

1977

Cotyledon and Primary Needle Variation in Loblolly Pine From Mesic and Xeric Seed Sources

M. Victor Bilan

Follow this and additional works at: <http://scholarworks.sfasu.edu/forestry>

Tell us how this article helped you.

Recommended Citation

Bilan, M. Victor, "Cotyledon and Primary Needle Variation in Loblolly Pine From Mesic and Xeric Seed Sources" (1977). *Faculty Publications*. Paper 358.

<http://scholarworks.sfasu.edu/forestry/358>

This Article is brought to you for free and open access by the Forestry at SFA ScholarWorks. It has been accepted for inclusion in Faculty Publications by an authorized administrator of SFA ScholarWorks. For more information, please contact cdsscholarworks@sfasu.edu.

- KAUFMANN, M. R. 1969. Effects of water potential on germination of lettuce, sunflower, and citrus seeds. *Can J Bot* 47: 1761-1764.
- , and K. J. ROSS. 1970. Water potential, temperature, and kinetin effects on seed germination in soil and solute systems. *Am J Bot* 57:413-419.
- KNIFE, O. E. 1973. Western wheatgrass germination as related to temperature, light, and moisture. *J Range Manage* 26:68-69.
- KOLLER, D. 1972. Environmental control of seed germination. *In Seed biology* (T. T. Kozlowski, ed.), vol. II, p. 1-101. Academic Press, N.Y.
- LARSON, M. M., and G. H. SCHUBERT. 1969. Effect of osmotic water stress on germination and initial development of ponderosa pine seedlings. *Forest Sci* 15:30-36.
- MICHEL, B. E., and M. R. KAUFMANN. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiol* 51:914-916.
- NOBLE, D. L. 1972. Effects of soil type and watering on germination, survival, and growth of Engelmann spruce: a greenhouse study. USDA Forest Serv Res Note RM-216, 4 p.
- . 1974. Natural regeneration of Engelmann spruce in clearcut openings in the central Rockies as affected by weather, aspect, seedbed, and biotic factors. Ph.D. Thesis, Colorado State University. 187 p. Univ. Microfilms No. 74-27,958.
- PAWLOSKI, M. C., and C. F. SHAYKEWICH. 1972. Germination of wheat as affected by soil water stress. *Can J Plant Sci* 52:619-623.
- SHARMA, M. L. 1973. Simulation of drought and its effect on germination of five pasture species. *Agron J* 65:982-987.
- TESCHE, M. 1975. Keimung von koniferensamen bei simulierter Trockenbelastung in Polyäthylenglycol (LUTROL). *Biochem Physiol Pflanzen* 67:577-584.
- WANJURA, D. F., and D. R. BUXTON. 1972. Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agron J* 64:427-431.
- USDA FOREST SERVICE. 1974. Seeds of woody plants in the United States. US Dep Agric, Agric Handb 450, 883 p.
- YOUNG, J. A., and R. A. EVANS. 1972. Germination and establishment of *Salsola* in relation to seedbed environment. I. Temperature after ripening, and moisture relations of *Salsola* seeds as determined by laboratory studies. *Agron J* 64:214-218.

Cotyledon and Primary Needle Variation in Loblolly Pine From Mesic and Xeric Seed Sources

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

ABSTRACT. Cotyledons and primary needles in loblolly pines (*Pinus taeda* L.) of Bastrop County, Texas, provenance have morphological modifications which should tend to conserve moisture during drought. *FOREST SCI.* 23:33-36.

ADDITIONAL KEY WORDS. Adaptations, drought resistance, needle morphology, provenance, *Pinus taeda*.

THE "LOST PINES" of Texas' Bastrop, Caldwell, and Fayette Counties, survive on about two-thirds the annual rainfall of the main population of loblolly pine (*Pinus taeda* L.) 100 miles to the east (Wahlenberg 1960). This has led to the conjecture that a drought resistant ecotype of loblolly pine exists in this area.

The authors are, respectively, Assistant Professor of Environmental Science, Ambassador College, Big Sandy, TX 75755, and Professor, School of Forestry, Stephen F. Austin State University, Nacogdoches, TX 75961. Research supported in part by funds provided under the McIntire-Stennis Act. Manuscript received July 15, 1976.

Work by Zobel (1955) and Brix (1959)¹ suggests that natural selection for drought resistance has taken place. Thames (1963) examined foliage of 2-year-old loblolly pine seedlings from four southern seed sources. "Lost Pines" trees differed more than the others, having more hypodermal cells, fewer stomates per unit length, greater cross-sectional needle area and also a greater perimeter. Knauf and Bilan (1974) compared needle morphology in 2- and 16-year-old trees of East Texas and Bastrop County seed sources and found more moisture-conserving adaptations in the younger trees than in the older ones. These adaptations included fewer stomates per needle and per unit of surface area, greater distance between rows of stomates, deeper stomates, and a thicker epidermis, cutinized epidermis and hypodermis.

The purpose of the present study was to determine whether cotyledons and primary needles of loblolly pine seedlings from the "Lost Pines" area possess morphological features which might account for their relatively greater drought resistance.

Methods.—We grew plants from East Texas and "Lost Pines" seed in a controlled environment in a greenhouse in Nacogdoches, Texas. The seeds were planted in plastic pots of 10 cm depth and diameter, and foliage was studied in September when the seedlings were between their second and third month of growth.

Three mature primary needles were taken from the base of the flush from each of five randomly selected trees of each seed source. A 1-cm section was cut from the middle of each of the three needles from each tree and thence into two 5-mm segments. The segments from each needle were killed, fixed, and stored in separate labeled vials with similar segments from the other two needles from the same plant. One set was processed with Carbowax, the other with paraffin. Samples processed with Carbowax were used for measurements wherever feasible because of their superior dimensional stability; paraffin-treated samples afforded better contrast between certain tissues when stained.

In the Carbowax treatment, the tissue was killed and fixed in 5 percent formalin for 24 hours. The specimens were then washed and placed in Carbowax 1000 at 45°–50°C for 3 days. The infiltrated tissue and Carbowax were then poured into chilled molds, and the molded blocks refrigerated at 0°–2°C until just before sectioning. The best sections were obtained with block and microtome blade temperatures between 0° and 2°C. Sections 19 μ thick proved best. The sections were affixed to labeled slides with Haupt's adhesive, stained for lipids with Sudan IV in polyethylene glycol (mol. wt. 200) and mounted in glycerin gelatin (Jensen, 1962).

Paraffin treatment followed the method described by Johansen (1940, p 22–39) and Sass (1958, p 22–39) and modified by Jensen (1962, p 80–83, 90, 258–263) except that tertiary butyl alcohol was substituted for ethanol. The tissue was infiltrated and embedded with Paraplast and sectioned at 15 μ . After removal of paraffin, sections were stained with Safranin O, counterstained with aniline blue and mounted in Permount.

Cotyledons were selected, cut, and processed in a manner identical to that used on the primary needles. Only cotyledons from plants with primary needles were sampled, insuring the maturity of the cotyledons.

Cuticle, cutinized epidermis, and dermal thickness were measured in three different places on each section from the three needles or cotyledons from each seedling. Thus, three mean values derived from nine measurements were available for each seedling.

Cellular and tissue dimensions, stomate depth, and needle and cotyledon dimensions were measured for three different sections of the same needle or cotyledon. These values were then averaged to give a mean value for each characteristic for each seedling. Variation between needles and cotyledons from the same plant was quite small.

For determination of number of cotyledons, all plants planted from May through October were examined—a total of 147 from each seed source.

Stomatal counts were performed on needles and cotyledons selected in a manner identical to the method used on foliage that was sectioned.

Since stomates on cotyledons and primary needles were highly variable, it was impossible to count them on a number-per-row or number-of-rows basis. All stomates were

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

TABLE 1. Mean values for morphological features of 2-month-old loblolly pine from two seed sources.

Morphological feature	Cotyledons		Primary needles	
	East Texas source	Bastrop County source	East Texas source	Bastrop County source
Cotyledon or needle dimensions				
Length, cm	3.5	4.2	2.0	2.5 ^b
Cross section, mm ²	.169	.224 ^b	.084	.099 ^b
Volume, mm ³	5.9	9.5 ^a	1.7	2.5 ^b
Surface area, mm ²	62.5	87.7 ^a	24.8	32.3 ^b
Surface/mm ³ volume, mm ²	10.6	9.2 ^a	14.6	13.5
Number of cotyledons	6.9	7.6 ^b		
Stomatal measurements				
Stomates per cotyledon or needle	1886.4	2183.2 ^b	2637.2	3209.4 ^b
Stomates/mm ² cotyledon or needle surface	30.5	24.9 ^a	107.4	101.9
Stomates/mm ³ mesophyll	516.5	318.9 ^a	3686.5	2782.3 ^a
Depth of stomates, μ	11.4	11.7	11.9	13.7 ^b
Cutinized epidermis, thickness, μ	2.3	2.9 ^b	2.4	2.8 ^b
Epidermis thickness, μ	17.9	17.2	14.8	14.9
Cuticle thickness, μ	1.9	2.4 ^b	1.3	1.4 ^b
Mesophyll volume, mm ³	3.7	6.7 ^b	.732	1.17 ^a
Endodermis area in cross section, mm ²	.032	.039 ^b	.030	.029
Phloem area in cross section, μ^2	1636.4	1184.9 ^b	1432.7	1217.5
Xylem area in cross section, μ^2	1258.6	1065.9	1661.3	1392.3 ^b
Transfusion tissue, area in cross section, mm ²	.029	.036 ^a	.023	.027

^a Values differ significantly at the 95 percent level.

^b Values differ significantly at the 99 percent level.

therefore counted on three 0.5-mm lengths located near the tip, base, and middle of the needle or cotyledon, and averaged. This was done on the adaxial and abaxial surfaces for primary needles, but only on the adaxial surfaces for cotyledons; they possessed no stomates on their abaxial surface.

Analysis for significance was accomplished by use of the paired *t* test.

Results and Discussion.—The data for the cotyledons and primary needles of this study are summarized in Table 1.

Cotyledons. The significantly larger number of cotyledons per seedling in Bastrop seedlings may be related to the slightly larger seeds in this provenance; 2.5 g of seed contained 93 seeds in the Bastrop and 107 seeds in the East Texas seed source. Mirov (1967, p 349–395) stated that there is a general tendency for pine species with larger seeds to have more cotyledons. The Bastrop ecotype also had cotyledons which significantly exceeded those of East Texas seedlings in volume, surface area, cross section area, and volume of mesophyll. These characteristics could also result from the larger seed size.

Bastrop seedlings averaged 24.9 stomates per mm² of cotyledon surface, significantly less than East Texas plants, which averaged 30.5 per mm², although the larger cotyledons of Bastrop seedlings had more total stomates. In relation to their size, therefore, Bastrop seedlings should be less exposed to water loss when stomates are open.

Thickness of cuticle and cutinized epidermis were both significantly greater at the 99 percent level, and epidermal thickness was significantly greater at the 90 percent level in cotyledons of Bastrop source. These thicker protective structures should permit less moisture loss through cuticular transpiration under conditions of moisture stress when stomates are closed.

Cotyledons of Bastrop seedlings contained significantly greater amounts of endodermal and transfusion tissue and significantly less phloem than those of East Texas seedlings. The utility of these relationships is not clear. Esau (1960, p 289-291) states that transfusion tissue may function in translocation between vascular bundles and mesophyll. Perhaps the greater amount of this tissue in Bastrop cotyledons plays a role in more effective conduction during periods of favorable moisture.

Primary Needles. Length of needles, area of needle cross section, needle surface area and volume, number of stomates per needle, and volume of mesophyll are all significantly greater in the primary needles of Bastrop seedlings. While these characteristics would lend no advantage during drought, the significantly smaller surface to volume ratio, thicker cuticle and cutinized epidermis, fewer stomates per mm³ of mesophyll and per mm² of surface do indicate the ability to conserve moisture when under stress.

Observations. Qualitatively, it was observed that the mesophyll of primary needles from the Bastrop source was more closely packed than in primary needles from East Texas seedlings. No differences in plication were observed, but greater internal space of East Texas seedlings definitely favors greater gas exchange and moisture loss when stomates are open.

Lumens in epidermal cells of Bastrop cotyledons and primary needles were more occluded than those of East Texas seedlings due to visibly thicker cell walls.

Resin canals were absent in cotyledons of both seed sources. In the primary needles two were always found—one within each acute angle of the needle cross sections, adjacent to the abaxial epidermis.

Both seed sources exhibited a pronounced palisadelike layer directly below the epidermis on the adaxial surfaces. The layer was recognizable, though less continuous, on the abaxial surface. This condition was not found in the cotyledons and is not as organized in mature secondary needles examined in a previous study. Also, certain moisture-conserving characteristics found in cotyledons and primary needles of "Lost Pines" were found in secondary needles of this ecotype (Knauf and Bilan 1974). These characteristics include fewer stomates per unit of surface area and per mm³ of mesophyll; thicker cuticle and cutinized epidermis; and greater depth of stomates.

LITERATURE CITED

- ESAU, K. 1960. Anatomy of seed plants. John Wiley & Sons, New York. 376 p.
- JENSEN, W. A. 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco. 408 p.
- JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill and Co., New York. 532 p.
- KNAUF, T. A., and M. V. BLAN. 1974. Needle variation in loblolly pine from mesic and xeric seed sources. *Forest Sci* 20:88-90.
- MIROV, N. T. 1967. The genus *Pinus*. Ronald Press, New York. 602 p.
- SASS, J. E. 1958. Botanical microtechnique. Iowa State University Press, Ames, Iowa. 228 p.
- THAMES, J. 1963. Needle variation in loblolly pine from four geographic seed sources. *Ecology* 44:168.
- WAHLENBERG, W. G. 1960. Loblolly pine. pp. 20-22. Duke University School of Forestry, Durham, N.C. 603 p.
- ZOBEL, B. J. 1955. Drought hardy tests of loblolly pines. *In Proc Third South Conf Forest Tree Improv*, p. 42-44. USDA Forest Serv, South Forest Exp Stn, New Orleans, La. 132 p.