STUDIES ON THE MORPHOGENESIS OF THE STORM RESISTANT CHARACTER IN STORMPROOF NO. 1 COTTON

By

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INTRODUCTION

The tendency of seed cotton to remain in the boll after maturity and the manner of boll dehiscence is of great significance to the cotton cultivator. Complete opening of the boll necessitates repeated harvesting during the cotton picking season. This aspect of the harvesting problem is of great economic value in the high plains of Texas and Western Oklahoma. According to Jones <u>et al</u> (12) and Shanklin <u>et al</u> (21) hand harvesting averages about $40 per bale.

Jones <u>et al</u> (12) and Smith <u>et al</u> (23, 24) point to the various aspects of the economic value of mechanical harvesting. Because more and more cotton farmers are becoming mindful of mechanical harvesting, better types of cotton plants suited for the mechanical harvester are of urgent need. This necessitated the need of a germ plasm pool that could yield the required characteristics for mechanical harvesting-type plants.

Fortunately, Mr. H. A. Macha, of Tahoka, Texas, noticed a cotton plant in his field that had not lost its cotton following a severe sandstorm in 1926. Mr. Macha saved, grew, and increased the seed from this plant. He distributed commercially in 1937 (under the name of Macha) the increased seed. The seeds of the original plant were used as a source of germ plasm in the breeding of storm-resistant type cotton plants. Stormproof No. 1, so called because of its storm-resistant character, was derived from Macha.

The present work was begun with an aim to the elucidation of the stormproof character. The diagnostic methods of anatomy and morphology were applied to determine the existence of variant characters that would be of aid in plant selection. The problem has a two-fold object; namely, to determine the morphogenesis of the storm-resistant character in Stormproof No. 1 and to aid in the solution of a plant breeding problem.

REVIEW OF LITERATURE

The mode of growth and the development of the cotton plant has been a subject of discussion by several investigators (2, 4, 6, 7, 10, 14, 15, 19). The development of the Stormproof No. 1 cotton plant does not differ significantly from the development of cotton plants (Gossypium hirsutum L.) discussed by these investigators. For this reason it is unnecessary to dwell upon that phase of the subject.

The stormproof character was described by Lynn (13) as follows:

> Strains of cotton pure for this character which open in October will firmly retain the seed cotton in the boll until January or later. The locks do not fluff as in normal bolled cotton, but are firmly packed into the burr and appear to be glued in the burr by a secretion. As the boll opens the strong attachment of the fibers to the burr causes a "stretched" appearance of the fibers between the carpel segments. When a lock of cotton is pulled from the burr, the fibers are seen to be entangled in cracks in the carpel walls.

The conclusion that the stormproof boll-type in G. hirsutum is due to simple inheritance was based by Lynn (13) on the analysis of the F_2 population, which could be segregated into 1:2:1 and 3:1 ratios.

The advantage in harvesting stormproof cotton is discussed by Parrott et al (16). Because the fibers of Stormproof No. 1 remain knitted in the bur and do not fall to the ground, the open bolls may be left in the field until after frost occurs and the entire crop harvested in one operation. For this reason, stormproof cotton is recommended for harvesting with a mechanical stripper (16).

A condition in Indian and Indian-American cotton which resembles the fiber adherence in stormproof cotton is reported by Singh (22). In all the bolls examined by Singh, most of the fibers were attached in the pits located at the base of the boll. Upon tearing the seed off the boll, a mass of fibers remain firmly adherent to the pit walls; also, quite a number of fibers remain attached to the sutures on the lower half of the boll.

Boll dehiscence of G. sanguineum, G. N. roseum, G. indicum, and the herbaceum groups of cotton was studied by Abraham (1). Faulty boll-opening in G. indicum was thought to be due to fission of the ventral bundles to a level above the position of the first basal ovule. This condition did not exist in the other cottons studied by Abraham. Therefore, he made genetic studies of faulty boll-opening using the following crosses and their reciprocals.

(a) G. indicum (N.14) x G. N. roseum

(b) G. indicum (N.14) x G. sanguineum

The analysis of the F_2 data showed that the character for "faulty-opening" segregated in a simple 3:1 ratio.

"Bad-opening" of the boll was investigated by Dastur (5) in the American cottons grown in India. There appeared to be a fundamental difference between "bad-opening" occurring in American cottons (G. hirsutum) in the Punjab and "bad-opening" that occurred in the Egyptian cotton (G. barbadense) in the Sudan. In one case, the seeds as well as the lint were immature; while in the other case, the lint alone was found to be immature, the seeds being generally quite hard and sound. In the Punjab and Sind, "bad-opening" or premature opening of the boll was found to be associated with two types of soils--viz., (i) sandy lands deficient in nitrogen, and (ii) soils with a high concentration of salt varying from 0.15 to 0.5 per cent in the sub-soil.

"Bad-opening" as described by Dastur (5) refers to the quality of the lint and seed, whereas Abraham (1) refers to the spreading of the carpels. There is some confusion as to the meaning of "bad-opening" as used by foreign investigators, and one should not consider the term comparable to the term stormproof as used by American investigators (3, 12, 13, 16).

A perusal of the literature on the subject of storm-resistance in cotton reveals the lack of anatomical and morphological studies.

MATERIALS AND METHODS

To determine the morphogenesis of storm-resistance in cotton, two cotton varieties, chosen because of their special characteristics, were studied at Stillwater, Oklahoma, during the years 1951-1953. The two varieties were Stormproof No. 1 and Oklahoma Special. Oklahoma Special was used as a check because it was considered to be the least storm-resistant variety in the Stillwater area.

The cotton plants were grown in 25-foot rows spaced 42 inches apart at the Oklahoma A. & M. agronomy farm, Perkins, Oklahoma. Cotton flowers of both varieties were tagged at anthesis for forty consecutive days during the 1951 season. Dated bolls were collected to obtain a continuous series from the first day of flowering to thirty-four days subsequent to this date. In order to observe the natural arrangement of the fibers within the boll, one- to six-day bolls were preserved whole; the remaining bolls in the series were cut longitudinally with a new single edge razor blade and immediately placed in formalin acetic acid-alcohol (FAA) reagent (20). All remaining bolls were left on the plants to mature for gross studies. The mature bolls were collected during the last week of November, 1952.

The microscope slides were prepared for anatomical examination by the following schedule. The bolls were transferred from FAA to a 70% n-butyl alcohol solution. Every two hours thereafter, dehydration was continued by the n-butyl alcohol technique.

Because infiltration of the whole boll by ordinary microtechnical methods would have required prolonged heating, low pressure infiltration was decided upon. The bolls were placed in vials containing 52-54° C melting point paraffin. A one-hole stopper, containing a glass tube with a rubber hose, was placed over the mouth of the vial and put into an electric heated oven (54-56° C). The unattached end of the hose was pushed through a hole in the side of the oven and connected to an aspirator. The pressure was reduced by slowly increasing the flow of water to capacity, then the bolls were allowed to aspirate 2.5 hours. The low pressure method of infiltration proved highly successful and was used in all subsequent infiltrations.

For embedding, approximately 20 grams of pure beeswax was added to 240 grams of paraffin (Texwax). The bolls were sectioned longitudinally and transversely at 15-20 microns and fixed on microscope slides with Szombathy's fixative. A combination of safranin O-fast green FCF (20) was used for differential staining. Sections of leaves and petioles were prepared for anatomical studies in the same manner as the bolls.

To differentiate the components of the mucilage found in the lysigenous glands, a combination of Harris hematoxylin (11) and orange G was employed. The staining schedule was as follows:

1. removed paraffin

2. hydrated to water in alcohol series
3. stained in Harris hematoxylin for 15-20 minutes
4. dehydrated to 100% alcohol
5. counterstained for 24 hours in a 1% solution of orange G in 100% alcohol
6. rinsed by dipping into two changes of 100% alcohol
7. cleared in xylene or toluene for 20 minutes
8. mounted coverslip with balsam

To facilitate the counting of the guard cells, sections of leaves 1.5 cm. square were cut from five different parts of the leaf. Each area was replicated from leaves of six different plants. The six sections from the same locations were placed for 20 minutes in five separate identifiable bottles containing FAA solution. The chlorophyll was removed by transferring the leaf sections into 100% ethyl alcohol heated by a hot water bath. Clearing was accomplished with a 5% sodium hydroxide solution. After being cleared for 72 hours, the sections were washed in tap water until all the sodium hydroxide was removed, then dehydrated to 70% alcohol. The sections were stained in a 1% solution of neutral red in 70% alcohol. The 70% alcoholic neutral red solution served two purposes; namely, to stain the epidermis and to preserve the material while the counts were being made. Leaf sections were removed from the solution, washed in 70% alcohol, placed on a Rafter counting cell, and covered with a coverslip. The number of guard cells occurring in each unit area of 0.5329 square millimeters was counted. Thirty-eight of these areas were chosen at random from each leaf section. Counts of these areas on the lower and upper epidermis were made and recorded.

The following method was used to observe the fibers that were adhered to the ovary wall of immature bolls. Four- and six-day bolls, which had been collected and preserved as described in a preceding page, were washed for two hours in running tap water. Each boll was removed from the water, blotted dry, and cut longitudinally into four quadrants. The adhered fibers inside the bolls were exposed for observation by carefully teasing the fibers away from the ovary wall. The fibers were classified as many, few, and none according to their degree of adhesion. These observations were recorded and later compared with mature open bolls of the same plants in the field.

The height of the vascular branching between the carpel suture was measured. The mature bolls used were cleared of the involucre and calyx. Divider points were placed between the edge of the receptacle at the base of the boll and at the point where cleavage between the two carpels ceased. This distance was recorded in millimeters.

To determine the solubility of the adhesive that held the fibers to the ovary wall, twenty mature Stormproof No. 1 bolls were placed in a 1% aqeous solution of neutral alkyl aryl sulfonate. Ten bolls were placed in each of the following solutions: acetone, xylene, ether, 100% ethyl alcohol, and a solution of equal volumes of alcohol and xylene. The bolls remained in the solutions twenty-four hours. They were removed, allowed to dry and then examined.

Raw cotton fibers were tested for the presence of protein by two different methods. Millon (8) reagent was used to test for tyrosine and Berg ninhydrin (8) reagent was used to test for the alpha-amino acid group.

The angle of boll spread was measured by the Smith et al (24) method.

All cell measurements were made by using a Bausch and Lomb research microscope with an ocular micrometer at high dry magnification; 645x.

The ratio between the rate of elongation of the cells that form the locule wall and the increase in circumference of the locule was determined by the following method. The length of the elongated cells was measured in one- and thirty-four-day bolls and the ratio determined. The heights of the isosceles triangle at median cross-section in one- and thirty-four-day bolls were measured and the ratio determined. Interpretation of the above data was based on the fact that in similar triangles, the perimeter varies directly with the perpendicular height of the triangle. Since the elongated cells form the perimeter of the triangle, these two ratios can be compared and deviations from a 1:1 ratio become significant.

EXPERIMENTAL RESULTS

No attempt will be made to discuss the morphological development of the cotton plants from the seedling stage to maturity. This aspect of the problem has been sufficiently reviewed by previous investigators. Since Stormproof No. 1 does not differ significantly from other cotton plants in its general development, this report concerns itself with the development of the square after flowering, the observation made on the mature plant and such variant characters as were observed.

Cyto-morphological studies

Well-developed cavities of lysigenous origin were found to exist in the pericarp and septum of one-day bolls of both varieties. The lysigenous cavities increased in size as the bolls matured. At ten days after anthesis these cavities extended throughout the entire length of the septum, leaving intact only the vascular elements. Smaller isolated cavities were scattered throughout the ovary and in the receptacle at the base of the pits.

The mucilage produced by these glands diffuses into the surrounding intercellular spaces and through the septum wall. The mucilage stained a dark red when fast green FCF-safranin O combination was used as the staining agent. However, when a combination of Harris hematoxylin was counter-stained with orange G, the mucilage of Stormproof No. 1 stained a purplish blue with a few yellow spots. Given the same treatment, the mucilage of Oklahoma Special stained yellow with a few purplish blue spots.

Cotton fibers within locules of three-day Stormproof No. 1 bolls were found to radiate from the seed coat and they formed clusters by joining at their periphery. Upon further elongation, the fibers forming these packets extend into the pit at the base of the carpel, around the dehiscent ridges, and between the placenta and septum. The fibers more distal from these structures align themselves along the carpel wall. Convolutions (Fig. 1) due to differential growth of the carpel wall occur in the storm-resistant variety. Cotton fibers aligned along the carpel wall become entrapped and are held in these convolutions. Further growth of the cotton fibers eventually fill the locule and press tightly against the locule wall. These results were somewhat consistent for the non-storm-resistant variety. However, fibers of the three-day bolls did not extend into the afore-mentioned structures, nor did the fibers become entrapped in convolutions.

At the base of each carpel of both varieties a pit (Fig. 2) is formed which extends into the receptacle. In cross section, the pit has a spherical triangular shape. The pit may extend straight downward or may curve away from the perpendicular (Fig. 3) as much as 45 degrees and the diameter and depth of these pits vary in size.

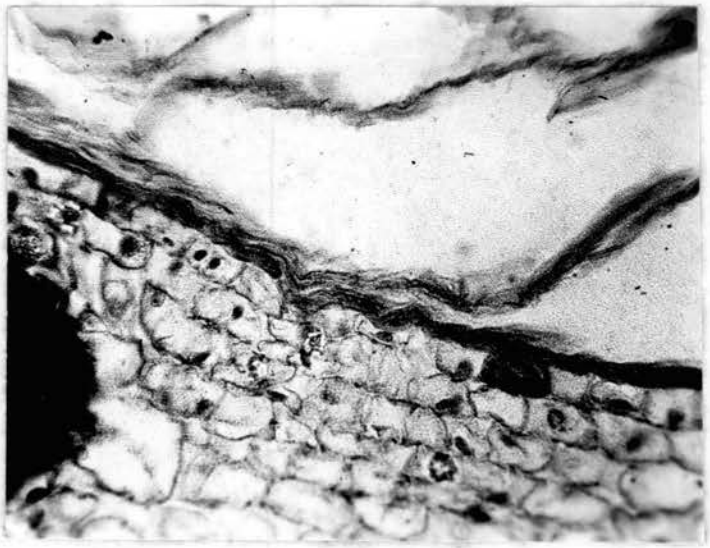


Figure 1. Transverse section of three-day Stormproof No. 1 boll showing early formation of convolutions with fibers adhered alongside locule wall.

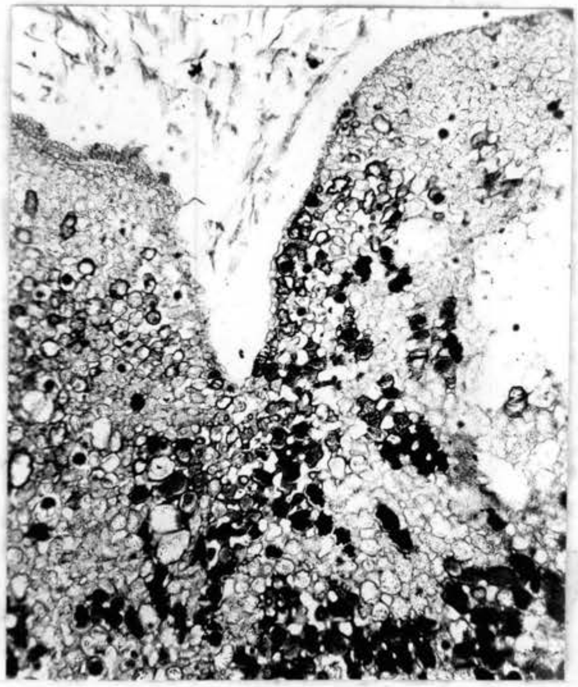


Figure 2. Oklahoma Special. Longitudinal section through pit extending into receptacle of six-day boll; x150.

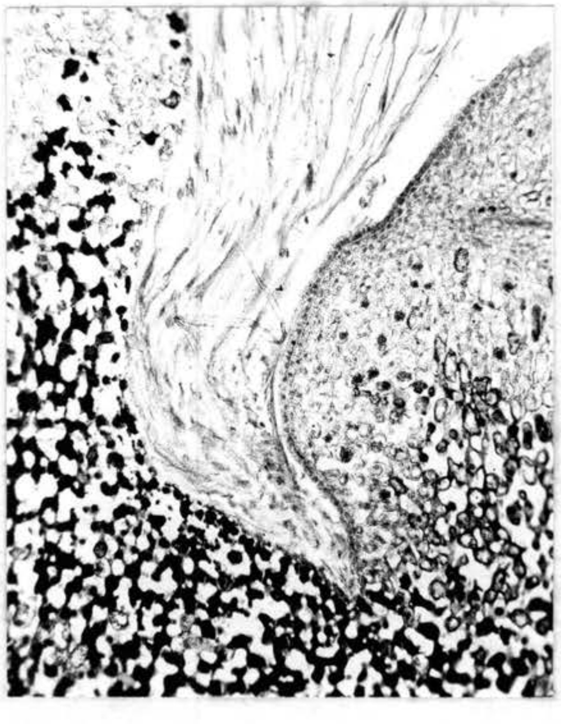


Figure 3. Stormproof No. 1. Longitudinal section through pit extending into receptacle of six-day boll; x150. Note curvature away from perpendicular.

Examination of one- to twelve-day bolls of Stormproof No. 1 showed many carpels, each possessing a small rudimentary structure located between the placenta ridges and below the basal ovules (Fig. 4). No differentiation of tissue was observed in these structures. They were composed of parenchymatous tissue, their size and shape varied and no epidermal outgrowths were observed from the epidermis. These structures can be recognized from the ovules by their smaller size, by their lack of differentiation and by their location between the placental ridges. These protuberances were not observed in Oklahoma Special.

Examination of longitudinal and cross sections of the storm-resistant receptacle showed a preponderance of glandular cells between the vascular elements and the base of the locule. These glands surround the pit (Fig. 5) which extended into the receptacle. The parenchymatous cells as well as the glandular cells were loosely arranged having an aerenchymous appearance, and they were connected by trabeculae (Fig. 6). A vastly different condition was found in the receptacle of the non-storm-resistant variety (Fig. 7). These cells were more compact and there were fewer glandular cells.

Fibers were found to penetrate not more than a cell in depth into the protuberance of the dehiscence ridge within the locule (Fig. 8). This condition was found to exist as early as three days after anthesis. In all the bolls examined, no evidence could be found of fibers penetrating the protuberance of the dehiscence ridges in Oklahoma Special.

Figure 4. Stormproof No. 1. Nearly median longitudinal section through rudimentary protuberance in six-day boll; x150.

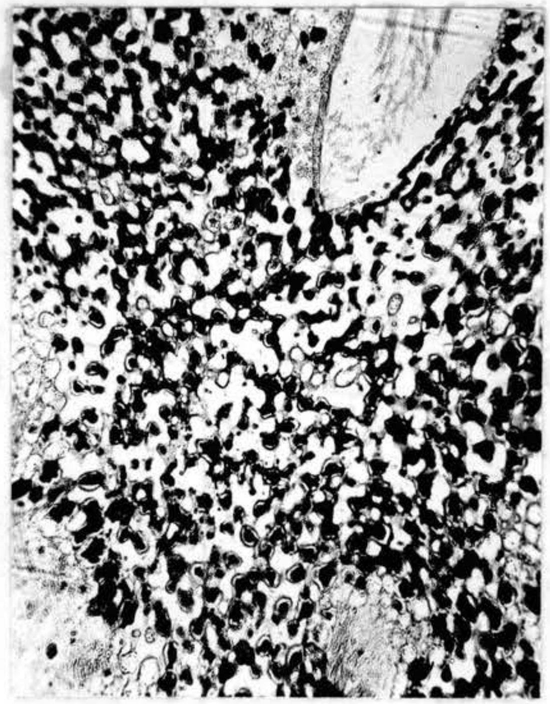


Figure 5. Longitudinal section of six-day Stormproof No. 1 boll showing glandular cells surrounding the pit; x150.

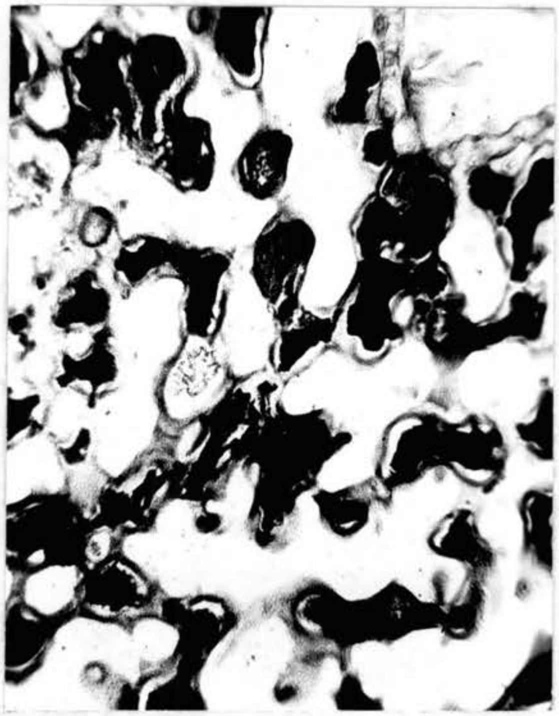


Figure 6. Longitudinal section showing trabeculae; x645.

Figure 7. Longitudinal section of Oklahoma Special Boll showing lack of glandular cells surrounding the pit and lack of intercellular spaces; x150.

Figure 8. Transverse section showing penetration of cotton fiber into dehiscence ridge of three-day Stormproof No. 1 boll; x645.

Development of the boll

Cytological examination of young squares of both varieties preceding anthesis revealed that growth of the ovary wall took place by cellular division. Mitotic figures were found in cross and longitudinal serial sections of young squares. In squares a few days before anthesis, mitotic figures decreased in number and became difficult to find. At anthesis, no figures could be found and cell division was assumed to cease. This period of quiescence ceased upon fertilization, and boll enlargement rapidly ensued.

In both varieties, the outline of the pericarp cells at median cross sections of one-day bolls were elliptical when viewed in a plane surface. The elliptical cells are considered to have two radii, i.e., a long and short radius. The longest radii of the pericarp cells in one-day bolls are aligned in the same plane as the diameter of the boll; thus, the shortest radii of the cells are perpendicular to the diameter of the boll. Examination of the pericarp cells of one- to ten-day bolls at median cross section showed these cells to become spherical as they enlarged. Upon further cellular enlargement (11-34 days after anthesis), these same cells became elliptical once again. However, the radii of the cells that were the shortest radii in the pericarp of one-day bolls became the longest radii in the pericarp cells of twenty-two- to thirty-four-day bolls. The axes translations, by as great as 90 degrees, was a feature of the maturation process. Intercellular spaces and the subsequent enlargement thereof occurred as the pericarp cells enlarged. The longest and the shortest diameters of the elliptical cells in the pericarp of one- to thirty-four-day bolls of Stormproof No. 1 were measured (Table 1). The cells of thirty-four-day bolls were approximately 2.5 times larger than those of one-day bolls.

The number of adjacent cells that made up the thickness of the pericarp was counted in each quadrant or lock of the above boll series. A summary of these findings is found in Table 2. The thickness of the pericarp at median cross sections of Stormproof No. 1 was found to be one millimeter at anthesis. Twenty-two days after anthesis the pericarp reached its maximum thickness of 1.5 mm. Thereafter, there was no appreciable increase in thickness.

The length of the elongated cells that lined the locule of one- to thirty-four-day bolls was measured. The length at anthesis was found to be 36.5 microns in the stormproof variety; those of thirty-four-day bolls were found to be 363.6 microns. These cells increased their length at a ratio of 1:10. The same cells of the non-storm-resistant variety were 40.8 microns at anthesis and 259.2 microns 34 days later. This is a ratio of 1:6.35.

The diameter of one-day bolls in the storm-resistant variety was between 6.0 mm. and 6.75 mm., and those of thirty-four-day bolls were between 34.0 mm. and 35.0 mm. The median cross sectional shape of the locule was essentially that of an isosceles triangle with a curved base. The length of the perpendicular height of the locule was 1.75 mm. at anthesis and 14.25 mm. 34 days subsequent to this date.

Table 1. Summary of the diameters in microns of the parenchymatous cells at median cross section of Stormproof No. 1 bolls.

	Age of bolls in days													
	1		4		6		10		17		22		34	
Diameters[1]	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Region I[2]	26.4	16.8	24.0	19.2	31.2	19.2	43.2	31.2	55.2	36.0	50.4	19.2	52.8	24.0
	28.8	15.6	36.0	21.6	40.8	21.6	43.2	33.6	57.6	43.2	76.8	28.8	76.8	33.6
	20.4	14.4	40.8	28.8	36.0	28.8	31.2	26.4	60.0	52.8	84.0	24.0	72.0	38.4
	19.2	12.0	40.8	36.0	48.0	36.0	52.8	36.0	62.4	45.6	52.8	31.2	38.4	24.0
	26.3	19.2	38.4	31.2	48.0	31.2	57.6	40.8	43.2	40.8	55.2	28.8	60.0	26.4
Sum	121.2	78.0	180.0	136.8	204.0	136.8	228.0	168.0	278.4	218.4	319.2	132.0	300.0	146.4
Average	24.2	15.6	36.0	27.4	40.8	27.4	47.6	33.6	55.7	43.7	63.8	26.4	60.0	29.3
Region II[2]	33.6	16.8	36.0	33.6	40.8	33.6	64.8	60.0	81.6	64.8	67.2	43.2	62.4	60.0
	36.0	16.8	43.2	28.8	40.8	45.6	42.4	36.0	72.0	60.0	60.0	50.4	76.8	48.0
	24.0	12.0	36.0	24.0	36.0	36.0	52.8	48.6	76.8	55.2	67.2	48.0	57.6	57.6
	24.0	14.4	36.0	26.4	43.2	40.8	40.8	40.8	69.6	62.4	63.8	60.0	76.8	60.0
	33.6	16.8	38.4	28.8	52.8	40.8	52.8	52.8	60.0	52.8	84.0	60.0	62.4	48.0
Sum	151.2	76.8	189.6	144.0	213.6	196.8	280.8	235.2	360.0	295.2	342.2	261.6	336.0	273.6
Average	30.2	15.4	37.9	28.8	42.7	39.4	56.2	47.0	72.0	59.0	69.4	52.3	76.2	54.7

[1] Diameters a and b refer to the longest and shortest respectively.

[2] The pericarp was divided into two parts for this study. Region I designates that area at median cross section between the locule wall and the vascular elements. Region II refers to that area between the vascular elements and the epidermis.

Somewhat similar measurements were obtained for the non-storm-resistant variety. The thickness of the pericarp varied between 0.75 mm. and 1 mm. for 1-day bolls. Twenty-two days after flowering, the pericarp reached its maximum thickness, 1.5 mm. The diameter of 1-day bolls was between 6.0- and 6.5 mm. at median cross section; and those of 34-day bolls were 35-36 mm. The length of the perpendicular height of the locule at median cross section was 1.75 mm. at anthesis and 14.50 mm. 34 days after anthesis.

Table 2. Number of adjacent cells at median cross section of Stormproof No. 1 pericarp.

	Age of Bolls in Days						
	1	4	6	10	17	22	34
Lock 1	50	49	49	45	42	41	42
Lock 2	42	38	44	38	49	43	40
Lock 3	39	40	40	42	37	42	41
Lock 4	37	39	47	41	38	45	39
Total	168	166	180	166	166	171	162
Average	42	41.5	45	41.5	41.5	42.8	40.5

The maximum enlargement of the boll in both varieties was attained between 22-25 days after anthesis, but a considerable period further elapsed (20-23 days) before the boll was ready to open.

In the pericarp a double row of special cells was differentiated during the development of the carpel. The vascular strands divided into two parallel branches and the dehiscence line developed between them. This branching takes place at the base of the carpel at a point where cleavage generally ceases. The height of the vascular branching in open bolls of Stormproof No. 1 was found to be between 3-5 mm. from the base of the receptacle. The height of the vascular branching in Oklahoma Special was found to be between 3-4.25 mm. (Table 3).

Table 3. Frequency table of the height of vascular branching in cotton bolls of Stormproof No. 1 and Oklahoma Special.

Height in mm.	No. of Bolls Stormproof No. 1	 Oklahoma Special
3.00	9	26
3.25	6	3
3.50	10	9
3.75	6	3
4.00	9	9
4.25	1	0
4.50	3	0
4.75	3	0
5.00	3	0
Total	50	50

Leaf and leaf petiole studies

A slight variation in length was found between the guard cells of the upper and lower epidermises in Stormproof No. 1 leaves. The length of the upper epidermal guard cells was approximately 28.8 microns; the length of the lower epidermal guard cells was approximately 26.4 microns. The two characteristics which the upper and lower epidermal guard cells have in common are the shape (in transverse section) and the thickness of the wall next to the aperture (Fig. 9, 10); the most noticeable difference is in their location. The guard cells in the upper epidermis are slightly sunken beneath the surface and are overarched by the ends of the epidermal cells adjacent to them. Those in the lower epidermis are on a level with the adjacent surface of the epidermal cells.

In general, the lengths of the guard cells in the upper and lower leaf epidermises of Oklahoma Special are similar to those of Stormproof No. 1. However, the guard cells are characteristically different in their abutment with the adjacent cells. Those on the upper epidermis are set on "oval-like" epidermal cells (in transverse section) which elevates them above the surface of the epidermis (Fig. 11). The guard cells in the lower leaf epidermis are similar to those in the storm-resistant variety (Fig. 12).

The cellular outline of the leaf epidermal cells is quite different in the two varieties. These differences are easily discernable upon examination of the photomicrographs of these cells (Fig. 13). In both varieties, each guard cell is flanked by only three epidermal cells.

A considerably larger quantity of mechanical tissue is present in the leaf petiole of Stormproof No. 1 (Fig. 14a) than in the leaf petiole of Oklahoma Special (Fig. 14b). The vascular bundles of the former are narrower in length and width. The walls of the mechanical tissue of Stormproof No. 1 are thicker; however, their diameters are shorter. In Oklahoma Special there is a larger amount of cortical tissue.

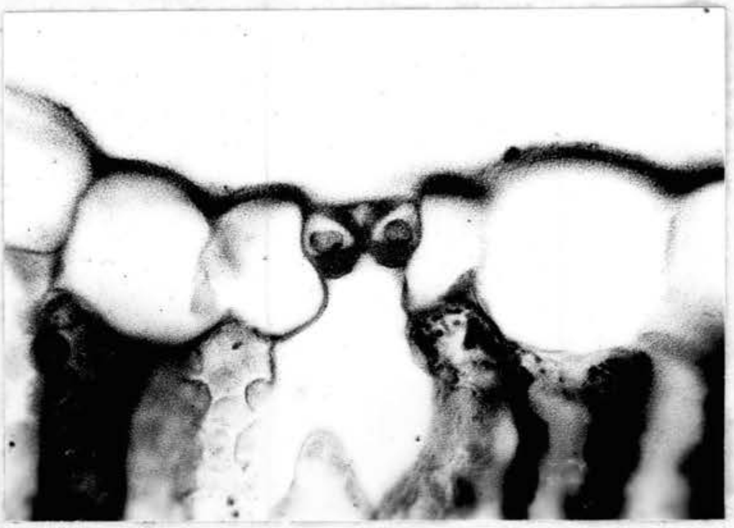


Figure 9. Transverse section through guard cell on upper leaf epidermis of Stormproof No. 1; x645.

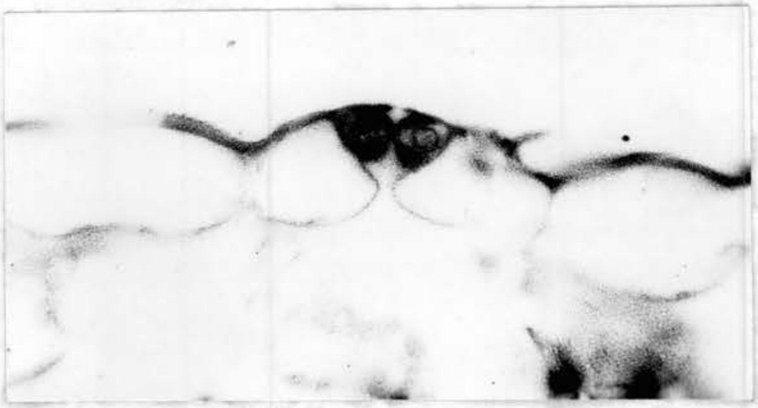


Figure 10. Transverse section through guard cells on lower leaf epidermis of Stormproof No. 1; x645.

Figure 11. Transverse section through guard cell of upper leaf epidermis of Oklahoma Special; x645.

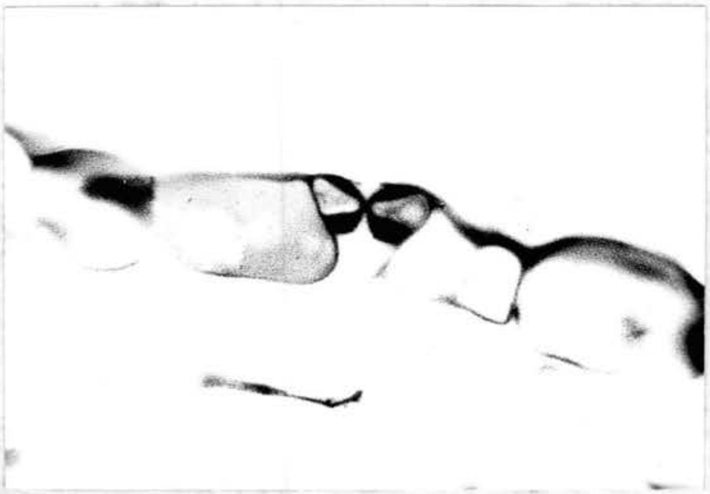
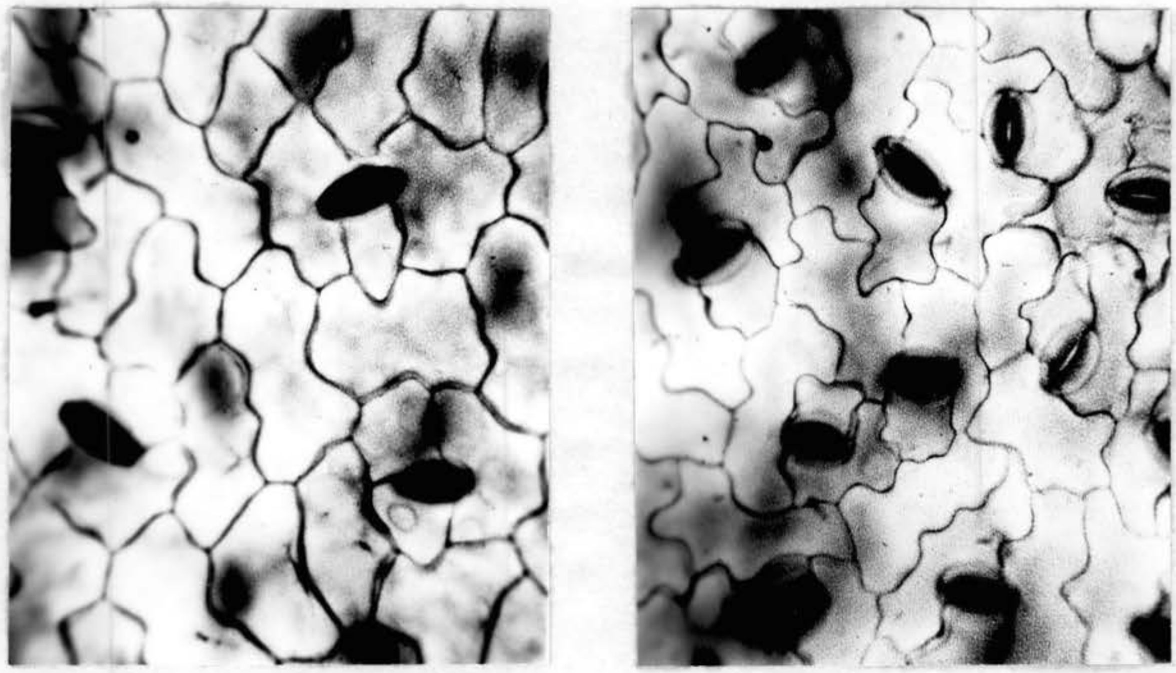
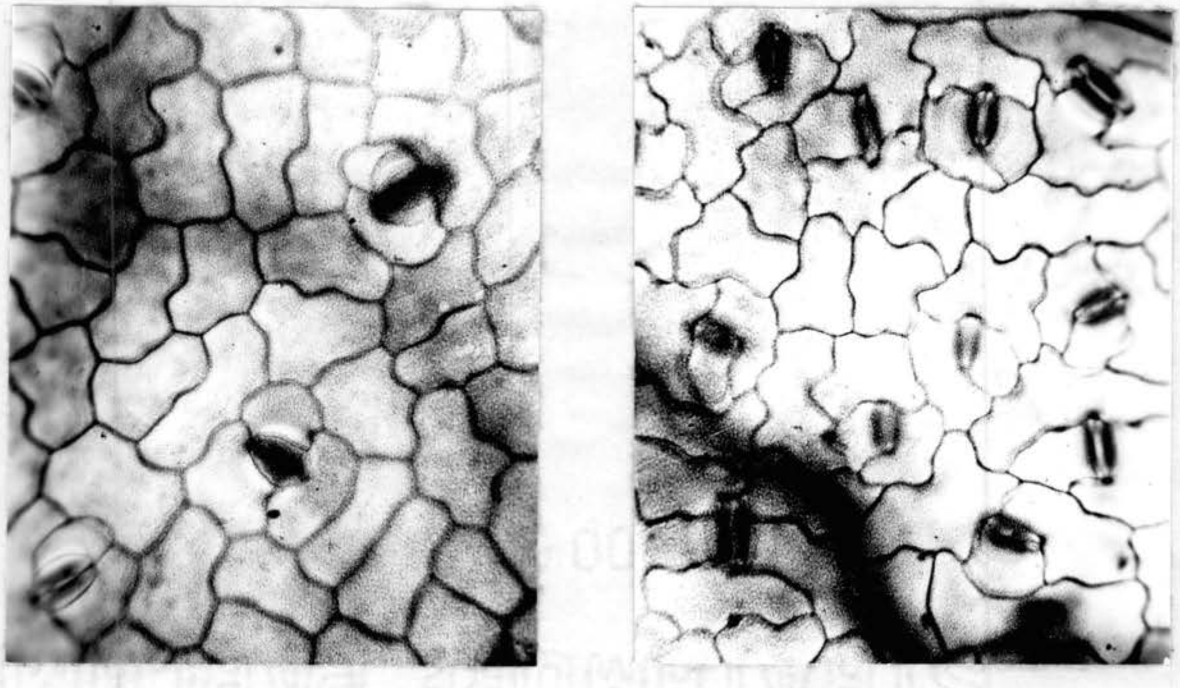


Figure 12. Transverse section through guard cell of lower leaf epidermis of Oklahoma Special; x645.



a. upper epidermis b. lower epidermis



c. upper epidermis d. lower epidermis

Figure 13. Stormproof No. 1 leaf epidermis (a, b); x645. Oklahoma Special leaf epidermis (c, d); x645.

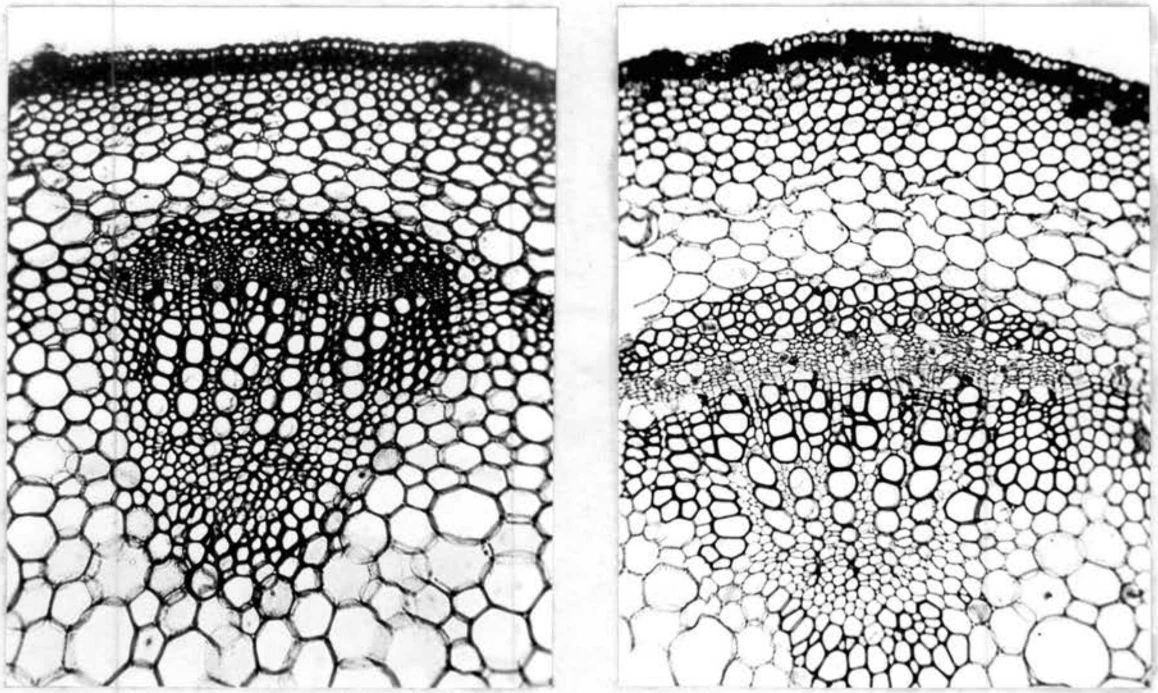


Figure 14. A. Nearly median transverse section from leaf petiole of Stormproof No. 1 showing a vascular bundle; x150. B. Approximately median transverse section from leaf petiole of Oklahoma Special showing vascular bundle; x150.

This condition may be associated, to a considerable extent, with the greater petiole diameter of Oklahoma Special.

No significant differences existed between stomata number per unit area between Stormproof No. 1 and Oklahoma Special. However, there was great variation between the number of stomata within a section of the leaf in both varieties. The preceding results are applicable for the upper and lower epidermises. The analysis of variance of stomata number is given in Table 4.

Table 4. Analysis of variance of stomata number per unit area on upper and lower leaf epidermises of Stormproof No. 1 and Oklahoma Special.

Upper Epidermis

Source of variation	d.f.	Sum of squares	Mean square	F
Varieties	1	22.87	22.87	1.52
Leaf Sections	4	24.46	6.12	0.41
Variety vs. Sections	4	14.81	3.70	0.25
Error	50	751.57	15.03	--
Total	59	813.71	---	--

Lower Epidermis

Source of variation	d.f.	Sum of squares	Mean square	F
Varieties	1	15.74	15.49	0.16
Leaf Sections	4	74.74	18.67	0.19
Variety vs. Sections	4	30.83	7.71	0.08
Error	50	4822.81	96.46	--
Total	59	4944.12	---	--

Mature boll studies

The mature bolls of Stormproof No. 1 and of Oklahoma Special are strikingly different (Fig. 15). The fibers in

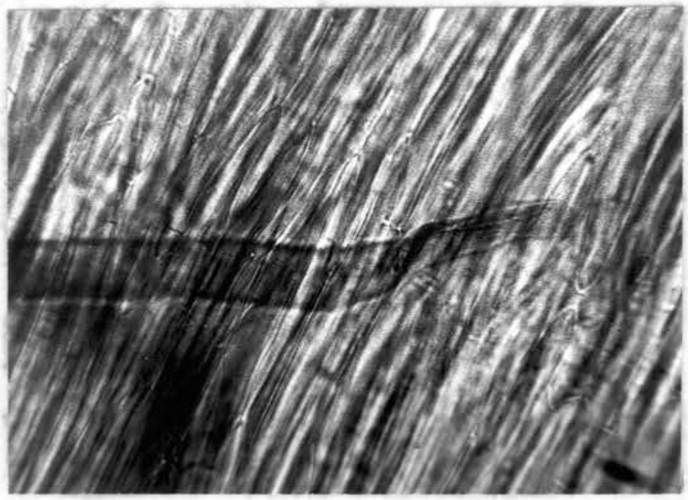


Figure 18. Cotton fiber of Stormproof No. 1 embedded between cells of the septum; x645.

Figure 19. Carpel of Oklahoma Special showing sheen and lack of convolutions in the septum.

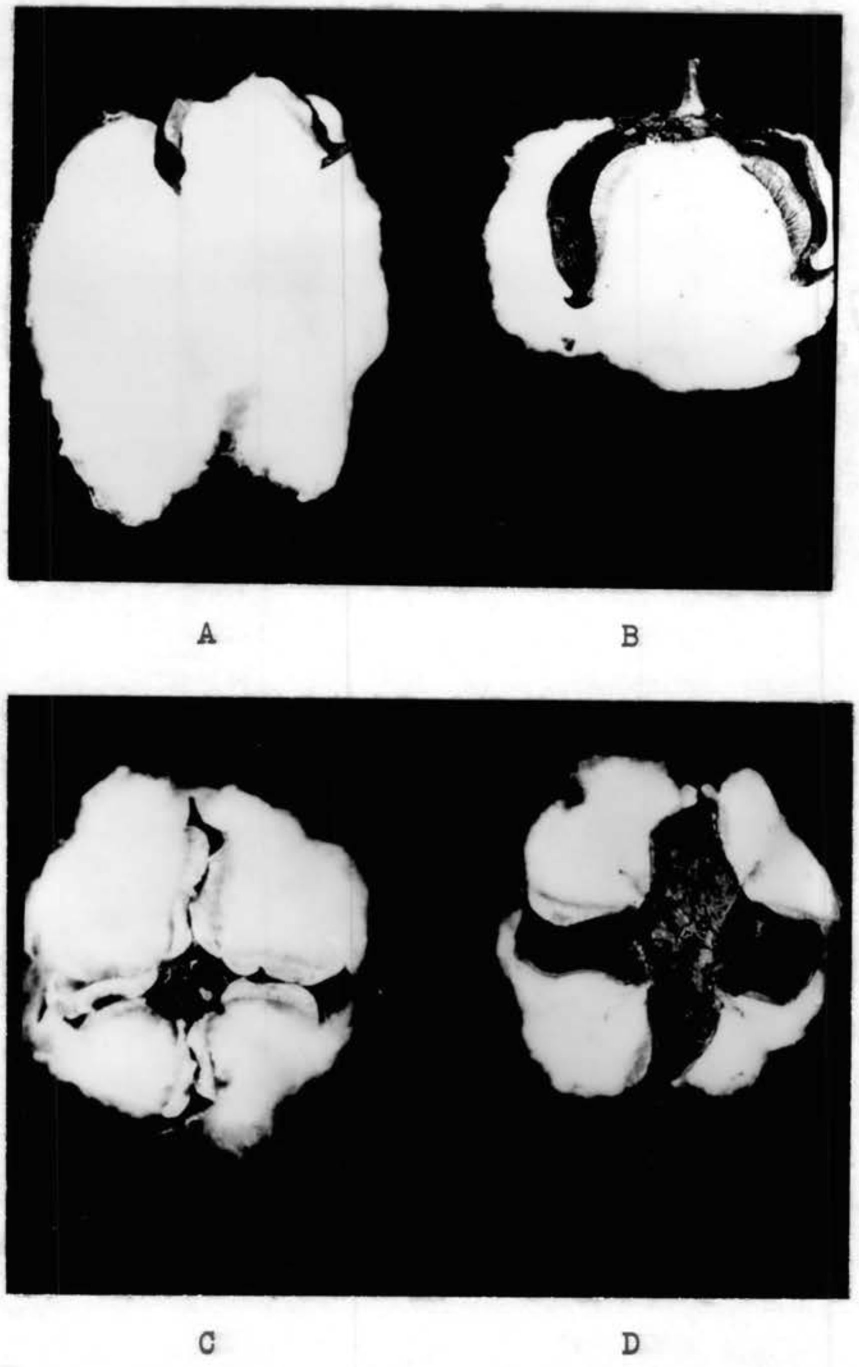


Figure 15. A and B. Side view of mature cotton bolls of Oklahoma Special and Stormproof No. 1 respectively. Note cupped appearance of B.

C and D. Top view of Oklahoma Special and Stormproof No. 1 bolls respectively. Note the slight backward reflexing of the carpel edges in D.

Stormproof No. 1 bolls do not string out and are retained within the bur. The carpel does not open fully, having a cupped appearance. The average angle of boll opening was 120 degrees. The carpels are broad with slight reflexing at the extreme margins of the pericarp (Fig. 15D). The inner surface of the carpels of the Stormproof No. 1 bolls was dull in appearance and had convolutions which were tightly compressed (Fig. 16). These convolutions were largest and deepest at the base of the carpel. Between each carpel septum and the pericarp, a deep crevice is formed. The dehiscence ridges of the placenta are reflexed tightly against the carpel septum. Investigation revealed that the cotton fibers were held tightly (i) between the convolution, particularly at the basal region of the carpel, (ii) between the placenta ridges and the carpel septum, and (iii) in the crevice formed at the peripheral margin of the lock septum (Fig. 17), as well as within the pits at the base of each carpel. A few cotton fibers were found that seemed to penetrate between the cells of the septum (Fig. 18). To prepare the material for the fiber penetration study was extremely difficult, therefore, no conclusions may be drawn from the limited observations made.

Many cotton fibers were also found to be adherent to the carpel walls by a secretion from the lysigenous glands. The adhesive substance was insoluble, to a great extent, in water, acetone, xylene, ether, 100% ethyl alcohol, or a solution of equal volumes of 100% ethyl alcohol and xylene, since few cotton fibers would loosen by this treatment.

Figure 16. Carpel of Stormproof No. 1 showing convolutions formed in the septum.

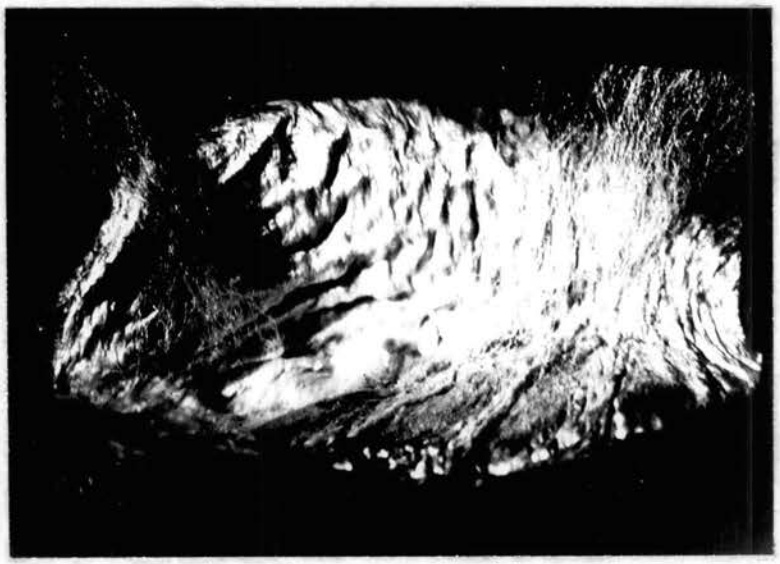


Figure 17. Carpel of Stormproof No. 1 showing crevices formed at peripheral margin of lock septum. Note trapped fibers.

The non-storm-resistant bolls of Oklahoma Special were quite unlike those of Stormproof No. 1. The angle of boll opening (average 140 degrees), the backward reflexing of the pericarp (Fig. 15c), and the lack of convolutions in the carpel (Fig. 19) as well as the lack of cotton fiber adhesion to the carpel wall caused the locks to string out (Fig. 15a). In contrast to the dull appearance of the carpel wall of Stormproof No. 1, the carpel wall of Oklahoma Special has a sheen.

Mature open bolls from Stormproof No. 1 plants were classified into three groups, depending on the degree of storm-resistance. Each classification of immature bolls from these plants was compared with the classification of the mature open bolls in the field of the same plant. These comparisons were based on the degree of fiber adhesion in the immature bolls to the degree of "storm-resistance" in the mature open bolls. The object of this comparison was to determine the relationship between the fiber adhesion in the immature bolls to the degree of storm-resistance in the open boll.

Out of 20 plants having immature bolls with many fibers adhered to the locule wall, 14 plants had storm-resistant mature bolls, and 2 plants had normal type mature bolls. Out of 47 plants having immature bolls with few fibers adhered to the locule wall, 19 plants had storm-resistant mature bolls, and 11 plants had normal type mature bolls. Out of 7 plants having immature bolls with no fibers adhered to the locule wall, 3 plants had storm-resistant mature bolls, 3 plants had intermediate storm-resistant mature bolls, and 1 plant had normal type mature bolls.

Histochemical studies

Histochemical tests for protein were performed on raw cotton fibers of both varieties to determine whether the mucilage which caused the fibers to adhere to the septum of Stormproof No. 1 differed in a protein group reaction. No indication of tyrosine in either variety was obtained using Millon reagent on the raw cotton fibers. Positive results were obtained for the alpha amino acid group in cotton fibers of both varieties when the Berg ninhydrin test was made. The Oklahoma Special cotton fibers stained a uniform red-purple color. The Stormproof No. 1 fibers ranged in color from a purplish-brown to a pale pink. Whether the reaction took place with the protein in the fibers or with the dried mucilage covering the fibers was not determined. Two reservations, however, should be kept in mind: (i) ninhydrin may show a positive reaction with ammonium salts of strong acids in high concentrations, (ii) some amines may give a positive test also. It may safely be assumed that there is no high concentration of an ammonium salt of a strong acid, but ammonium salts of a weak acid might be present in the mucilaginous material.

DISCUSSION

The results obtained in this study indicate that there are several contributing factors that influence the stormproof character of the boll. These factors are as follows: the restricted boll opening, the slight backward reflexing of the carpel edges, the convolutions formed within the bur that entrap the cotton fibers between them, and the adhesion of the cotton fibers to the locule wall. Which of these factors is the most influential could not be determined. Upon examination of a typical storm-resistant boll, it becomes evident that all these factors contribute to the character. Examination of intermediate stormproof bolls revealed at least one of these factors to be missing; and, in extreme cases, the lack of any one of these characters may render the resulting boll non-storm-resistant.

The early occurrence of the fibers adhering to the locule wall and the role of the mucilage in this connection is still uncertain. Since both varieties produce mucilage, the question as to why they should not behave similarly may be asked. A partial answer to this is found in the differential staining of the mucilage with the Harris hematoxylin-orange G staining technique. However, this merely indicates that the mucilage reacts differently with the stains. The variation in color obtained from the staining reaction may lead one to assume that other differences may exist between the mucilages produced by the two plants, one of which is the adhesive property of the mucilage produced by the Stormproof No. 1 plant.

In all microscopic investigations of the developing fibers, no indications were found that the fibers themselves were involved in the production of the adhesive. To further elucidate whether the cotton fibers were involved in the production of the adhesive, histochemical studies for protein were carried out. Although the reaction between the cotton fibers of the two varieties differed, no parallelism can be drawn as to the correlation of the stormproof character, since this test was performed on two cotton varieties only. A dyeing technique described by Goldthwait et al (9) illustrates the use of dyeing to determine cotton fiber maturity. The ninhydrin test may prove fruitful in this respect.

The retention of the fibers within the pit is not an exclusive feature of stormproof cotton. Singh (22) reported the role of these pits in fiber retention in Indian and Indian-American cottons which were studied by him. Many non-storm-resistant bolls examined at Stillwater had fibers held within their pits, but this feature alone is not sufficient to retain the fibers within the bur.

The penetration of the fibers into the pericarp is interesting, but its role in fiber retention within the bur is insignificant when compared with the other factors. These fibers are not to be mistaken for the intra-carpellary hairs reported by Peebles (18) and Youngmen <u>et al</u> (25). Intra-carpellary hairs were found at the margins of the dehiscence ridges (Fig. 20) of Stormproof No. 1 bolls.

Pearson (17) reported rudimentary bodies existing in sixteen cotton varieties. She referred to these structures as "basal bodies" and the motelike structures into which they develop as "false motes." The rudimentary bodies found in the Stormproof No. 1 bolls differed from Pearson's "false motes" in that there is no differentiation of tissue within the rudimentary bodies. Pearson's "false motes" show traces of vasculation and signs of elongation of epidermal cells on the day of flowering. These somewhat similar structures in Stormproof No. 1 bolls show neither vasculation nor elongation of the epidermal cells six days after anthesis (Fig. 4).

The boll enlarges as follows: by the enlargment of the developing vascular system, by the 90 degree translation of the axes of the ellipsoid cells, by the formation of the inter-cellular spaces, and by the parenchyatous cells increasing 2.5 times their size from anthesis to boll maturity. The translation of the cellular axes not only increases the circumference of the boll, but also limits the pericarp to increase 1.5 times its original thickness from anthesis to boll maturity. The cells of the locule wall, however, increase in number by cellular division. Mitotic figures were found in these cells as late as eight days after anthesis.

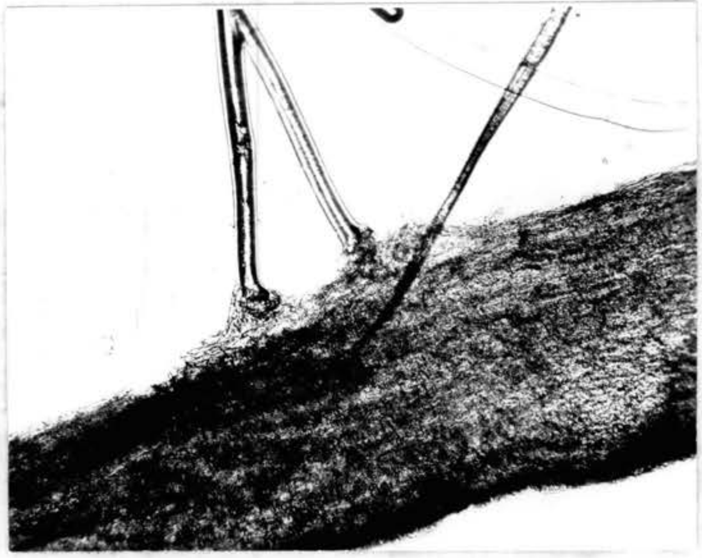


Figure 20. Intra-carpellary hairs from the dehiscence ridge of Stormproof No. 1; x150.

The cells that form the locule wall elongate at a greater rate than the increase in circumference of the locule. In this differential rate (5:4) lies the foundation for the formation of the convolutions present in the bur. The expression of the convolutions in open bolls results from the restricted boll opening and the lack of backward reflexing of the carpel edges. In Oklahoma Special, the ratio of the elongation of the cells of the locule wall and the increase in the circumference of the locule was 1:1. Since the circumference keeps pace with the elongation of the cells and the extreme backward reflexing of the carpel edges occurs, no convolutions are formed.

Environmental conditions considerably affect the stormproof character. Bolls developed during the early part of the growing season were characteristically stormproof. Those that developed during the latter half of the growing season resembled stormproof type bolls shortly after boll opening; however, after they remained in the field several weeks, they resembled the intermediate types. Both stormproof and intermediate type bolls were found on the same plants. As was to be expected, the stormproof types occurred on the lower fruiting branches; the intermediate types were found on the upper half of the plant.

This dual condition may be attributed in part to the water tension in the plant during the fruiting period. The early maturing bolls did not develop during periods of extreme water tension. Those that developed during the latter period suffered from long periods of drought.

These findings are in agreement with Lynn (13) who reports that there is less gluing effect if the plants are subjected to extreme drought conditions during the period of boll development.

Dastur (5) suggests that "bad opening" of bolls, but not premature opening of the boll, is merely due to reduction of boll size and that the carpels can not open fully because of a smaller number of seeds present inside (usually 5-8). Unfortunately, this point of view was discovered too late to carry out a study of the number of seeds present inside the bolls of Stormproof No. 1 and Oklahoma Special. However, it seems unlikely that this is the case in Stormproof No. 1.

Abraham (1) reported that fusion of the adaxial bundle extended to a very high level in G. indicum and its allied forms. This, together with the mechanical tissue formed around the vascular tissue, prevented complete opening of the boll. In the two varieties reported in this study, the level of fusion of the abaxial bundles paralleled each other so that it could not be inferred that it hindered cleavage (Table 3). The backward recurving of the carpel edges appears to be associated with the shape of the boll as well as the loss of water caused by the drying of the boll. The bolls of Stormproof No. 1 are roundish in shape and mostly four locked with the result that the carpels are too broad to allow backward recurving of the carpel edges. In Oklahoma Special where the bolls are fairly long and tapering and mostly five locked, the shape of the carpels apparently does not affect boll opening.

Stormproof No. 1 plants may be easily tested with good results for the stormproof character. A four-day or older boll is carefully cut longitudinally with a sharp razor blade and tested as previously described. If many fibers trail behind as the fibers are teased away from the locule wall, it is a good indication of the presence of the stormproof character.

SUMMARY

1. Stormproof No. 1 cotton plants were used to study the development of the stormproof character of the boll.

2. Oklahoma Special, a non-storm-resistant cotton variety, was used as the control.

3. Variant characters that exist between the two varieties are reported.

4. The primary factors in fiber retention within the bolls of Stormproof No. 1 are the incomplete opening of the boll, the slight backward reflexing of the carpel edges, the formation of the convolutions within the carpel, and the adhesion of the fibers to the locule wall.

5. Differential staining the mucilage indicated a difference in the mucilage produced by the two plants.

6. Secondary factors in fiber retention are reported.

7. The convolutions are formed due to the differential rate of elongation between the cells of the locule wall and the circumference of the locule.

8. The development of the boll, the formation of intercellular spaces, and the translation of cellular axes are discussed.

9. A possible color test is suggested for determining the "maturity" of cotton fibers.

10. A simple method for testing immature Stormproof No. 1 bolls for the stormproof character is described.

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