

Use of Transpiration Decline Curves to Identify Drought-Tolerant Cotton Germplasm¹

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ABSTRACT

Traditional plant breeding approaches have not been very effective in the development of higher yielding cotton (*Gossypium hirsutum* L.) cultivars for growth under water-deficient field conditions. The purpose of this research was to evaluate the ability of a laboratory technique to identify cotton genotypes with higher growth rates under water stress conditions in the field. The technique consisted of detaching cotton leaves from the plant at sunset and hydrating those leaves overnight. The following day, the leaves were placed under a light-bank in the laboratory and weighed every 2 min as they dried. From that data, transpiration decline curves were plotted. The curves exhibited stomatal and cuticular transpiration phases. The point where those two linear phases intersected was termed "mean stomatal closure" (MSC), and both time and relative water content (RWC) at that point were estimated. Sixteen entries were evaluated for transpiration decline curve components and growth rates in irrigated and dryland field plots in 1978 at Big Spring, Tex. In 1980, 12 different entries were evaluated for the same traits under dryland conditions at the same location.

In 1978, entry differences occurred for all transpiration decline curve components in the irrigated test and for all components, except stomatal transpiration rates, in the dryland test. The interaction between entries and water levels was significant for all components. In 1980, only RWC at MSC and cuticular transpiration rates were different among entries. Growth rates among entries could not be separated statistically within any experiment. However, large negative correlation coefficients were measured between mean RWC at MSC and mean growth rates in the dryland tests in both 1978 and 1980. This relationship indicated that growth rates tended to be higher when the stomates remained open to a lower RWC before closure. The relationship was not significant in the 1978 irrigated test. Transpiration decline curves appear to be useful in screening cotton genotypes for differences in growth rates under conditions of water stress.

Additional index words: *Gossypium hirsutum* L., Osmotic adjustment, Stomatal conductance, Dry matter accumulation, Drought resistance, Relative water content, Detached leaves, Mean stomatal closure.

COTTON (*Gossypium hirsutum* L.) is grown on approximately one million hectares of the Southern Great

Plains under semiarid, nonirrigated conditions. Another million hectares of cotton are grown in the region with some supplemental irrigation. Due to the rapidly declining Ogallala aquifer and the rising cost of energy to pump irrigation water, many of the hectares now irrigated will soon be used to grow cotton without irrigation water. Cotton cultivars which could produce more yield with the available rainfall would be highly beneficial to producers in the region. Cotton breeders have attempted to use traditional selection techniques to increase yield in semiarid, nonirrigated environments. For the most part, these attempts have been unsuccessful because of low yield levels relative to experimental error and the large interaction components between genotype and environment (Quisenberry et al., 1980).

Several other approaches to cultivar development in a semiarid region have been suggested (Hurd, 1976; Quisenberry, 1981). Quisenberry (1981) proposed that the breeding approach toward increasing yield in a semiarid region should be comparable with approaches traditionally followed in the development of disease or insect-resistant cultivars, i.e., initial efforts should be made to discover traits or germplasm which offers resistance (regardless of source). In the case of drought resistance or increased water-use efficiency, screening would be for germplasm with higher dry-matter accumulation (growth rates) under water-stressed field conditions. As for yield, growth rates may be difficult to statistically separate. It may be necessary to identify traits correlated with growth rates and to make selections based on those traits and not on growth rates per se.

A potentially useful trait, stomatal behavior, affects rates of CO₂ uptake by the plant and of water and heat loss from the plant. Much of the reduction in CO₂ assimilation in light during water stress is due to partial stomatal closure impeding the inward passage of CO₂ (Hsiao et al., 1976). Roark and Quisenberry (1977) demonstrated heritable differences in stomatal behavior which also suggested that genetic improvement in the trait should be feasible.

One method used to determine stomatal behavior on a

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large number of plants, a major consideration in any screening technique, is to monitor the rate of water loss from detached leaves under known or constant environmental conditions. These measurements determine, in an indirect manner, the response of stomata as they experience stress resulting from drying of the leaf tissue. Several studies have been conducted using this method of determining water loss (Hygen, 1951, 1953; Slavik, 1958a, 1958b). Cetl (1953, 1957) used the cuticular loss of water from detached leaves after hydroactive stomatal closure to test for drought resistance in barley (*Hordeum vulgare* L.). Roark et al. (1975) divided detached-leaf drying curves (transpiration decline curves) into stomatal and cuticular phases and estimated the time and relative water content at stomatal closure. Those researchers identified intraspecific variation in upland cotton and concluded that leaves of these cultivars developed in semiarid environments reached a lower relative water content before stomatal closure than did leaves from cultivars developed in higher rainfall or irrigated environments.

The purposes of this research were *i*) to estimate the variability among cotton germplasms for drying rates of detached leaves, *ii*) to define the relationship between drying rates of detached leaves and growth rates under water-optimal and water-stressed field conditions and *iii*) to determine the feasibility of using the method to screen cotton germplasm for growth rates under water stress.

MATERIALS AND METHODS

In 1978, 15 photoperiodic (short-day) stocks and one cultivar, 'Blightmaster,' were planted in randomized, complete block designs at two water levels near Big Spring, Tex. The 15 non-flowering stocks were from the World Collection of *Gossypium hirsutum* L. maintained by USDA-ARS scientists at College Station, Tex. These particular exotic stocks were chosen because of their differential growth rates as based on visual observations made over several years of testing numerous photoperiodic stocks at Big Spring.

The two soil-water regimes consisted of fully-irrigated vs. dryland tests located in the same field about 30 m apart. The soil type, previously described by Burnett et al. (1962), was an Amarillo sandy clay loam (Aridic Paleustalfs).

Water was applied to the irrigated test when tensiometer readings in any plot fell below -0.5 bar at a soil depth of 46 cm. This irrigation frequency was chosen to minimize growth inhibition caused by soil-water deficits. About 100 mm of irrigation water was applied at planting and at 45, 56, and 65 days after planting (DAP) for a total of about 400 mm during the test. For more information about the stocks used and the experimental design, the reader is referred to Quisenberry et al. (1981).

A different group of 12 exotic, nonflowering stocks were grown in a dryland test in 1980 at Big Spring. These stocks were randomly selected from the World Collection and had not previously been observed by us when grown under water-stressed field conditions.

Detached leaves from plants grown in 1978 and 1980 were used to evaluate the rate and amount of water loss during the stomatal and cuticular phases of transpiration. Single, uppermost, fully expanded leaves were excised from field-grown plants just prior to sunset (2000 to 2100). Immediately after the leaf was removed from the plant, its petiole was placed in water with its leaf blade in air at 100% relative humidity (RH). The leaves were allowed to hydrate for about 8 hours in the dark. The following day, the leaves were exposed to 340 microeinsteins/m²/sec

photosynthetically active radiation (PAR) for 90 min in air at 100% RH to open the stomates. After this period, the petiole was excised; and the leaf blade was placed under a 340 microeinsteins/m²/sec (PAR) light-bank in the laboratory at 31 C and about 46% RH (range 43–51% RH during different runs). In the lighted area, rate of water loss was determined for each leaf blade by weighing it at 2-min intervals over a 2-hour period. The leaf blades were attached to a moving chain so that each moved in a circle exposing each one to as close to the same temperature and RH as possible. After 2 hours, the leaf blade was removed; and its area and dry weight were determined. These data were used to calculate a transpiration decline curve for each leaf. The curve exhibited what could be interpreted as two distinctive linear slopes: *i*) rate of water loss during the stomatal phase of transpiration and *ii*) rate of water loss during the cuticular phase of transpiration after the stomates had closed (Fig. 1) The intercept of the two slopes was defined as the point of "mean stomatal closure" (MSC). The relative water content (RWC) at MSC and time required to reach MSC were determined for each leaf.

Leaves for the transpiration decline data were taken 72 days after planting (DAP) in 1978 and 64 DAP in 1980. From the field plots, plant dry weights were determined 58 and 86 DAP in 1978 and 50 and 78 DAP in 1980. Growth rates were estimated by subtracting initial dry weights from final dry weights and dividing by the 28 days between harvests.

A small, separate experiment was conducted on a few cotton leaves to evaluate the response of stomates and leaf-water status to leaf drying. A Lambda L65 diffusion porometer³ was used to measure both top and bottom leaf conductances on several detached leaves during a drying cycle. These conductance values were compared with leaf water-loss data to determine the validity of the MSC point. Leaf cutter thermocouple psychrometers were used to measure leaf-water and osmotic potentials during leaf drying. Turgor potentials were then estimated by calculating the differences between total leaf-water potential and the osmotic potentials obtained at each sampling time. Those values were then used to determine the relationship between MSC and zero turgor.

RESULTS AND DISCUSSION

Significant differences among entries occurred in the 1978 tests for all transpiration decline components except for stomatal transpiration rates in the dryland plots (Table 1). Although the uppermost fully expanded leaf was used to collect these data, differences in growth rates between the two water levels (Table 3) would suggest that the physiological age of the leaves tested may have been different. In spite of potential differences in leaf age, overall means for stomatal transpiration rates, RWC at MSC, and time to MSC were not different between the irrigated and dryland plots. Cuticular transpiration rates were greater in the irrigated leaves than in the dryland leaves. The ranges of entry means from the dryland plots were greater for all traits than the ranges of values from the irrigated tests.

Linear correlation coefficients between mean entry values from the irrigated vs. dryland plots were nonsignificant for all four components which suggested that a significant interaction existed between water levels and entries. In fact, analyses of variance across water levels

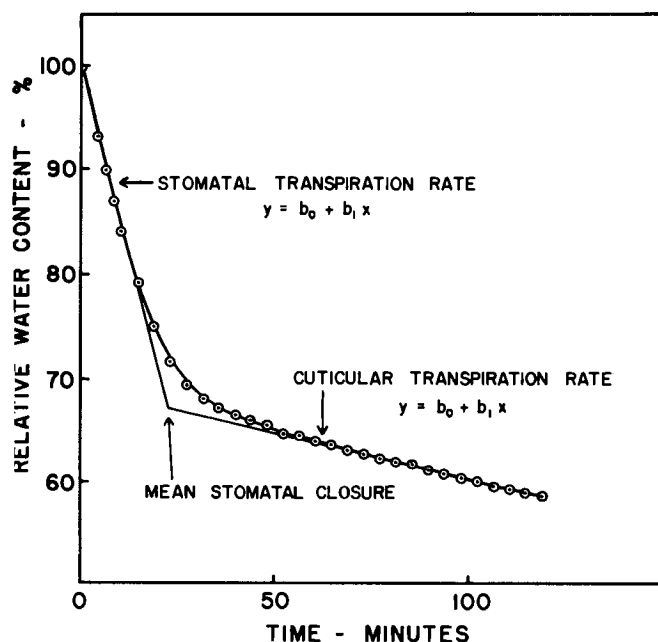
³Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Analyses of transpiration decline curve components for two water levels and 16 cotton entries grown at Big Spring, Tex. during 1978.

Source	Mean squares									
	df		Stomatal transpiration rates		RWC at MSC		Time to MSC		Cuticular transpiration rates	
	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
Reps	2	3	266**	202	52*	102	14	29	1.23	0.94
Entries	15	15	24*	177	53**	367**	29**	104**	2.28**	2.67**
Error	30	45	11	95	18	75	7	23	0.78	0.55
Coefficient of variation (%)			8.2	25.2	6.7	12.7	12.4	20.6	24.2	30.7
			— mg/dm ² /min —		— % —		— min —		— mg/dm ² /min —	
Mean			40.8a†	38.6a	65.1a	68.5a	21.6a	23.3a	3.7a	2.4b
Range			46.1–34.6	48.0–24.7	73.1–57.3	85.3–52.1	26.9–17.0	33.8–14.3	5.5–2.6	5.0–1.5
r between irrigated vs. dryland means			0.12		0.14		0.08		0.03	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

† Means between water levels for a trait followed by different letters were significantly different at the 0.05 probability level based on a Duncan's Multiple Range Test.

**Fig. 1. Transpiration decline curve components defined (actual data points from a typical leaf measurement).**

showed significant water level \times entry interactions for all traits.

In the 1980 dryland test, entries differed in RWC at MSC and in cuticular transpiration rates (Table 2). Coefficients of variation were generally small in 1980 compared to 1978.

Statistically, growth rates among entries within a test could not be separated by analysis of variance techniques in 1978 or 1980 (Table 3). Comparisons among growth rates showed that the dryland plots were significantly more stressed than the irrigated plots in 1978. However, a significant linear correlation coefficient was detached between growth rates for the irrigated vs. dryland entry means during the 1978 tests. The size of the coefficient suggests that 29% of the variation in dryland growth rates among entries could be explained by growth potential as expressed in the irrigated plots (and vice versa).

Table 2. Analyses of transpiration decline curve components for one water level and 12 cotton entries grown at Big Spring, Tex. during 1980.

Source	df	Mean squares			
		Stomatal transpiration rates	RWC at MSC	Time to MSC	Cuticular transpiration rates
Reps	2	64	67**	17*	1.0
Entries	11	18	29*	8	0.9*
Error	22	20	10	4	0.3
Coefficient of variation (%)		9.1	5.1	9.1	16.4
		mg/dm ² /min	%	min	mg/dm ² /min
Mean		48.7	63.2	23.0	3.3
Range		52.2–44.4	70.8–60.2	26.2–21.2	4.3–2.4

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

Linear correlation coefficients between mean entry growth rates and their mean transpiration decline curve components are shown in Table 4. In both dryland tests, RWC at MSC correlated inversely with growth rates (Fig. 2 and 3). This relationship in the 1978 irrigated test was not significant.

A typical relationship between leaf-water content and stomatal conductance is shown in Fig. 4. When leaves were taken from lighted, 100% RH chambers and placed under the light-bank at 46% RH, an initially rapid reduction in conductance occurred followed by a leveling off while the rate of water loss from the leaf continued (but at a reduced rate). As the stomates began to close, the rate of water loss from the leaf blade was reduced. When the stomates had closed, water loss from the leaf should have been primarily through the cuticle.

Turgor pressure reached zero just prior to complete stomatal closure (data not shown). The under-estimation of osmotic potential associated with no estimate of matrix potential was probably responsible for this result. When the nonosmotic volume was estimated at 8%, zero turgor pressure occurred near the point of stomatal closure. In general, it appeared that our estimate of RWC at MSC occurred at zero turgor pressure at conductances less than 0.1 cm/sec.

Turgor pressure appears to be a major factor in deter-

Table 3. Analyses of growth rates for two water levels and 16 cotton entries during 1978 and one water level and 12 entries during 1980 grown at Big Spring, Tex.

Source	df			Mean squares		
	1978		1980	1978		1980
	Irrigated	Dryland	Dryland	Irrigated	Dryland	Dryland
Reps	2	3	2	0.0093	0.0009	0.0191*
Entries	15	15	11	0.0509	0.0024	0.0102
Error	30	45	22	0.0686	0.0037	0.0071
Coefficient of variation (%)				55.3	94.4	46.2
Mean				g/plant/day		
Range				0.47a†	0.06b	0.18
r between irrigated vs. dryland means				0.71-0.24	0.11-0.03	0.26-0.07
				0.54*		

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

† Means between water levels in 1978 followed by different letters were significantly different at the 0.05 probability level based on a Duncan's Multiple Range Test.

Table 4. Linear correlation coefficients between mean growth rates and mean transpiration decline curve components for two water levels and 16 cotton entries during 1978 and one water level and 12 entries during 1980 grown at Big Spring, Tex.

Traits†	1978		1980
	Irrigated	Dryland	Dryland
Stomatal transpiration rates	0.33	0.35	0.20
RWC at MSC	0.32	-0.76**	-0.65*
Time to MSC	-0.23	0.47	0.48
Cuticular transpiration rates	0.13	0.27	0.17

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

† Degrees of freedom were 14, 14, and 10 for the 1978 irrigated, 1978 dryland, and 1980 dryland experiments, respectively.

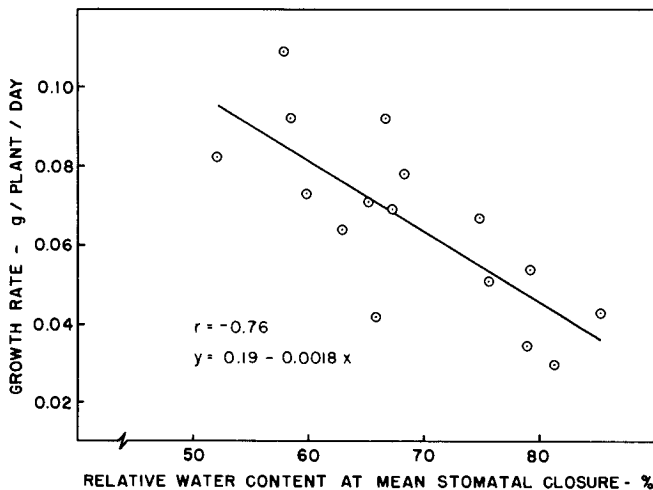


Fig. 2. Relationship between growth rates and relative water content at mean stomatal closure for 16 cotton entries grown on dryland at Big Spring, Tex. during 1978.

mining stomatal action and closure. It has been well documented (Turner, 1974; Hsiao et al., 1976) that to maintain turgor pressure under conditions of water stress, either a reduction in tissue osmotic potential or an increase in cell wall elasticity is necessary. The increase in osmotic potential is from an accumulation of solutes either by concentration, absorption, or synthesis. If the stomates remain open longer in one genotype than in another as water stress develops, then the genotype that keeps its sto-

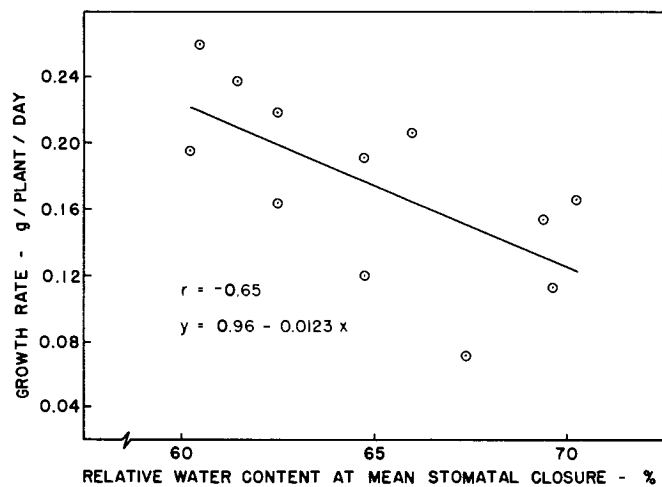


Fig. 3. Relationship between growth rates and relative water content at mean stomatal closure for 12 cotton entries grown on dryland at Big Spring, Tex. during 1980.

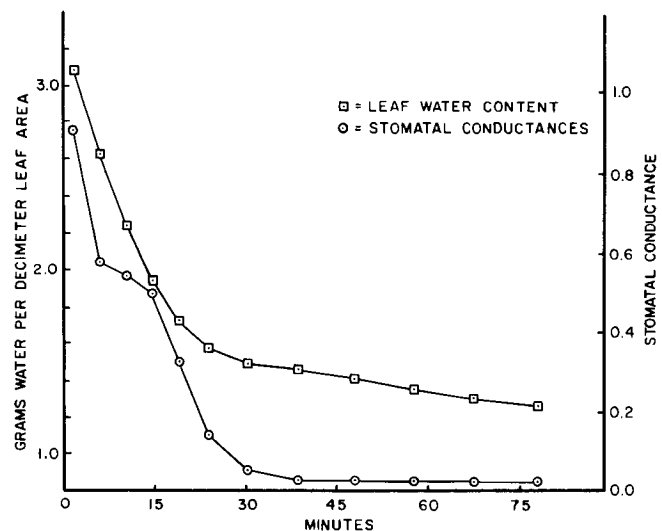


Fig. 4. Relationship between stomatal conductance and detached leaf-water content (actual data points from a typical leaf measurement).

mates open may be able to remain photosynthetically active for a longer period of time and thus result in increased productivity. Ludlow and Ng (1976) have shown evidence that *Panicum maximum* Jacq. var. *trichoglume* plants, that had been conditioned to osmotically adjust, maintained photosynthetic rates at higher (i.e., more negative) water potentials. Brown et al. (1976) have shown that stomates of greenhouse-grown cotton plants closed at higher leaf-water potentials when the plants had been subjected to several wetting and drying cycles. Whether estimates of RWC as MSC is an adequate indicator of osmotic adjustment in the field cannot be conclusively determined from our present data.

In our studies, we have demonstrated that:

i) There were significant differences among cotton entries for RWC at MSC using transpiration decline curves for detached leaves. This variability indicated that under laboratory conditions, the stomata of some entries were closing at lower RWC's than other entries. RWC may estimate some degree of osmotic adjustment,

ii) There was a significant correlation between RWC at MSC and growth rates of the entries when grown under water-stressed field conditions. This correlation demonstrated that field productivity (dry matter accumulation) tended to be greater in those entries which closed their stomata at lower RWC values under laboratory conditions, and

iii) There was not a correlation between RWC at MSC and growth rates under optimal water conditions. This lack of correlation suggests that under favorable water conditions, the ability of a genotype to keep its stomates open at low RWC was not an advantage. Therefore, the germplasm must be grown under water-stressed field conditions to obtain effective separation among entries using this technique.

Based on these results, we conclude that transpiration decline curves appear useful in screening cotton germplasm for differences in growth rates under water stress. If

those higher growth rates can be converted through plant breeding into higher lint yields, it should be possible to develop cotton cultivars which produce higher yields than are now possible under water stress.

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