mean of Group 3 seedlings; $r^2 = 0.34$).

respiration rates at high temperatures than those from northern sources. He suggested that trees from southern sources may have greater heat tolerance.

In the present study, no differences in heat tolerance were found for seedling roots from Texas, South Carolina, and New Jersey sources. Changes in respiration with changes in temperature from 30°, to 35°, 40°, and back to 30° C are given in Table 4. Respiratory responses to tem-

Table 4. — Effect of temperature (°C) on respiration of roots.

Source	Respiration at:			
	30°	35°	40°	30°
Percent ¹⁾				
New Jersey	100	153	145	100
South Carolina	100	154	152	100
Texas	100	151	144	104

¹⁾ Expressed in percent of respiration at 30° C before temperature was raised.

perature changes were not significant among sources, and all sources appeared to recover equally from the effects of high temperatures.

Discussion

The study was designed to determine whether there is significant variation in the physiological characteristics of shortleaf pine roots attributable to geographic origin of seed. The data show that there is. However, the differences found are not necessarily due to inherent differences in the roots; they may merely reflect differences among sources in top characteristics. It has not yet been demonstrated that there is geographic variation in roots *per se* among the sources studied (ALLEN, this issue).

Summary

In the study reported here, which was conducted near Gulfport, Mississippi, very young shortleaf pine (*Pinus echinata* MILL.) seedlings from eight geographic seed sources exhibited racial variation in root characteristics. Significant differences attributable to seed origin were found in root respiration and in amount of acetone powder, indoleacetic acid (IAA) oxidase, peroxidase, an inhibitor of IAA oxidase, an elongation inhibitor, and a pigment. Respiration was positively correlated with mean annual temperature at seed origin. No differences among sources were found in

respiration response to high temperature or recovery from effects of high temperature.

Literature Cited

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The Inheritance of Compression Wood and its Genetic and Phenotypic Correlations with Six Other Traits in Five-Year-Old Loblolly Pine

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Introduction

The inheritance of compression wood has not previously been directly studied or for that matter, even seriously considered. It is the common belief that compression wood is formed as a direct response to inclination of the tree bole from the vertical. This explanation presupposes that trees within a species will not differ appreciably in their response to a given amount of inclination from the vertical. In fact, WESTING (1965, p. 434), in his comprehensive review of all aspects of compression wood, went so far as to say:

"Little would be gained by such a study, because structure, pattern of occurrence, and extent of formation of compression wood are remarkably uniform from individual to individual and from species to species."

This statement appears to be too sweeping. Evidence is presented in this paper that direct as well as indirect genetic control of compression wood formation exists and considerable inter- and intra-specific variation in proportions of compression wood occurs.

There is good evidence that bole straightness is quite strongly inherited, at least in Southern pines. PERRY (1960) and GODDARD and STRICKLAND (1964) both showed that spiral crook in loblolly pine is heritable; McWILLIAM and FLORENCE (1955) and NIKLES (1966) reported large gains in straightness for open and control pollinated progenies from select parents. In a study of six-year-old open pollinated progenies of select trees, WOESSNER (1965) found straightness to be one of the most strongly inherited characteristics. Evidence from a well designed heritability study of loblolly pine (SHELBOURNE, 1966) also supports these findings. On these grounds there is a strong probability of indirect inheritance

of compression wood through genetic control of bole straightness.

Compression wood is also associated with branch development. It is found in the stem below the points of branch insertion (ZOBEL and HAUGHT, 1962). The amounts and intensity of such compression wood development are believed to be related to branch angle which itself has been shown to be moderately heritable (e. g. EHRENBERG, 1963; CAMPBELL, 1958).

Inter-generic differences in response to a given amount of inclination of the stem were demonstrated as early as 1888 by KONUNCHUK (WESTING, 1965), who found that Norway spruce formed compression wood more strongly than Scots pine. Similar differences in response to slight inclination were observed for white spruce and red pine by RENDLE (1956). Large intra-specific differences in compression wood percentage by volume (up to 50%) were reported by LOW (1964). He found that differences between trees in compression wood percent were only very weakly correlated with size and number of stem deviations, indicating that compression wood percent was in fact behaving as a phenotypically variable, independent trait. It is not unreasonable to infer from this that, in direct contradiction to WESTING's statement, study of compression wood might well yield evidence of inheritance of this characteristic.

The investigation⁴) reported here involved a study of compression wood percentage, specific gravity, and growth rate in a number of open pollinated (half sib) families of loblolly pine. This investigation is one of a series (SHELBOURNE loc. cit.) dealing with the inheritance of bole straightness, with the effect of differing intensities of compression wood on specific gravity, tracheid length and spiral checking of tracheids, and also the variation and relationships among compression wood, bole straightness and other traits. The latter study revealed that the widely-held assumption that amounts of compression wood formed

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