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ABSTRACT. Relationships among percentage of open stomates, transpiration, and needle moisture content in seedlings of loblolly pine of two Texas provenances were studied under changing soil moisture conditions. Needle moisture content correlated very well with transpiration under favorable moisture conditions, and with percentage of open stomates under soil moisture stress. Transpiration and percentage of open stomates were correlated under a wide range of moisture conditions. Foliage moisture content was still relatively high in both ecotypes when stomates closed and transpiration drastically declined. The "Lost Pines" seed source appeared to have superior ability to conserve moisture under droughty conditions by closure of stomates and reduction of transpiration. FOREST SCI. 23: 457–462.

ADDITIONAL KEY WORDS. Ecotype, drought resistance, moisture relations, stomates, provenance.

IN BASTROP, FAYETTE, AND CALDWELL COUNTIES of central Texas, isolated stands of loblolly pine (*Pinus taeda* L.) exist more than 100 miles west of the continuous range of that species. Known as the "Lost Pines," they generally receive 10–20 inches less annual and 4–6 inches less July–August rainfall than trees in the pine belt of East Texas.

In field experiments, seedlings of Lost Pines provenance survived better than seedlings from East Texas when grown under droughty conditions (Zobel and Goddard 1955, Goddard and Brown 1959). Natural selection in this semiarid environment has apparently produced a more drought resistant strain of loblolly pine.

Drought resistance in a plant may result from drought avoidance—ability to maintain moisture in its tissues, or drought tolerance—ability to survive moisture stress within the plant tissues, or from both (Hopkins 1971). Plants may avoid drought by restricting water loss, by developing extensive or deep root systems, or by both. Some drought tolerant plants are able to maintain life activities when absorbing almost no water (Oppenheimer 1960).

There is some evidence that Bastrop pines survive somewhat higher moisture stress than pines of East Texas provenance.[1] They also produce deeper roots which

[1] Brix, H. 1959. Some aspects of drought resistance in loblolly pine seedlings. Ph D Diss, Tex A&M Univ, College Station, Texas. 94 p.

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may afford access to greater water supplies,[2] but their drought resistance is probably due primarily to ability to conserve water by reduced transpiration (van Buijtenen, Bilan, and Zimmerman 1976). Brix[1] attributed variation in drought resistance of loblolly pines to factors affecting their water economy. Other workers have reported that seedlings from the Lost Pines area have a thicker cuticle and fewer stomates per unit of surface area than similar seedlings from East Texas (Thames 1963, Knauf and Bilan 1972 and 1977). In addition, Haugen[3] found that their stomates began to close at lower levels of moisture stress.

Because stomatal movement is the chief regulator of water loss (Slatyer 1967), especially where leaves have a well-cutinized epidermis, this study simultaneously determined transpiration rates and proportion of open stomates on leaves of loblolly pines of Bastrop and East Texas provenance, as affected by progressive drought.

Procedure

Loblolly pine seeds collected in Polk County, East Texas, and Bastrop County, Lost Pines area, were planted in plastic pots uniformly filled with sandy soil and were grown with frequent watering under 50 percent full sunlight in an air-conditioned greenhouse.

Groups of seedlings from each source, aged 6, 10, and 12 months, were studied in separate experiments. On the youngest seedlings, primary needles constituted 30 percent of foliage surface; at age 10 and 12 months proportions of primary needles were negligible.

The apparatus used for transpiration measurements is similar to one developed by Bierhuizen and Slatyer (1964), and modified by Unterschuetz and others (1974). Cold and warm water baths, each containing a copper tubing coil and an aspirator bottle, regulate temperature and relative humidity within the system. A seedling is enclosed in a plexiglas water-jacketed cuvette equipped with an externally driven fan. When installed in the cuvette, the shoot of the seedling and its potted root system are in separate air- and water-tight compartments. A third constant temperature bath equipped with a water pump helps to regulate temperature within the cuvette. Four 1250 watt self-ballasted mercury vapor lamps light the seedling. Air is driven through the system at a rate measured by two flowmeters which, due to their location, indicate if the cuvette is properly sealed. The relative humidity of the air before and after passing over the seedling is measured by two electric lithium chloride temperature-compensated hygrosensors (Hygrodynamics Type 15-1811) and recorded on a hygrosensor recorder. Hygrosensors are sealed in separate plexiglas chambers and submerged in the water bath which maintains them at a constant temperature. Tygon tubing was used in limited amounts for flexible connections.

Seedlings were organized into experimental pairs (one Lost Pines, one East Texas), based on similarity of shape and size. Five pairs of each age class were used in the experiments. Each pair was acclimated with daily irrigations at 8:00 a.m. in a growth chamber for 1 week prior to the beginning of measurements. The environmental conditions maintained in the growth chamber and cuvette are listed in Table 1.

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[2] Davies, G. 1973. Response of loblolly pine seedlings from two seed sources to favorable and unfavorable moisture regimes. Unpublished M S thesis, Stephen F. Austin State Univ. 76 p.

[3] Haugen, R. M. 1972. Effect of some environmental factors on the stomatal reaction of loblolly pine seedlings from two seed sources. Unpublished M S thesis, Stephen F. Austin State Univ. 53 p.

TABLE 1. Environmental conditions within the growth chamber and the cuvette.

Item	Cuvette	Growth Chamber	
		light	dark
Temperature ..... °C	31	29–35	18–21
Relative humidity ..... percent	45–50	40–50	90–100
Light source	mercury vapor	incandescent/fluorescent	——
Light intensity ..... foot-candles	1000	1000	——
Caron dioxide ..... percent	0.05	0.05	——
Turbulence ..... cm/sec	9.14 cm/sec	——	——
Photoperiod (7 a.m.–8 p.m.) ..... hours	——	13	——
Thermoperiod (7 a.m.–8 p.m) ..... hours	——	13	——

Each pair was subjected to drought by withholding irrigation for 7 to 8 days. Percentage of open stomates and rate of transpiration were determined at 2-hour intervals for 6- and 12-month-old plants and at 3-hour intervals for 10-month-old plants. Monitoring began at 8:00 a.m., 1 hour after commencement of the light period, on selected days during the dry-down period. For each pair, determinations began 24 hours after the latest watering. Except as noted, needle moisture content was determined at 8:00 a.m., and 6:00 p.m. on the same days as the other determinations.

Oppenheimer and Engelbergs' (1965) dye infiltration method was used to observe open stomates. At each observation, before the seedling was placed in the cuvette, one healthy secondary needle from the base of the crown was immersed for 2 minutes in a dye of the following proportions: 9 ml ether, 9 ml chloroform, 0.6 gm methyl violet. After washing in secondary butanol, needles were rinsed in water and the percentage of open stomates was estimated under a binocular microscope to the nearest 1 percent.

Transpiration rate was computed from the increase in relative humidity of the air stream after passing the test plant, and expressed on the basis of total foliage surface area progressively corrected for the removed samples. Foliage surface was computed as the product of needle perimeter at midpoint, needle length, and the number of needles on the seedling. Air flow through the system was 2000 ml/min for seedlings of all age classes.

Readings were taken after each seedling had been in the cuvette for 20 minutes. Since transpiration could be measured on only one seedling at a time, the first plant of each pair was installed in the cuvette on the hour, and the second about 40 minutes later. A minimum of 20 minutes was allowed between seedlings of the pair for equilibration of the apparatus.

Needle moisture content (percent) was determined gravimetrically from one needle from each seedling as follows:

$$\text{NMC} = \frac{\text{fresh weight} - \text{dry weight} \times 100}{\text{dry weight}}$$

Needle samples for moisture content determination were collected from the same position on the crown as the samples for open stomate percentage determination.

Results and Discussion

Because separate analyses of data for each of the three experiments produced similar results, data of all 15 pairs of seedlings were combined for one common statistical

TABLE 2. Average needle moisture content, percent open stomates, and transpiration rate in 1-year-old loblolly pines, by days since last watering.

Day since watering and seed source	Needle moisture content		Open stomates		Transpiration	
	Percent	*t value*	*Percent*	*t value*	*μg/min/cm²*	*t value*
Day 1						
East Texas	224 ± 4	−0.89	22.9 ± 1.1	−1.32	10.77 ± 0.73	0.52
Lost Pines	220 ± 3		20.7 ± 1.2		11.30 ± 0.72	
Day 3						
East Texas	219 ± 4	−1.10	16.8 ± 1.0	−2.38*	6.78 ± 0.35	0.52
Lost Pines	213 ± 4		13.5 ± 1.0		7.08 ± 0.47	
Day 5						
East Texas	213 ± 4	−1.59	9.4 ± 0.9	−3.80***	3.12 ± 0.14	−0.80
Lost Pines	201 ± 4		5.5 ± 0.6		2.92 ± 0.21	
Day 7						
East Texas	193 ± 5	−1.58	4.8 ± 0.5	−3.33***	1.77 ± 0.17	−2.46*
Lost Pines	180 ± 7		2.3 ± 0.5		1.20 ± 0.16	
Entire dry-down period						
East Texas	211 ± 2	−2.31*	12.9 ± 0.6	−3.69***	5.38 ± 0.27	0.80
Lost Pines	204 ± 3		9.9 ± 0.6		5.41 ± 0.30	

* Values differ significantly at the 5 percent level.
*** Values differ significantly at the 0.1 percent level.

analysis. Thus daily averages in Table 2 are based on 30–90 values depending on the number of monitorings per day.

Average needle moisture content for the entire dry-down period was significantly higher (211 percent) in East Texas than in Lost Pines (204 percent) seedlings, but differences for individual days were not significant. General pattern of needle moisture decline from about 225 percent on the first day to 180 percent on the seventh day after the last waterings was similar in both seed sources.

Average percentage of open stomates for the dry-down period as a whole was very highly significantly greater in the East Texas plants than in the Lost Pines plants. Differences significant at the 5 percent level were detected on the third day; on the fifth and seventh day of the dry-down period they were significant at the 0.1 percent level. Average percentage of open stomates declined from 23 on the first day to 5 percent on the seventh day in the East Texas pines and from 21 to 2 percent in Lost Pines pines.

Rate of transpiration was measured at about 11 $\mu g/min/cm^2$ for plants of both seed sources on the first day, but decreased to 1.77 $\mu g/min/cm^2$ in East Texas plants and to 1.20 $\mu g/min/cm^2$ in Lost Pines plants on the seventh day, at which time the difference between seed sources was significant at the 5 percent level. Average transpiration rate for the dry-down period as a whole was about the same; 5.38 $\mu g/min/cm^2$ for East Texas and 5.41 $\mu g/min/cm^2$ for Lost Pines seed sources.

For the dry-down period as a whole, the percentage of open stomates was very highly significantly correlated with needle moisture content and with the rate of transpiration in both seed sources (Table 3). Significant correlations between percentage of open stomates and needle moisture content were observed on days 1 and 7 in East Texas plants and on days 5 and 7 in Lost Pines plants. The highest value of correlation (0.36) existed on the seventh day of the dry-down period.

TABLE 3. Pearson coefficients of correlation among needle moisture content, percent open stomates, and transpiration rate of loblolly pine seedlings from East Texas and Lost Pines seed source, by days since last watering.

Days since last watering and monitored variable	Seed source	Percent open stomates	Transpiration $\mu g/min/cm^2$
Day 1			
Needle moisture content percent	East Texas	–0.3590 (.017)[1]	–0.5578 (.001)
	Lost Pines	–0.0497 (.388)	–0.5682 (.001)
Percent open stomates	East Texas		0.6523 (.001)
	Lost Pines		0.4298 (.001)
Day 3			
Needle moisture content percent	East Texas	–0.1792 (.152)	–0.4407 (.004)
	Lost Pines	0.0253 (.443)	–0.3167 (.032)
Percent open stomates	East Texas		0.4274 (.001)
	Lost Pines		0.2398 (.016)
Day 5			
Needle moisture content percent	East Texas	0.1831 (.129)	–0.0208 (.449)
	Lost Pines	0.2482 (.061)	–0.0039 (.490)
Percent open stomates	East Texas		0.1725 (.047)
	Lost Pines		0.4374 (.001)
Day 7			
Needle moisture content percent	East Texas	0.3655 (.015)	–0.1005 (.289)
	Lost Pines	0.3630 (.016)	0.0417 (.412)
Percent open stomates	East Texas		0.2156 (.031)
	Lost Pines		0.2574 (.015)
Entire dry-down period			
Needle moisture content percent	East Texas	0.2332 (.002)	0.0094 (.454)
	Lost Pines	0.3272 (.001)	0.1207 (.070)
Percent open stomates	East Texas		0.7008 (.001)
	Lost Pines		0.6683 (.001)

[1] Level of confidence.

Significant correlation between percentage of open stomates and the rate of transpiration was detected for every day in both provenances, with the highest coefficients (0.70 for East Texas; 0.67 for Lost Pines) for the dry-down period as a whole.

Needle moisture content and transpiration rate were significantly correlated only on the first and third days in both seed sources. The coefficients were highest on the first day; 0.56 for East Texas and 0.57 for Lost Pines seedlings.

It seems that needle moisture content of loblolly pine seedlings correlates very well with the rate of transpiration under favorable moisture conditions, and with percentage of open stomates under moisture stress. Percentage of open stomates is very well correlated with the rate of transpiration under a wide range of moisture conditions. It was of interest to note that the stomates practically closed and transpiration was drastically reduced at the needle moisture content of 180–190 percent, almost twice that of lethal moisture stress reported by Brix (1960), Stransky (1963), and Davies.[2]

Lower percentage of open stomates and more drastic reduction of transpiration at inceptive moisture stress in the foliage of Lost Pines seedlings are two, of probably many, characteristics which enable this ecotype to maintain itself in the Lost Pines area.

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