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Note by T. A. Knauf and M. V. Bilan

Abstract. Anatomical modifications in protective layers and stomatal characteristics of needles which would have moisture conserving effects were found in 2-yr-old seedlings of loblolly pines of the Bastrop County, Texas, provenance. Most of these modifications were absent from needles of 16-yr-old trees of the same provenance. **Forest Sci. 20:88–90.**

Additional key words. Pinus taeda L., xeric ecotype, needle morphology, stomates, moisture relations.

LOBLOLLY PINE (Pinus taeda L.) ranges from south central Texas to southern Delaware, a testimonial to its ability to adapt to widely differing environments. The isolated westernmost limit of this natural range, known as the "Lost Pines," receives 10-20 inches of rainfall a year less than the continuous range 200 miles to the east. Somehow, the "Lost Pines" of semi-arid Bastrop, Caldwell, and Fayette Counties, Texas survive on about one-half the rainfall of the main population (Wahlenberg 1960). This evidence has led to the conjecture that a drought resistant variety of loblolly pine exists in the "Lost Pines" area. Work by Zobel (1955) and Brix¹ suggests some natural selection for drought resistance has taken place.

Thames (1963) examined foliage of 2-yrold loblolly pine seedlings from four seed sources. "Lost Pines" trees differed the most, having more hypodermal cells, fewer stomates per unit length, greater cross-sectional needle area, and greater perimeter.

To further study possible modifications for drought resistance through reduction of transpiration, we compared needle structure in loblolly pines of two ages from two seed sources.²

Methods. Needles from 2-yr-old trees were obtained from a nursery at Nacogdoches,

Texas. Needles from 16-yr-old trees came from the Arthur H. Temple, Sr. Research Area, at Fastrill in Cherokee County, Texas At both sites, trees of Bastrop County provenance were available, as well as comparable trees of East Texas provenance, which were grown from Polk County seed. At Fastrill, needles were obtained from trees of unspecified East Texas provenance.

In June and early July, 1970, mature needle fascicles (previous year's growth) were collected from each of 5 randomly selected trees of each provenance and each tree age.

A 5mm section was cut from the middle of one fascicle from each tree, and the resulting three needle sections were soaked for 24 hr in individual test tubes of distilled water. They were then placed in test tubes containing melted polyethylene glycol, mol wt 1000 (Carbowax 1000). Each tube was placed in a warming oven at $45^{\circ}-50^{\circ}$ C for 3 days while the tissues were infiltrated with the medium. Mounting, embedding, microtoming, and slidemaking were performed as described in Jensen (1962). Sections were cut at thicknesses varying from 15 to 25 microns.

Carbowax 1000 was chosen over paraffin Having a high affinity for water, it dehydrates and infiltrates at the same time. Lipids and waxes are left intact because Carbowax is not a lipid solvent. Another set of the same sections was stained with safranin and mounted in Canada balsam (Johansen 1940).

Anatomical dimensions, stomate depth, needle dimensions, and size of epidermal openings were measured on each of three different sections of each needle. These results were then averaged to minimize any distortion which might have occurred in microtoming and mounting. No real distortions appeared, however, and the data for each section of each needle varied less than the data for each needle of the same three-needle fascicle.

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¹Brix, H. 1959. Some aspects of drought resistance in loblolly pine seedlings. Ph.D. Dissertation. Texas A & M University. College Station, Texas. 94 p.

² Knauf, T. A. 1972. Needle characteristics of loblolly pines from two Texas seed sources. Thesis presented to Stephen F. Austin State University in partial fulfillment of requirements for the Degree of Master of Science in Forestry. 133 p.

	2-yr-old trees		16-yr-old trees	
	East Texas source	Bastrop County source	East Texas source	Bastrop County source
Needle dimensions				
Length, cm	14.6	14.2	15.4	15.0
Cross section, mm ²	0.36	0.33	0.72	0.58
Volume, mm ³	51.8	46.8	110.0	87.1*
Surface area, mm ²	358.4	330.2	565.6	484.3
Abaxial width, μ	1235.0	1145.0	1792.0	1638.0
Adaxial width, μ	610 .0	590.0	945.0	791.0
Surface/mm ³ volume, mm ²	6.9	7.1	5.1	5.6
Stomatal measurements				
Stomates per needle	30,770	23,867*	64,136	59,436
Stomates/mm ² of needle surface	85.5	72.3*	113.3	123.5
Stomatal rows, abaxial surface	8.7	6.8**	14.0	11.8
Stomatal rows, adaxial surface	9.1	6.6**	14.5	13.1
Distance between stomatal rows,				
abaxial surface, μ	100.2	118.2	112.3	94.2
Distance between stomatal rows,				
adaxial surface, μ	87.6	119.8**	84.4	94.0
Stomates per mm of row, abaxial				
surface	11.4	11.8	14.8	15.6
Stomates per mm of row, adaxial				
surface	11.4	12.3**	14.8	15.6
Depth of stomatal crater, abaxial				
surface, μ	10.8	11.0	13.6	12.2
Depth of stomatal crater, adaxial				
surface, μ	10.4	11.8*	12.4	11.0**
Cutinized epidermis				
Thickness, abaxial surface, μ	5.0	5.8**	7.2	7.6
Thickness, adaxial surface, μ	4.8	4.9	7.0	7.6
Epidermis plus hypodermis				
Thickness, abaxial surface, μ	22.2	23.6*	49.1	46.1
Thickness, adaxial surface, μ	21.5	24.0**	38.6	36.4
Cuticle				
Thickness, abaxial surface, μ	None	None	1.02	1.24
Thickness, adaxial surface, μ	None	None	0.96	0.98

TABLE 1. Mean values for anatomical features of loblolly pine needles from trees of two ages and two seed sources.

* Values from East Texas and Bastrop trees of equivalent age differ significantly at the 95 percent level.

** Values from East Texas and Bastrop trees of equivalent age differ significantly at the 99 percent level.

Curved needle dimensions were measured in short segments by manipulation of the slides.

Stomate counts, measurement of stomatal craters, and determination of distances between stomate rows were performed on a separate fascicle from each sample tree.

Data were analyzed by analysis of variance.

Results and Discussion. Results of measurements and counts are summarized in Table 1.

Average needle lengths for the two seed sources did not differ significantly at either age. Bastrop needles had smaller cross sections and volumes than East Texas needles, although differences were significant only for the older trees. Differences in surface area were not significant at either age, and the absence of significant differences in abaxial and adaxial dimensions indicates no substantial differences in shape. This is confirmed by the lack of significant differences in the ratio of surface area to volume. Thus, there is no indication that needle size or shape has been modified in a way that would conserve moisture.

Distribution and dimensions of stomates on seedling needles differed between sources in ways that do suggest adaptation to limit transpiration. Number of stomates per needle and per mm² of surface area were significantly less in Bastrop seedlings despite significantly more stomates per millimeter of row on the adaxial surface. This smaller number resulted from significantly fewer rows of stomates at wider spacing on both abaxial and adaxial surfaces. Concentration of stomates on adaxial surfaces may tend to conserve moisture, since these surfaces are somewhat less exposed. On needles from the 16-vr-old trees. difference between sources were not significant for any of these attributes.

The reduced number of stomates per unit of needle surface in young seedlings would tend to reduce transpiration and thereby improve chances for survival during the critical early years of seedling establishment. More numerous stomates on older trees would permit increased metabolism after extensive root systems had made drought effects less critical.

Depth of stomatal crater on adaxial surfaces differed significantly at both ages, being greater on needles of Bastrop provenance at 2 yr but greater on those from East Texas sources at 16 yr. These differences are associated with and perhaps caused by corresponding differences in thickness of epidermis plus hypodermis (discussed below). Both seedling modifications would tend to conserve internal moisture; why they are reversed in the older trees is not clear.

Seedlings of Bastrop provenance had

needles with significantly thicker layers of cutinized epidermis on abaxial, but not on adaxial, surfaces than East Texas seedlings There were similar differences in combined thickness of epidermis and hypodermis, which were significant on both abaxial and adaxial surfaces. Thus, needles of Bastrop seedlings are better protected from loss of moisture through surface layers than those of East Texas seedlings.

On needles from 16-yr-old trees there were no significant differences in cuticle thickness in the two provenances.

Conclusion. Needles from loblolly pine seedlings of Bastrop provenance are adapted for survival in a dry climate by having thicker protective layers and fewer stomates per unit of surface area than those from East Texas. These adaptations should be of greatest survival value to the plant while it is young and establishing an extensive root system; they appear to be lost when they have served their purpose.

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