Published July, 1974

Variability in Leaf Anatomy in Primitive and Commercial Stocks of Cotton[1]

P. R. Morey[2], J. E. Quisenberry, and B. Roark[3]

ABSTRACT

The comparative anatomy of the uppermost leaf having near-complete lamina expansion was studied in primitive and commercial stocks of Upland cotton (*Gossypium hirsutum* L.). Significant between-stock variation for lamina thickness and palisade height occurred when plants were two months old and later when these same plants were six months old. Leaves formed when plants were six months old had a thicker lamina, a taller palisade layer, and wider palisade cells than leaves from two-month-old plants.

Additional index words: **Cotton, Palisade, Spongy parenchyma.**

INTERSPECIFIC differences in leaf mesophyll structure are well documented in the genus *Gossypium*. Among American cottons, *G. barbadense* and *G. tomentosum* are characterized by the presence of palisade tissue underlying both the upper and lower epidermis (11). In *G. hirsutum*, where palisade tissue usually underlies only the upper leaf surface (11), a lower palisade sometimes develops in leaves from the middle level of large plants (1). In the Old World cultigen, *G. herbaceum*, well-defined palisade layers underlie both leaf surfaces even in young plants (1).

[1] Joint contribution from Texas Tech University and ARS-USDA in cooperation with the Texas Agricultural Experiment Station. Supported in part by the Institute for University Research of Texas Tech University. Received Feb. 12, 1974.

[2] Associate Professor, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409.

[3] Research Geneticist and Plant Physiologist, respectively, ARS-USDA, Texas A & M University Agricultural Research and Extension Center at Lubbock, Route 3, Lubbock, TX 79401.

The occurrence of intraspecific differences in leaf anatomy in *Gossypium* is less well documented and is difficult to separate from structural changes induced by variation in light intensity, water stress, and position of the leaf itself on the plant axis. The purpose of this study is to determine if significant variation in leaf mesophyll structure occurs in primitive and commercial stocks of *G. hirsutum.*

MATERIALS AND METHODS

Two commercial cultivars, 'Deltapine 16' and 'Western Stormproof,' were studied. Deltapine 16 (DPL-16) has a wide range of adaptability and is grown throughout most of the U. S. Cotton Belt. Western Stormproof (W.S.) is somewhat limited in cultivated distribution to certain semiarid portions of the Cotton Belt. Seventeen primitive stocks also were investigated. These included representatives of each of the perennial races of *G. hirsutum* L., and as such should have represented a wide range of phenotypic variation within this species (3). Representative plants of all stocks were grown singly in clay pots and arranged in a randomized block design on greenhouse benches. Two plants of each stock were examined anatomically.

Although leaf structure is genetically determined, it is known to vary phenotypically with position on the plant (4). Accordingly, leaf tissue used in this study was sampled when the plants were 2 months old (February) and again when the same plants were 6 months old (June). All leaves sampled were developmentally similar, i.e., each was the uppermost leaf on the main axis having near-complete lamina expansion. Leaves sampled when the plants were 2 months old were located approximately at the fifth node. Leaves sampled when the plants were 6 months old were located approximately at the 25th node above the cotyledons. A 2 $\times$ 2 cm tissue sample that included the extrafloral nectary of the midvein was taken from each leaf for histological examination. Leaf tissue was fixed in formalin-acetic acid-alcohol, embedded in paraffin, sectioned transversely, and stained with safranin-fast green.

All quantitative measurements were made from portions of interveinal leaf tissue that was entirely devoid of trichomes and gossypol glands. For each anatomical character a total of 50 or more measurements were made from sections taken throughout the 2 $\times$ 2 cm tissue sample from each leaf. Tissue sections were microtomed at a thickness of 10 and 5 μ for leaves of 2-month- and 6-month-old plants, respectively. The dense packing of cells of the mesophyll in leaves from 6-month-old plants necessitated the use of thinner sections in order that cell boundaries might be adequately defined. *Lamina thickness* was measured from the upper to the lower cuticular surface. The length of palisade cells medianly along their long axis was defined as *palisade height. Palisade cell width* was then determined at a point halfway along the long axis. *'Tissue ratio'* (12) was the summed thickness of the spongy parenchyma and the epidermal layers divided by palisade height. Low tissue ratios have been suggested to be indicative of leaf xeromorphy (12). *Substomatal chamber depth* was measured for the lower leaf surface only. A vertical line was drawn through the open pore of a stoma to the point of intersection with the first underlying spongy parenchyma cell. Substomatal chamber depth was defined as the straight line distance between the inner periclinal walls of the two guard cells and the surface of the spongy parenchyma cell. Substomatal chamber depth was not measured for leaves of 6-month-old plants because of the presence of a loosely developed lower palisade. *Epidermal wall thickness* was measured as a straight line across both the primary cell wall and the cuticle. *Raised and sunken stomata,* respectively, were defined as stomata where both guard cells were elevated above or depressed below a line drawn through the midplane of the epidermal layer. Epidermal wall thickness and percent raised and sunken stomata were determined for both epidermal surfaces.

RESULTS AND DISCUSSION

Variations in Leaves of 2-Month-Old-Plants. Significant between-stock differences occurred both for lamina thickness, palisade height, and tissue ratio (Table 1). Lamina thickness was correlated (r= .83) with palisade height. Thus stocks with thick leaves tended to have a tall palisade. Lamina thickness and palisade height have also been positively correlated among *Citrus* sp. (2). A thick lamina, a tall palisade, and a low tissue ratio have been suggested as indications of leaf xeromorphy (7). In dicotyledonous leaves, a taller palisade was positively correlated with a greater exposed internal surface and a higher rate of transpiration (9).

Table 1. Structural characteristics of leaves from 2-month-old cotton plants.

Stock†	Lamina thickness	Palisade height	Palisade width	Tissue ratio
	μ			
W. S. (lat)	139	49	9	1.9
DPL-16 (lat)	112	40	8	1.8
T1 (pal)	119	43	8	1.8
T25 (pun)	141	55	9	1.6
T45 (pun)	132	47	8	1.8
T111 (mg)	130	48	9	1.7
T125 (mor)	128	49	9	1.6
T143 (lat)	126	46	9	1.7
T144 (pun)	136	50	9	1.7
T145 (ric)	109	39	9	1.8
T169 (lat)	106	42	7	1.5
T179 (lat)	123	43	8	1.9
T192 (mor)	133	53	9	1.5
T223 (lat)	133	43	8	2.1
T252 (-)	148	60	10	1.5
T256 (ric)	125	50	9	1.5
T292 (mor)	128	45	10	1.9
T488 (pun)	134	49	9	1.7
T1236 (yuc)	146	50	9	1.9
LSD .05	24	10	1	.4
LSD .01	31	14	1	.5

† Racial identity: lat = latifolium, mor = morrilli, ric = richmondi, pal = palmeri, pun = punctatum, yuc = yucatanense, mg = marie-galante. T252 is unclassified. Authority: Regional Research Project S-77, Gossypium Germ Plasm Catalogue.

Considerable variation in palisade cell width occurred between stocks (Table 1). Palisade cell width was correlated with lamina thickness (r= .69) and palisade height (r= .69).

The intercellular space system of the spongy parenchyma as estimated by substomatal chamber depth varied significantly between stocks. In contrast, in a study of leaves of *Lolium* sp. little difference was found in the proportion of air space within the spongy mesophyll (6). Differences in substomatal chamber depth in cotton may be meaningful both in terms of the amount of interveinal translocation between spongy parenchyma cells in the plane of the lamina and the amount of internal cell wall surface available for gaseous exchange. Substomatal chamber depth showed little correlation (r $<$ .50) with other structural characteristics including palisade height and lamina thickness.

In 2-month-old plants, variation was minimal between stocks for the thickness of the epidermal cell wall on both surfaces and the percentage of raised and sunken stomata in both epidermal layers.

Variations in Leaves of 6-Month-Old Plants. Leaves of 6-month-old plants exhibited strong between-stock variation for lamina thickness, upper palisade height, palisade cell width, and tissue ratio (Table 2). It was of interest that lamina thickness, as in leaves from younger stocks, was correlated both with upper palisade height (r= .96) and palisade cell width (r= .65).

A palisade layer was found along the lower surface in leaves of all 6-month-old plants. Considerable variation, however, was found in palisade height and the density of parenchyma cells in this layer even in leaves of plants from the same stock. The number of pali-

Table 2. Structural characteristics of leaves from 6-month-old cotton plants.

Stock	Lamina thickness	Upper palisade height	Upper palisade width	Tissue ratio
	μ	μ	μ	
W.S. (lat)	214	91	11	1.3
DPL-16 (lat)	223	95	10	1.3
T1 (pal)	142	58	10	1.5
T25 (pun)	148	65	10	1.3
T111 (mg)	124	48	8	1.6
T125 (mor)	150	60	9	1.5
T145 (ric)	167	65	9	1.7
T179 (lat)	159	65	9	1.5
T192 (mor)	193	80	9	1.5
T252 (-)	198	80	10	1.5
T292 (mor)	145	60	9	1.4
T1236 (yuc)	214	77	10	1.8
LSD .05	58	31	2	.4
LSD .01	71	38	2	.5

Table 3. Structural comparisons between leaves of 2-month-old and 6-month-old cotton plants.

Anatomical character	2-month-old plants Mean ± S.E.	6-month-old plants Mean ± S.E.	t values
Lamina thickness, μ	130 ± 7.6	173 ± 18.5	5.20***
Upper palisade height, μ	48 ± 3.4	70 ± 10.0	6.13***
Upper palisade width, μ	8.8 ± .3	9.6 ± .6	3.23**
Tissue ratio	1.7 ± .13	1.5 ± .13	4.35***

,* indicate statistically significant differences for an anatomical character between 2-month-old plants and 6-month-old plants at the 1 and .1 percentage levels, respectively, as compared by a paired t-test.

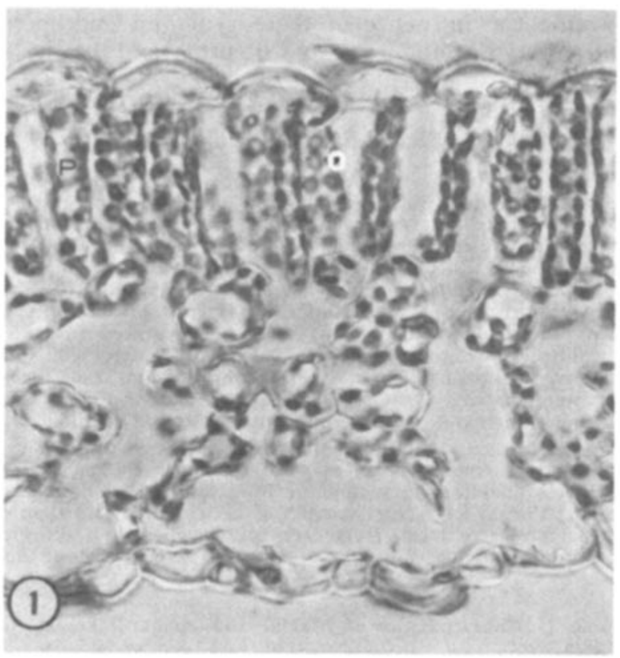

Fig. 1. Transverse section of leaf from 2-month-old primitive stock (T192) of *G. hirsutum* showing upper palisade (P) and extensive intercellular space system of spongy parenchyma. Phase contrast micrograph. (565 ×).

sade parenchyma cells per millimeter of length of lower epidermal surface was consistently higher below minor veins without bundle sheath extensions.

Comparisons of Leaves Produced on 2-Month and 6-Month-Old Plants. Leaves from 6-month-old plants were consistently more xeromorphic than leaves of 2-

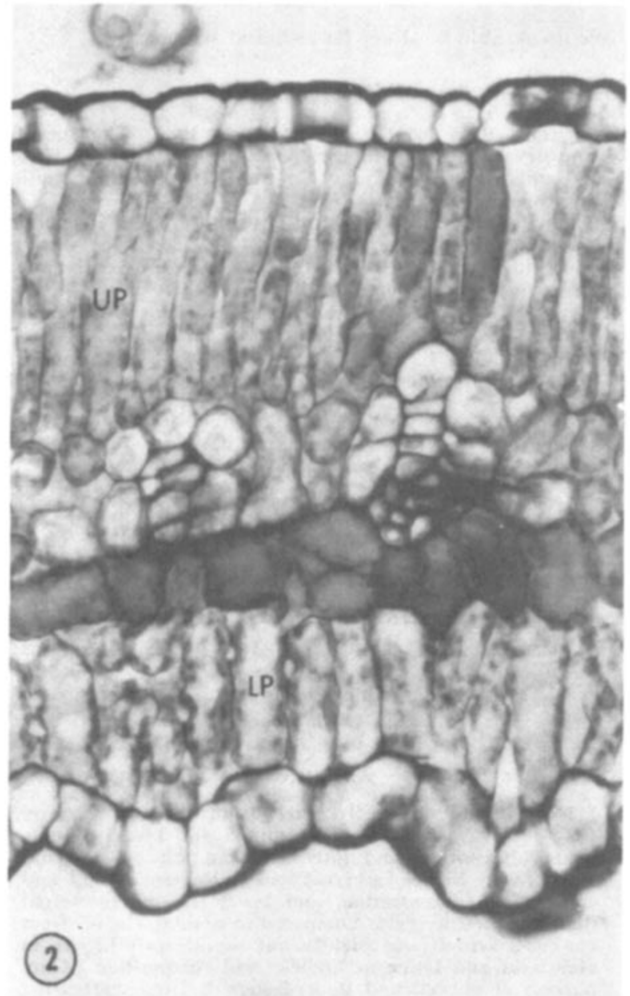

Fig. 2. Transverse section from 6-month-old primitive stock (T192), showing a tall upper palisade (UP) and a short, less dense, lower palisade (LP). Lamina thickness and palisade height were consistently greater in leaves from 6-month-old stocks (cf. with Fig. 1). Bright field micrograph. (565 ×).

month-old plants. By xeromorphic we mean that the leaves of older plants had a thicker lamina, a taller palisade, and a lower tissue ratio (Table 3). In addition, a lower palisade layer was present only in leaves from 6-month-old plants (cf. Fig. 1 and 2). These differences collectively suggest that leaves developed later in ontogeny were more xeromorphic than leaves formed during the earlier growth stage. Water stress and high light intensity have been implicated as causing leaf xeromorphy and specifically as environmental factors promoting the development of a taller palisade (5,7,8,9). In cotton plants subjected to chloride salinity, taller than normal palisade cells were developed (10). Whether or not such anatomical characters as a tall palisade, a thick lamina, and a low tissue ratio imply a degree of drought tolerance in cotton, however, should be interpreted with caution since xeromorphic structure by itself does not necessarily imply xeromorphic adaptation (7).

ACKNOWLEDGMENT

We thank Elsie D. Morey for technical assistance.

REFERENCES

1. Baranov, P. A., and A. M. Maltzen. 1937. The structure and development of the cotton plant. Laboratory of Cytology and Anatomy of the Central Plant-breeding Station, Moscow-Leningrad.
2. Halma, F. F. 1929. Quantitative differences in palisade tissue in *Citrus* leaves. Bot. Gaz. 87:319-324.
3. Hutchinson, J. B. 1951. Intra-specific differentiation in *Gossypium hirsutum*. Heredity 5:161-193.
4. Levitt, J. 1972. Responses of plants to environmental stresses. Academic Press, New York, 697 p.
5. Penfound, W. T. 1931. Plant anatomy as conditioned by light intensity and soil moisture. Amer. J. Bot. 18:558-572.
6. Sant, F. I. 1969. A comparison of the morphology and anatomy of seedling leaves of *Lolium maltiflorum* Lam. and *L. perenne* L. Ann. Bot. 33:303-311.
7. Shields, L. M. 1950. Leaf xeromorphy as related to physiological and structural influences. Bot. Rev. 16:399-447.
8. Transeau, E. N. 1904. On the development of palisade tissue and resinous deposits in leaves. Science 19:866-867.
9. Turrell, F. M. 1944. Correlation between internal surface and transpiration rate in mesomorphic and xeromorphic leaves grown under artificial light. Bot. Gaz. 105:413-425.
10. Waisel, Y. 1972. Biology of halophytes. Academic Press, New York, 395 p.
11. Webber, I. E. 1938. Anatomy of the leaf and stem of *Gossypium*. J. Agr. Res. 57: 269-286.
12. Wylie, R. B. 1951. Principles of foliar organization shown by sunshade leaves from ten species of deciduous dicotyledonous trees. Amer. J. Bot. 38:355-361.