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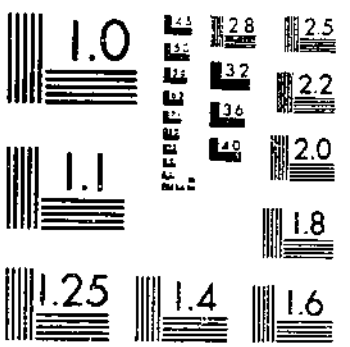

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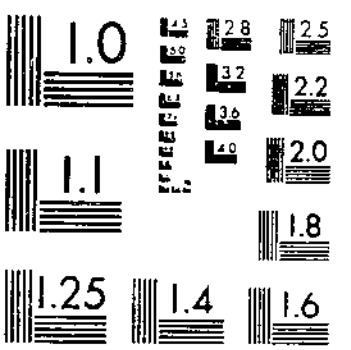

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Anatomical Structure of the Cottonseed Coat as Related to Problems of Germination [1]

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CONTENTS

INTRODUCTION

In the production of cotton, adequate stands of healthy seedlings are necessary if the full possibilities for yield are to be realized. Present methods of seed testing frequently are inadequate for the detection of seed weaknesses that may cause poor stands in the field. In this investigation it has become apparent that the failure of some seeds to produce healthy seedlings in the field is due to certain structural characteristics of the seeds, which under adverse conditions may hinder or prevent the normal physiological processes necessary for germination.

The mature cottonseed embryo is enclosed in a semihard covering or seed coat. The general anatomical structure of the seed coat has been studied and described by numerous investigators, but no detailed study of the specialized areas near the micropyle and chalaza has been reported. The structure of these areas appears to be of importance in interpreting some of the physiological phases of seed germination.

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[2] The authors are indebted to N. I. Hancock, of the Agricultural Experiment Station of the University of Tennessee, for the data presented in table 3.

[3] Since the preparation and submission of this manuscript, a contribution entitled "Relation of the Structure of the Chalazal Portion of the Cotton Seed Coat to Rupture During Ginning," by Dr. Norma L. Pearson, has been published in the Journal of Agricultural Research, Vol. 58, No. 11, p. 865-873, June 1939. Inasmuch as the present paper was written without opportunity of reference to Dr. Pearson's article, the latter is not included in the list of literature cited.

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REVIEW OF LITERATURE

Among the papers concerning the anatomical structure of the seed coat of cotton are those of Von Bretfeld (*1*),[4] Hanausek (*4, 5*), Reeves (*10*), Reeves and Valle (*11*), and Winton (*16, 17*). The five layers—epidermal, outer pigment, colorless, palisade, and inner pigment—have been described and illustrated. Most recent investigators (*8, 10*) describe the palisade layer as being one cell in thickness, the cells containing the remains of a nucleus and apparently divided by transverse thickenings radiating from the walls inward. Reeves and Valle (*11*) and Kondo (*8*) found varieties of cottonseed to differ in the proportion of cellulose and lignin in the palisade cells. Kondo discussed the structure of the funiculus. Reeves (*9*) concluded that the fringe cells arise from the inner epidermis of the inner integument. Von Bretfeld (*1*) referred to the "polster" and Hanausek (*5*) "polsterchen" at the chalazal region as an enlarged area of the inner colored layers composed of spongy or star-shaped parenchymalike cells, but did not describe the region in detail. Von Bretfeld illustrated the membrane and the inner colored and outer colored layers as thicker at both the chalazal and micropyle regions, and the palisade layer as continuous over the micropyle and chalaza, but with shorter cells in these regions.

The present investigation was planned with reference to the problems of cottonseed germination, including seed viability, dormancy, induced dormancy, and deterioration. It has been found with seeds of other plants that germination may be affected by (1) inherent qualities in the embryo itself; (2) anatomy of the seed coat mechanically influencing the opening or splitting at the time of germination; (3) mechanical structures or chemical inclusions in the seed coat which influence permeability; (4) permeability of special membranes to water and other liquids; and (5) exclusion of oxygen by the seed coat or membranes (*2, 6, 12, 14*).

STRUCTURE OF THE SEED COAT

The general anatomy of the mature seed coat of cotton (fig. 1) on the whole is similar in all species and varieties studied, although slight differences are found in the volume of certain tissues and in the detail of the types of cells. The variations in the structure of the micropyle and chalazal regions appear important as points of water absorption and gas exchange, and as focal points of infection. Therefore, detailed descriptions of their structure are of interest.

Anatomy of the Micropyle Region

The micropyle is located at the small pointed end of the seed and is completely covered by the funiculus, which extends out from the seed and is continuous with the raphe. The epidermis is continuous over the seed around the funiculus into which the bundle of the raphe extends. The cells of the outer pigment layer make up the greater part of the funiculus (fig. 2, *A*, *b*; *B*, *b*). This layer, composed of compact brown tissue, is thicker at the micropyle than at the sides of the

[4] Italic numbers in parentheses refer to Literature Cited, p. 23.

seed (fig. 2, *B*, *b*). The colorless cells continue to the tip and extend into the funiculus (fig. 2, *A*, *c*; *B*, *c*).

The palisade cells are shorter toward the micropyle (fig. 2, *B*, *d*), the rows, with their long axis perpendicular to the seed surface, curving

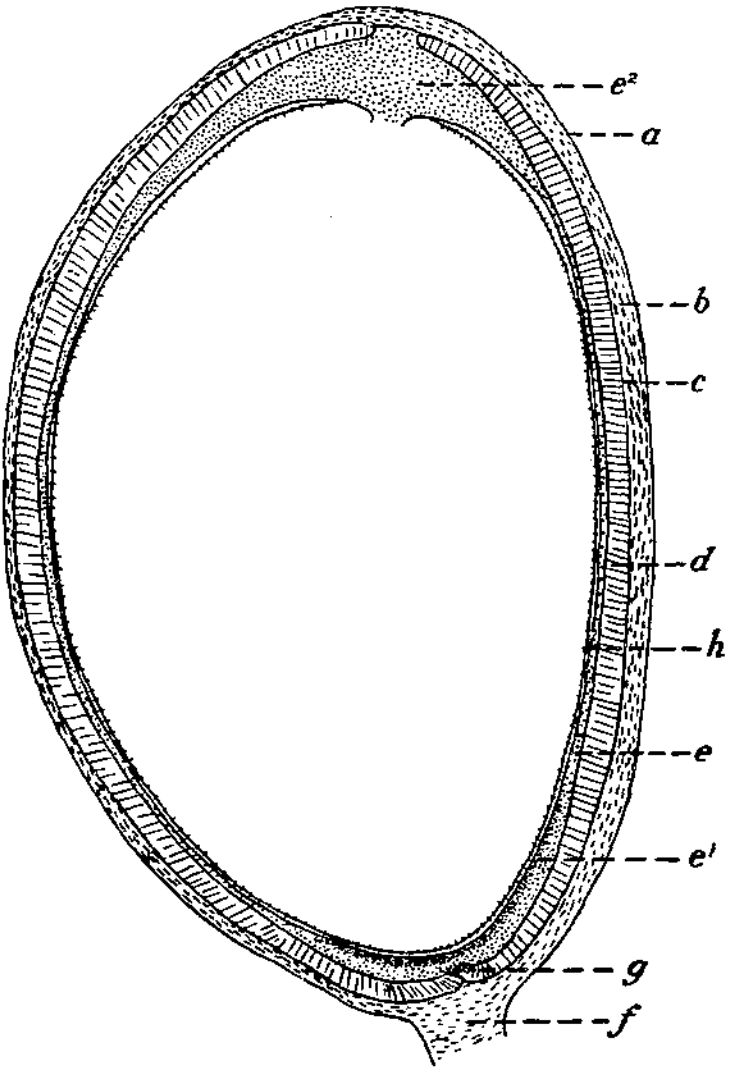

FIGURE 1.—Diagram of the layers of the cottonseed coat: *a*, Epidermis; *b*, outer pigment; *c*, colorless; *d*, palisade; *e*, inner pigment; e^1, differentiated inner pigment; e^2, chalazal cap; *f*, funiculus; *g*, micropyle; *h*, fringe.

inward to one point. Thus the micropyle is lined with incurved rows of palisade cells, the outer walls being in contact (fig. 2, *A*, *d*; *B*, *d*) and completely closing the micropyle.

At the termination of the palisade layer the cells are very short and flattened. The irregularity is probably caused by the pressure of the

cells on all sides. The palisade cells at the micropyle are broader than those at the sides of the seed and have thinner walls. Lumen occurs throughout the full length of the cells near the micropyle, in contrast to those toward the side where the lumen occurs only in the upper third of the cells. The cells lining the micropyle are embryonic; those surrounding it are in various stages of development.

At the base of the palisade cells (fig. 2, *B*, *e*) around the micropyle, a group of specialized cells stand out from the adjacent inner pigment

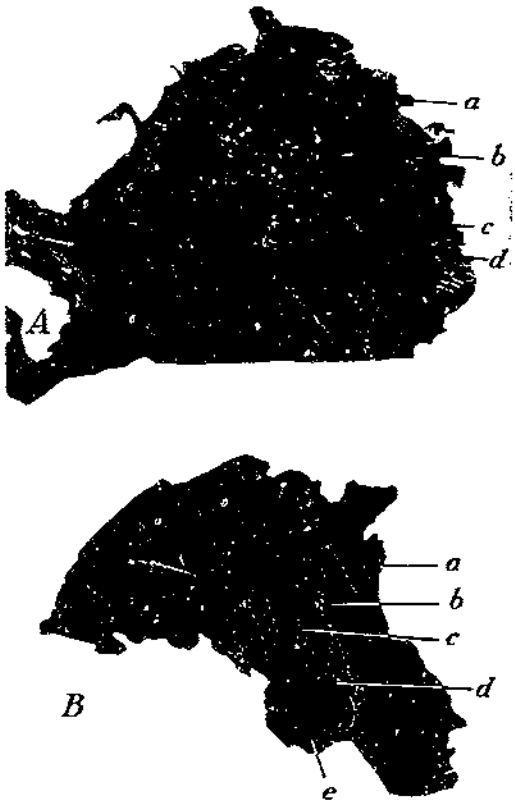

FIGURE 2.—*A*, Cross section of the base of the funiculus and the tip of the micropyle: *a*, Epidermis; *b*, outer pigment tissue; *c*, colorless layer; *d*, palisade cells. × 70. *B*, Median section of the micropyle region: *a*, Funiculus; *b*, outer pigment layer; *c*, colorless layer; *d*, palisade cells; *e*, specialized group of cells. × 60.

cells. They have thicker walls, lack the brown pigment, and stain much more readily with safranin. These cells are definitely a part of the inner pigment layer; in many ways they appear more embryonic.

The inner-pigment layer under the micropyle forms a wide cap over the whole tip of the embryo (fig. 1, *e*, *e*[1]). The outer six to eight rows are made up of large, regularly shaped cells, almost square in cross section, filled with brown pigment and protoplasm. The inner three or four rows are less pigmented, lack contents, are long and narrow, are arranged in strands, and are closely appressed laterally.

In the mature cottonseed the fringe cells, which compose the outer row of cells of the inner membrane, break away from the inner pigment layer of the seed coat at the sides of the seed and in the micropyle

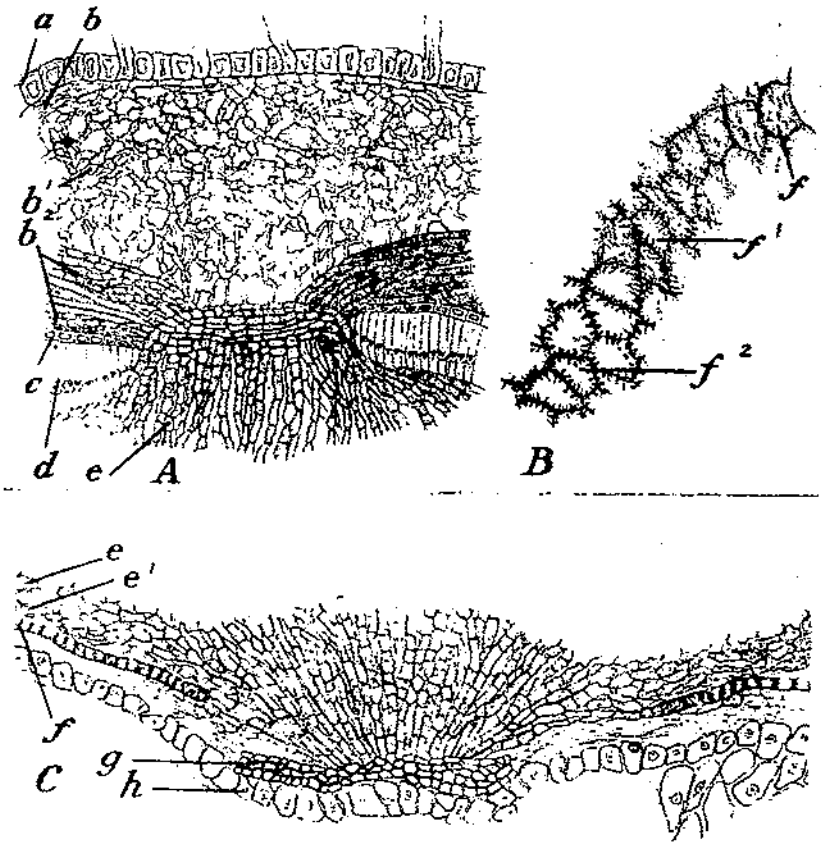

FIGURE 3.—A, Median section of the chalazal cap tip and the tissues covering it: *a*, Epidermis with hair cells; *b*, dense outer pigment cells; b^1, light outer pigment cells with large intercellular spaces; b^2, radiating compact outer pigment; *c*, colorless layer; *d*, palisade cells; *e*, chalazal cap tip. ×90. *B*, Surface view of fringe cells in three stages of development: f, f^1, and f^2. ×370. *C*, Median section of chalazal cap base: *e*, Compact pigment cells of cap; e^1, lower differentiated pigment cells; *f*, fringe cells; *g*, specialized group of cells at base of cap; *h*, membrane. ×90.

region. Thus the membrane enclosing the embryo is attached to the seed coat only at the chalazal cap.

ANATOMY OF THE CHALAZAL REGION

The broad end of the cottonseed at the termination of the raphe may be termed the chalazal region. The layers of the seed coat and inner membrane at this point are illustrated in figure 3. Over this region

the epidermal layer is continuous (fig. 3, *A*, *a*) and is similar to the epidermis of the side wall. The outer pigment layer is thickened and is differentiated into two areas. The inner cells (fig. 3, *A*, b^2) retain the compact, regular arrangement of those of the side wall, contain the characteristic brown pigment of the layer, and terminate at the edge of the chalazal tip. The outer portion (fig. 3, *A*, *b*, b^1) is continuous over the chalaza and is composed of loosely arranged intertangled strands of cells with very large intercellular spaces. Other areas of delicate, loosely arranged cells are present in the outer pigment layer over the vascular bundles and raphe. The cells of the colorless layer (fig. 3, *A*, *c*) tend to be smaller and have thinner walls than those of this layer in the side walls.

The palisade layer extends from the side-wall areas into the chalazal region but terminates with the colorless layer at the tip of the chalazal

FIGURE 4.—Cross section through chalazal region: *a*, Chalazal cap; *b*, cap tip; *c*, inner pigment layer; *d*, palisade layer. × 15.

cap. The layer is composed of long cells oriented so that their long axis is perpendicular to the surface of the seed. Palisade cells are shorter over the cap than in a median section of the seed. They are progressively shorter from the sides of the cap toward the center. At the region of the cap tip the palisade cells are approximately one-third the length of those of the side wall and are broader in proportion to their length, the lumen extends to the base of the cell, and the wall thickenings are lacking (fig. 3, *A*, *d*). The staining reaction is that of cellulose in contrast to the lignin in the palisade layer of the side wall.

Directly underlying the palisade layer at the chalaza is the enlarged area of the inner pigment, the "polster" of Von Bretfeld (*1*) and Hanausek (*5*), which may be referred to as the chalazal cap. The nearly circular cap is convex on the outer surface and concave on the inner, much thickened in the center, and gradually becoming more narrow toward the sides, where it is continuous with and indistinguishable from the inner pigment layer (fig. 4, *c*) of the side walls. A disklike tip (fig. 4, *b*) is present near the center of the outer surface.

At the center base of the chalazal cap the rows of pigment cells extend into a special group (fig. 3, *C*, *g*) compactly arranged, bricklike, regular cells. Whether this specialized group of cells is a part of the membrane or of the inner pigment could not be determined. The cells seem to be in more direct contact with the pigment cells than with the cells of the membrane. They may be considered undeveloped cells that have lost their contents. This group is similar to the group under the micropyle. The cap is directly attached to the membrane (fig. 3, *C*, *h*) in this region.

The general anatomical structure of the chalazal region of the seed coat differs from that of the side walls principally in the looser arrange-

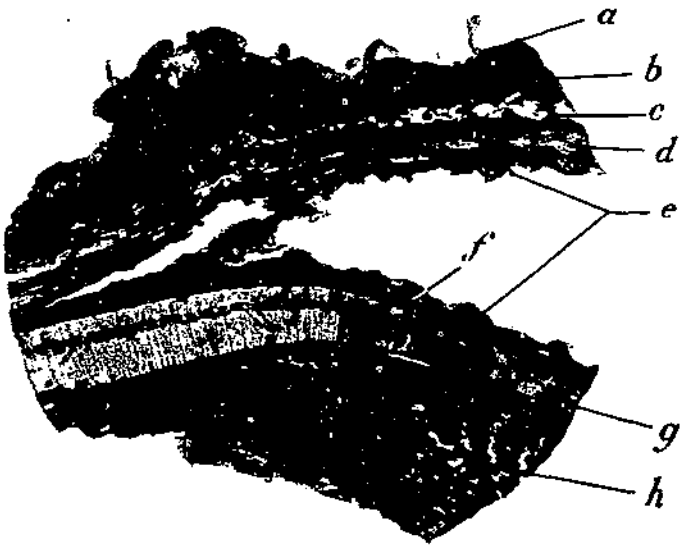

FIGURE 5.—Cross section of the raphe side of the seed coat over the chalazal cap: *a*, Epidermal cells; *b*, compact outer pigment cells; *c*, loose outer pigment over vascular bundle; *d*, vascular bundle; *e*, loose outer pigment tissue broken apart; *f*, colorless layer; *g*, palisade cells; *h*, side of chalazal cap. × 70.

ment of the cells of the outer pigment, the absence of the colorless and palisade layers over the chalaza, the presence of thin-walled nonlignified palisade cells around the chalaza, and the thickened caplike differentiation of the inner pigment layer.

A small portion of the cap and the tissues covering it are shown in detail in figure 3. The chalazal cap is composed of dark-brown pigmented cells. In longitudinal view the cells are somewhat rectangular, united end to end in long rows, or strands. In the upper several layers of the cap the cells are compact and closely appressed. Rows, or strands, of cells extend from the base to the tip; others radiate from the center outward, curved similarly to the shape of the cap.

Toward the outer edges of the cap the cells are less compact, loosely arranged with intercellular spaces similar to spongy parenchyma. The cells in cross section are irregular, lobed, or star-shaped. The lower two or three rows of the cap extending outward contain less pigment than the other cells of the cap and react to the stain differently. The cells are narrower and longer and the rows appressed.

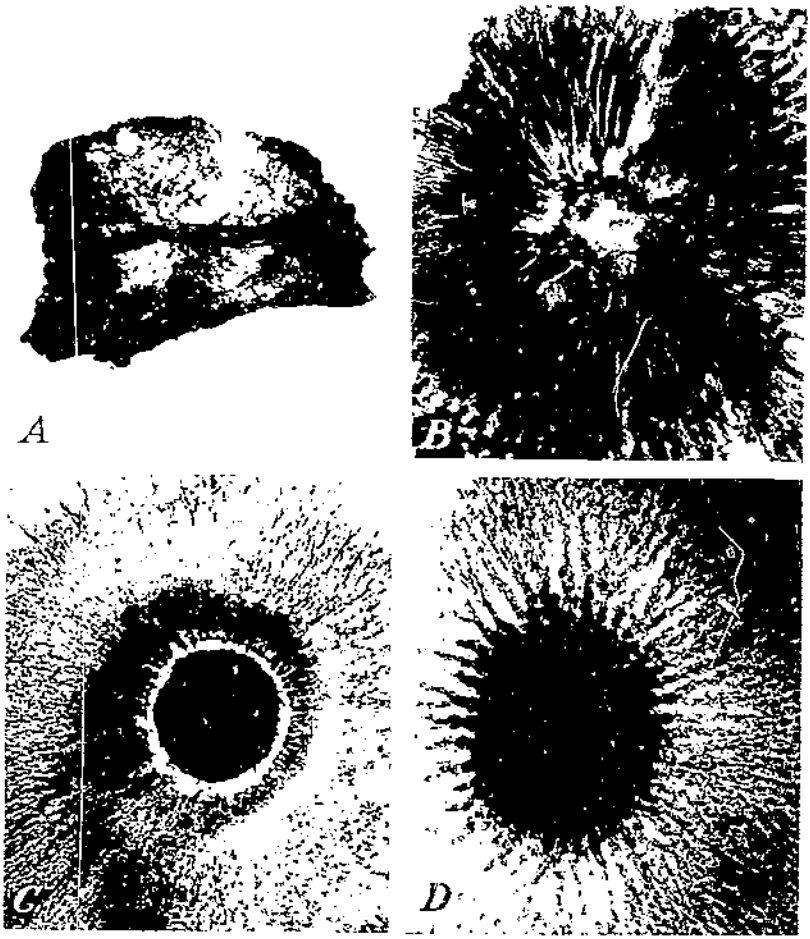

FIGURE 6.—A, [illegible] cap. B, Outer [illegible] cells, × 20. C, [illegible] cells, × 60. D, [illegible] Chalazal [illegible] × 70.

Fringe cells are differentiated from the sides of the chalazal cap slightly above its base (fig. 3, *C*, *I*). The stages in development are shown in figure 3, *B*. In *f* the cells exhibit the thickenings of the walls, the breaking down of protoplasm; in *f'* the contents have disintegrated, side walls are irregularly thickened, and in *f''* the upper walls have dissolved away, and fringelike thickenings extend from the lower and side walls.

As is shown in figure 5, *e*, the loosely arranged outer cells over the chalazal cap break apart readily when subjected to moisture. In figure 6 is shown a series of sections cut tangentially at the broad end of the seed directly across in relation to the cap. The vascular bundles (fig. 6, *A*) in the pigment layer join in this area of the seed and are surrounded by loosely arranged cells. Cross sections (fig. 6, *B*) show the compact pigment cells adjacent to the palisade layer, which converge from the central loose area, and (fig. 6, *C*) the palisade surrounding the cap tip. Underneath the palisade cells, pigment cells (fig. 6, *D*) of the cap converge toward the tip.

In the presence of sufficient moisture the chalazal end tends to open. The opening may be in the form of a pinprick hole or a larger aperture caused by the splitting apart or dissolving away of the epidermal and outer pigment tissue. The epidermis and the outer pigment cells in this area separate from each other and the surrounding tissue and easily disintegrate or flake off. Likewise, the outer pigment in the raphe region is very loose and separates readily (fig. 5, *c*). In many seeds the tissue over the cap is a powderlike mass of disconnected cells held in place by the epidermis. When the epidermal cells loosen, the cells underneath drop out and leave a small smooth hole over the cap tip.

The palisade layer tends to break away from the upper part of the cap (fig. 4). In fact, separation of the layers may take place in a mature seed before any treatment or further breaking is noticeable. When wet, the palisade and outer pigment cells, which converge toward the tip, may split apart in groups (fig. 6, *B*). In a cross section placed in water the swelling and extending of the rows of cells and the splitting and breaking apart laterally may be observed. Swelling and splitting may continue until a large cavity is formed over the cap. The pigment tissue of this swollen area will further disintegrate. In fuzzy seeds the initial loosening of the tissue is apparent as the epidermal cells disintegrate and slough off, leaving a small area without fuzz.

Effect of Acid Delinting on the Seed Coat

Delinting with sulfuric acid is an effective method of removing external contamination from cottonseed, and frequently is recommended as a means of seedling disease control. In addition to the removal of the fuzz and sterilization of the external parts, there is considerable chemical action upon the seed coat.

Some effects of acid delinting on the structure of the seed coat are illustrated in figure 7. With short applications of acid, the fibers and their basal cells are dissolved and the epidermal and outer pigment layers are broken and distorted. The colorless layer is affected only after longer exposure. With normal acid delinting of from 5 to 8 minutes' immersion in the acid, the tissues of the palisade layer apparently are unaffected. The action of the acid is particularly noticeable on the thick pigment layer over the chalazal cap. In many cases this tissue is disintegrated and sloughs off, leaving the chalazal opening exposed; frequently, the palisade layer splits open and curls back, exposing the chalazal cap. Seed lots differ in the manner and degree of opening, depending upon the firmness of the tissue around

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the chalaza. Seeds with open chalazas absorb water faster than those in which the chalazas are closed.

Under most conditions of soil moisture, acid-delinted seeds germinate in 2 or 3 days less time than normal fuzzy seeds. Rapidity of

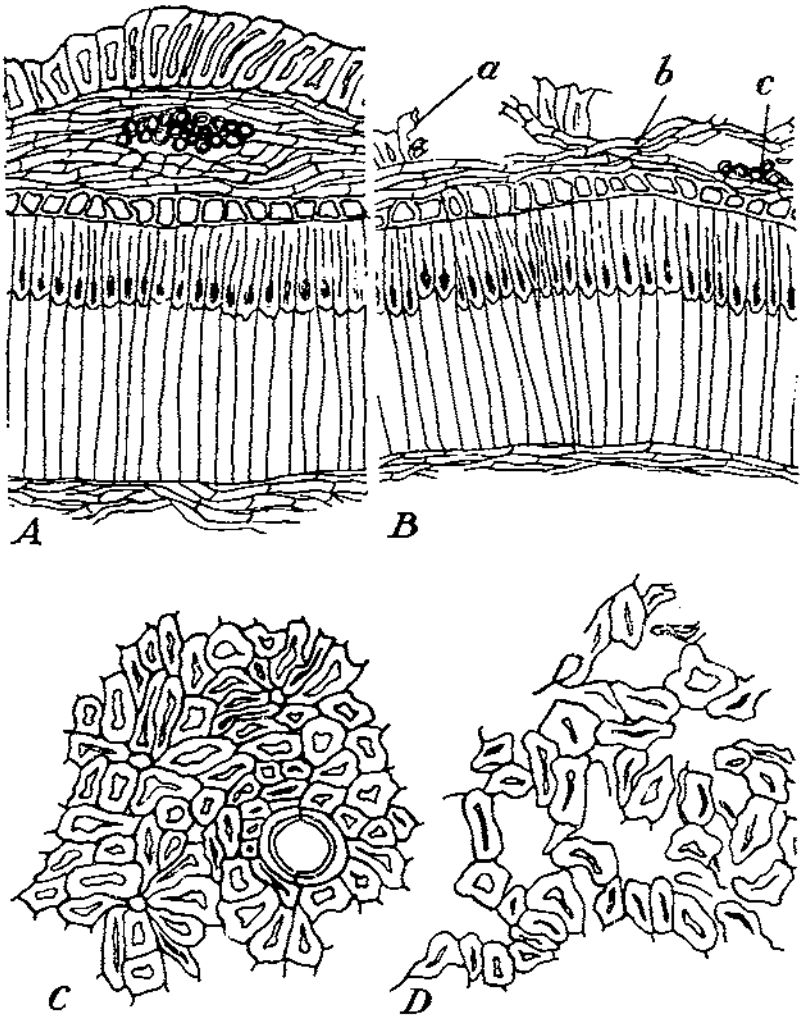

FIGURE 7. *A*, Diagram of a section of seed coat of normal cottonseed. *B*, Section of acid-delinted seed coat showing (*a*) epidermis broken or gone, (*b*) outer pigment disturbed in patches, (*c*) vascular bundle distorted and cells disintegrated. *C*, Cellular detail of the surface of a normal seed coat. *D*, Surface of acid-delinted seed coat showing disintegration of epidermal cells. ×90.

germination primarily is due to the more rapid absorption of water. Closer contact with the substrata is possible because of the absence of fuzz. The absence of fuzz appears to play a more important part in facilitating water absorption than does the disintegration of the epidermal and pigment layers, as naked seeds not acid treated ger-

minate almost as fast as those treated with acid. Seed lots are occasionally encountered in which germination is apparently injured by acid delinting.

ROLE OF THE SEED COAT IN GERMINATION

A recent text on general botany (7) outlines certain conditions that must prevail before seed germination can occur. Most prominent among these are a supply of water, a supply of oxygen, and a favorable temperature. Germination begins with the absorption of water. This initial absorption and digestion of food is necessary to provide the materials for increased respiration. In other words, the seed can use oxygen only after it has absorbed sufficient moisture. Too much water, however, interferes with the free passage of air into the seed and may prevent complete germination. It is reasonable to believe that these general conditions are applicable to cottonseed.

Course and Rate of Water Absorption Through the Seed Coat

From the study of the structure and nature of the chalazal and micropyle regions, it would seem that these regions of the seed may greatly influence the passage of water and exchange of gases and be an important point of entrance for micro-organisms.

In order that the relations of the various sections of the seed coat to water absorption might be studied, an experiment was designed to determine the course and rate of absorption in normal fuzzy, normal smooth, and acid-delinted cottonseed. Seeds of five varieties of cotton were used. To show the course of absorption, aqueous solutions of safranin were used in place of water, as the stain could be identified readily in the cells of the seed coat. In this experiment, seeds of each lot were sealed at various sections with paraffin or cellulose waterproofing to differentiate the points and rate of absorption. After sealing, the seeds were placed in dishes of the staining solution.

At intervals, seeds were removed from the staining solution and sectioned in several regions with a dry razor. From numerous examples of each seed lot, diagrams were made showing the location of the stain. It was found that in the five lots of seed, both acid delinted and untreated, the course of absorption was the same, although differences in the time required for the absorption were noted among the seeds of different varieties and between acid-delinted and untreated seeds.

In unsealed seeds (fig. 8) the presence of stain was observed in the seed-coat tissues in the following order: 1, Epidermis; 2, chalazal and micropyle regions of the outer pigment layer (fig. 8, *A*); 3, chalazal cap and outer pigment (fig. 8, *B*); 4, inner-pigment layer, spreading from the chalazal cap or micropyle region (fig. 8, *C*); 5, fringe cells (fig. 8, *D*), the region of stained fringe cells following closely the staining in the adjacent stained inner pigment cells.

In seeds sealed at the chalazal end only, the stain entered through the micropyle, spreading from this point to the inner pigment and fringe cells and toward the chalazal end of the seeds. In these cases the stain also entered the exposed epidermal cells and passed laterally in the outer pigment under the seal and toward the chalazal end.

In seeds sealed at the micropyle only, initial entrance of the stain was at the chalaza, from which it spread through the chalazal cap

into the inner pigment and fringe layers. Some lateral movement of the stain was traced under the seal through the outer pigment layer toward the micropyle, but this movement was noticeably slower than

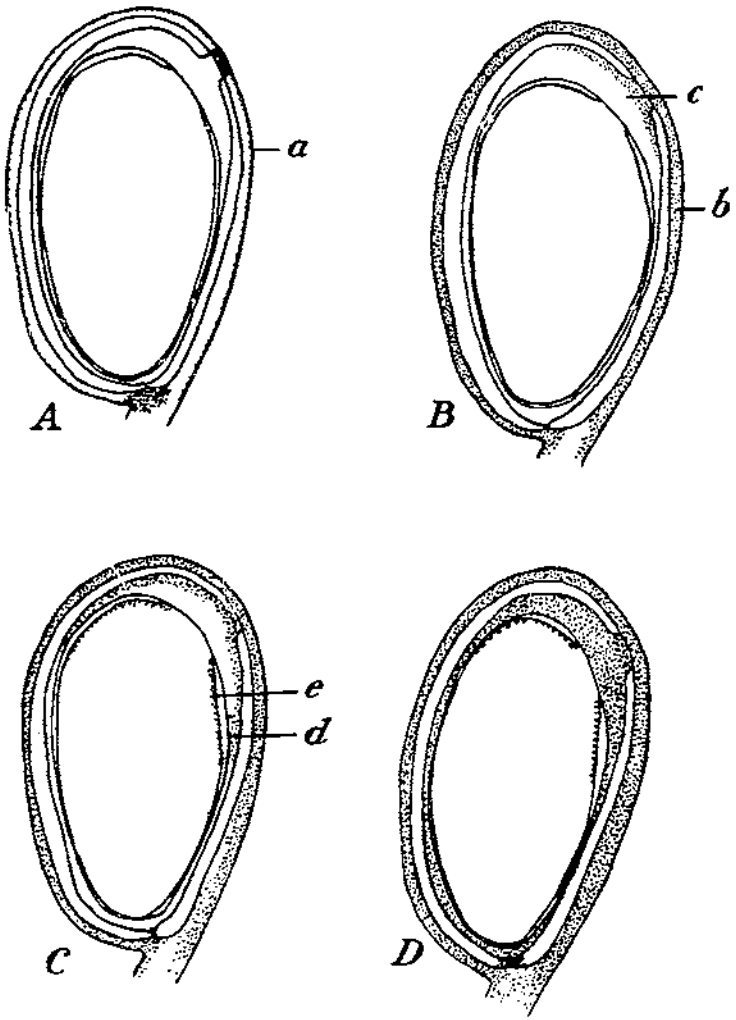

FIGURE 8.—*A*, *B*, *C*, *D*: Diagrams of longitudinal sections of the cottonseed coat and membrane showing the progressive course of water absorption as indicated by presence of stain (stain represented by shading): *a*, Epidermis; *b*, outer pigment; *c*, chalazal cap; *d*, inner pigment; *e*, membrane.

the lateral movement from the chalazal cap through the inner pigment and fringe layers.

When both ends of the seed were sealed, the stain entered the seed coat through the exposed epidermal cells of the side wall and passed, by means of the outer pigment layer, toward the chalazal end. From the chalazal cap the stain spread to the inner pigment layer and membrane. Movement also occurred in the outer pigment layer toward

the micropyle, but at a much slower rate. The outer colorless and palisade layers did not become stained, a fact indicating that water does not readily pass directly through the side walls.

Although all the seeds studied in the above experiments showed similarities in the course of stain, there appeared differences in the rate of absorption. An approximation of the time required for the penetration of the stain to the various layers of the seed coat was obtained by placing fuzzy seeds between filter papers saturated with an aqueous staining solution (table 1).

TABLE 1.—*Staining of seed-coat tissues at various time intervals in 5 lots of cottonseed* [1]

Time interval	Seed coat tissues listed in order of staining and percentage of germination for—				
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
2 hours	Fuzz. Epidermis.	Fuzz.	Fuzz.	Fuzz.	Fuzz.
5 hours.	Fuzz. Epidermis. Outer pigment. Chalaza.	do. Epidermis. Outer pigment.	do. Epidermis. Outer pigment.	do. Epidermis.	Do.
24 hours.	Fuzz. Epidermis. Outer pigment. Chalaza. Inner pigment.	Fuzz. Epidermis. Outer pigment. Chalaza.	Fuzz. Epidermis. Outer pigment. Chalaza.	Fuzz. Epidermis. Outer pigment.	Do. Epidermis.
36 hours	90 percent germinated.	30 percent germinated.	50 percent germinated.	Fuzz. Epidermis. Outer pigment. Chalaza.	Fuzz. Epidermis. Outer pigment. Chalaza.
4 days.	100 percent germinated.	100 percent germinated.	100 percent germinated.	80 percent germinated.	Fuzz. Epidermis. Outer pigment. Chalaza. Inner pigment.
5 days					100 percent germinated.

[1] Lots 1 to 5, inclusive, refer to samples taken from Sea Island, Coker Wilds, Cleveland, Farm Relief, and Stoneville 5, respectively. They are referred to as lots rather than varieties because of improbability that these limited samples necessarily represent varietal traits.

When seeds were immersed directly in the staining solution, the staining was much more rapid in all seed lots, and differences between varieties were more distinct. Fuzzy seeds absorbed the stain more slowly than acid-delinted seeds. At the end of 5 hours, most delinted seeds showed the stain all the way through the membrane, whereas fuzzy seeds showed stain in most cases only in the epidermis and outer pigment and at the micropyle and chalazal ends. Fuzzy seeds required from 12 to 24 hours for the stains to reach the inner membrane, except in lot 1, which had little fuzz. Differences between the seed lots were noted in rate of absorption. This may be attributed to structural characteristics in the seed coats or to differences in structure due to degree of maturity, field exposure, or treatment of the various seed lots used.

RESPIRATION DURING THE PERIOD OF GERMINATION

Studies of the respiration of cottonseed show that carbon dioxide is evolved in considerable quantities during the period of germination. In recent tests, germinating seed at 30° C. constant temperature, during a period of 72 hours, gave off as much as 120 mg. of car-

bon dioxide per gram of seed. This gas at atmospheric pressure would have a volume equivalent to approximately 75 times the volume of the seed. In order that germination may take place, there must be an exchange of oxygen and carbon dioxide through the seed coat. If cottonseeds are placed on a moist substratum in a suitable container and the air is withdrawn from around the seed, germination will not take place so long as oxygen is excluded.

Toole and Drummond (*15, p. 287*) observed in their studies of sensitive seeds that:

> A few exceptional samples have been encountered which behave in a peculiar manner when tested by the prewetting method. In these samples, many of the seeds remain in a dormant condition in the germinator, as they do when soaked too long in water. In these cases, the total germination is greater by the standard method.[5]

As poor germination may be brought about in the laboratory by prewetting or oversoaking, it is logical to assume that in these seed lots the too rapid absorption of water, with the consequent water-logged condition of the seed coat, prevents the normal interchange of gases and induces a type of dormancy or retards germination. With ample moisture and a favorable temperature for the development of seed-borne or soil micro-organisms, retarded germination of the seed would offer a very nearly perfect set-up for seed decay.

Relation of Seed-Coat Maturity to Molds

The rapid development of rhizopus and penicillium molds on samples showing low germination and on sensitive seeds is of common occurrence, but samples from vigorous germinating lots may be unaffected even when germinated in towels heavily contaminated with such micro-organisms. Toole and Drummond (*15*) and Del Curto (*3*) ascribe the susceptibility or resistance of the seeds to the relative vigor of germination of the seed lots.

At Knoxville, Tenn., seeds were obtained from bolls of known age, varying from 35 to 54 days from flowering. These seeds were dried, acid-delinted, and placed in small dishes for germination. The very immature seeds failed to germinate, but promptly developed heavy growths of molds. The mature seeds germinated normally, with very little fungus growth. In the series of seed lots the molds were progressively more active on the more immature seeds. Full maturity of the seeds in this instance was reached in from 48 to 54 days after flowering, the time varying for different varieties.

Immaturity or undevelopment of seeds may result from drought, insect attack, frost damage, or other factors causing the cessation of growth or premature opening of bolls. Immaturity of the seed coat may contribute to susceptibility to molds. In the development of

[5] The standard technic for the germination of cottonseed, as adopted by the Association of Seed Analysts of North America, stated briefly, is as follows: From a thoroughly mixed sample of cottonseed, 2 lots of 50 seeds each are taken at random. The dry seeds are put between folds of moist cotton flannel or absorbent paper toweling and placed in the germinator at the usual alternating temperatures of 20° to 30° C. The test should be kept in the 30° temperature for 6 to 8 hours and in the 20° temperature for the remaining part of the day. The sprouted seeds should be counted and removed on the third and fifth days. Tests are considered completed at the end of 7 days. The prewetting method differs from that outlined above in that the seed samples are stirred or shaken in water until the fuzzy covering of the seeds is wet, and the seeds are then placed on the moist substrata for germination. The vacuum method of testing as used by the authors differs from the standard technic as follows: (1) The seed samples are first delinted with sulfuric acid and thoroughly washed and dried. (2) The delinted seeds are placed in a suitable container and covered with water; the aperture of the container is then attached to a suction line and the contents subjected to a negative pressure of 27 inches of mercury for 5 minutes. (3) The seeds are removed from the container, drained of surplus water, and placed immediately on moist paper toweling for germination in the chambers at the usual 20° to 30° alternate temperatures, as in the standard method.

the seed coat the hardening or lignification of the cell structure takes place as the seed matures. The softer seed coat of an immature seed would be more easily penetrated by micro-organisms than would the fully developed and hardened seed coat of a mature seed.

The seed coat of an immature seed may furnish nutrients more suitable for the growth of micro-organisms than are found in a mature seed. Reeves (*10*) found large quantities of starch in the two integuments of the developing ovule, but it disappeared before the ovule reached maturity. He stated that the cell walls of the epidermis are composed chiefly of cellulose, but that they also contain varying proportions of lignin and cork. Further, that the walls of the fringe tissue and colorless layer are highly lignified at maturity, and that the two pigment layers, at maturity, are highly suberized. He found that they also frequently give tests for small quantities of lignin and cellulose, and that the palisade cells of the seed coats consist chiefly of cell walls, the lumina being small and their contents scanty. Their walls are composed chiefly of lignin, cellulose, and cork.

Dormancy

Cottonseed from freshly opened bolls usually show a degree of dormancy (*13*) and must pass through an after-ripening process before prompt germination can be obtained. This initial dormancy usually is removed after drying and a short period of storage. Under some conditions, however, dormancy may persist in certain varieties for several months after harvest.

In 1937, at Knoxville, Tenn., boll samples were obtained from several varieties for seed-germination tests. All bolls were tagged on the day of beginning of opening and harvested 14 days later (October 15). Boll samples were dried and stored under laboratory conditions until the latter part of February, when germination tests (table 2) were made of each variety, with normal fuzzy seed, acid-delinted seed, acid-delinted seed vacuum treated in water, and on embryos with seed coats removed. Striking differences in the rate of germination were apparent between the varieties, although the total germinations after 14 days in the germinator were approximately the same. Acid delinting hastened initial germination but did not materially affect the rate of germination in other respects. Seeds that were acid delinted and subjected to reduced atmospheric pressure while immersed in water showed initial germination in 2 days, but the tendency to delayed germination or dormancy was actually increased. In contrast to these results, embryos from which the seed coats had been removed gave complete germination in 2 days irrespective of variety or previous tendency to delayed germination.

Further evidence of varietal differences in dormancy was obtained from boll samples of these same varieties collected at Lubbock, Tex. Farm Relief and Stoneville 5 had many dormant seeds, but Wilds 5 and Cleveland (Wann.) germinated very promptly, similarly to seeds of these varieties grown at Knoxville. These data indicate that dormancy may be a condition imposed by the seed coat upon the embryo.

TABLE 2.—*Effect of acid delinting, acid delinting and vacuum treatment, and seed-coat removal on dormancy and rate of germination in nine varieties of cottonseed*

NORMAL FUZZY SEED

Variety	Seed germinated after being in the germinator for—											
	2 days	3 days	4 days	5 days	7 days	8 days	9 days	10 days	11 days	12 days	14 days	Total
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Farm Relief			15	13	15	8	10	9	7	11	6	94
Stoneville 5			18	8	26	13	16	4	6	2	1	94
Mexican Big Boll			21	28	24	9	5	1	1	1	1	91
Qualla			15	16	30	10	7	7	3	1		89
Cook 912			30	22	19	13	6	3	1	1		95
Rowden 2088			20	33	27	9	2	2				93
Startex			30	15	20	12	12					89
Wilds 5			86	6	1	1						94
Cleveland (Wann.)			84	11	2							97
ACID-DELINTED SEED												
Farm Relief		23	6	6	22	10	7	7	4	5	5	95
Stoneville 5		8	3	5	18	15	16	12	6	5	9	97
Mexican Big Boll		30	15	17	17	9	2	2	1	1		94
Qualla		17	6	11	29	11	10	4	4			92
Cook 912		32	19	15	19	8	3					96
Rowden 2088		20	30	20	16	8	3	1				98
Startex		27	12	11	19	13	9	4	1			96
Wilds 5		96	1									97
Cleveland (Wann.)		90	5	1								96
ACID-DELINTED SEED VACUUM-TREATED IN WATER												
Farm Relief	18	20	0	8	14	10	4	6	6	2	0	[1] 88
Stoneville 5	4	4	0	6	10	4	16	10	20	6	10	[1] 92
Mexican Big Boll	22	8	20	10	18	2						80
Qualla	10	2	14	4	16	10	10	18	8	3		94
Cook 912	6	14	26	14	20	6	2	8	4			100
Rowden 2088	22	4	22	20	22	4						94
Startex	28	4	4	4	22	8	8	8	6	2		94
Wilds 5	84	2	2	2	2							92
Cleveland (Wann.)	66	8	8	6	8							96
EMBRYO WITH SEED COAT REMOVED												
Farm Relief	96											96
Stoneville 5	100											100
Mexican Big Boll	100											100
Qualla	100											100
Cook 912	100											100
Rowden 2088	100											100
Startex	100											100
Wilds 5	100											100
Cleveland (Wann.)	100											100

[1] 10 percent of seed remained apparently sound but ungerminated at end of 14 days.

SCARIFIED SEEDS

Injury to seed germination by modification of the seed-coat structure is shown by the behavior of scarified cottonseed in seed-treatment tests at Jackson, Tenn., in 1938. Normal fuzzy, acid-delinted, and acid-delinted scarified seed of Acala 44–5 and Deltapine were used. Scarification was done after acid delinting on a commercial seed scarifier. Crushed or broken seeds were removed from the scarified lots before planting. The germination percentages (table 3) were calculated from actual counts of the number of seeds planted and of

the seedlings surviving just prior to thinning, May 24. In both varieties, seed scarification resulted in highly significant reductions in field germination.

TABLE 3.—*Germination percentages from Acala 44–5 and Deltapine fuzzy, acid-delinted, and scarified seeds in seed-treatment tests, Jackson, Tenn., 1938*

Treatment	Germination[1]		Treatment	Germination[1]	
	Acala 44–5	Deltapine		Acala 44–5	Deltapine
	Percent	*Percent*		*Percent*	*Percent*
Normal fuzzy+lime	74.5	56.3	Acid delinted and scarified	34.0	20.5
Normal fuzzy+mercury chloride	79.3	61.3	Acid delinted and scarified+lime	36.5	25.0
Normal fuzzy+bordeaux	72.5	46.3	Acid delinted and scarified+mercury chloride	37.5	28.3
Acid delinted	75.3	54.0	Acid delinted and scarified+bordeaux	37.3	13.5
Acid delinted+lime	71.0	71.0			
Acid delinted+mercury chloride	75.5	60.0			
Acid delinted+bordeaux	75.5	53.0			

[1] Difference required for significance at odds of 99 to 1, 11.25.

Similar but less marked reductions in field germinations were obtained at Jackson in 1937 from mechanically delinted seed of a lot of Deltapine. Of normal fuzzy, acid-delinted, and mechanically delinted seed of the same lot (table 4), mechanically delinted seed gave lowest field germination. Under the conditions at Jackson, significant improvement in germination was obtained from mercury dust treatment. In a planting of these same seed at Knoxville (table 4), approximately equal germinations were obtained from fuzzy and mechanically delinted seeds, and germination of acid-delinted seed was significantly higher. Under the conditions at Knoxville no benefit was obtained from treatment with mercury dust.

TABLE 4.—*Field germination of normal fuzzy, acid-delinted, and mechanically-delinted seed in seed-treatment tests at Jackson and Knoxville, Tenn., 1937*

Type of seed	Jackson				Knoxville			
	Field germination				Field germination			
	Untreated	Ethyl mercury chloride	Ethyl mercury phosphate	Type average	Untreated	Ethyl mercury chloride	Ethyl mercury phosphate	Type average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Normal fuzzy	17.2	47.4	57.0	40.5	57.9	59.0	59.8	58.9
Mechanically delinted	10.3	33.7	42.4	28.8	57.0	61.2	59.0	59.1
Acid-delinted	20.2	44.7	49.1	38.0	64.7	67.8	68.4	67.0
Average	15.9	41.9	49.5		59.9	62.7	62.4	
Difference required for significance at odds of 99 to 1. Between treatment average				7.7		4.3		
Between type average				7.7		4.3		
Between treatments within types				13.4		7.5		

The results of the experiments with scarified seed and with mechanically delinted seed indicate that changes in the structure or mechanical injury to the seed coat, under certain conditions, may contribute to lowered field germination. These experiments illustrate the impor-

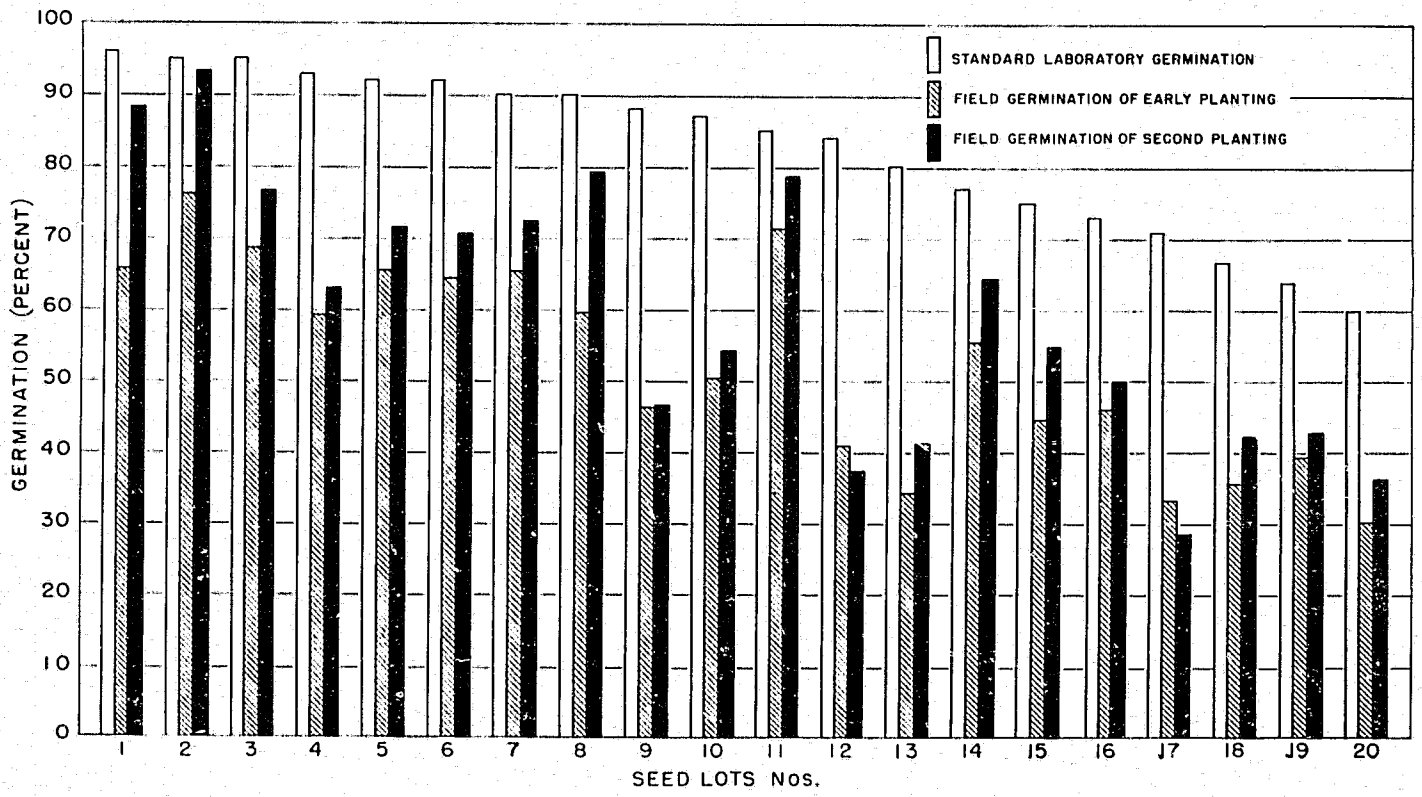

FIGURE 9.—Comparison of laboratory- and field-germination percentages of 20 lots of cottonseed.

tant role of the seed coat in the protection of the embryo during the period of germination.

Seed-germination percentages obtained in the laboratory under optimum conditions closely approximate the total percentage of viable seed in the sample. Figure 9 illustrates the comparative germination of a number of cottonseed lots in the laboratory and in the field. It may be observed that the seed lots usually give lower germination percentages in the field and that seed lots of the same laboratory-germination percentages differ in their ability to produce seedlings under field conditions. These differences in field efficiency are due to seed weaknesses not detectable by the standard method of germination. The similarity of the field behavior of the individual seed lots at the two dates of planting indicate that the differences between seed lots are due to qualities within the seeds and not to soil or climatic conditions.

DETECTION OF SENSITIVITY IN COTTONSEED

In germination tests with cottonseed, samples occasionally are encountered that germinate well under optimum conditions in the laboratory but that are practically worthless when planted under adverse conditions in the field. These types of cottonseed have been studied and described by Toole and Drummond (*15*) and Del Curto (*3*) and are termed "sensitive." The presence of sensitive or weak seed in ordinary lots of cottonseed, however, has not been generally recognized, because lesser degrees of sensitivity are difficult to detect in the laboratory by the standard method of seed germination and usually are obscured in the field by the high seedling mortality from other causes. If seeds that are comparatively free from disease contamination are planted on relatively clean soil, weaknesses due to physiological causes become apparent. Favorable conditions for determining physiological weaknesses of seed lots have existed at Knoxville during the course of these studies, as land not previously planted in cotton has been available for field experiments.

It has been shown that germinating cottonseed are sensitive to excess water under certain conditions of soil and temperature, and that sensitivity may be increased by injury to the seed coat. Observations of germinating seed under various laboratory conditions have indicated that the water-oxygen relationship is extremely important in germination and that any interruption of the normal process of water absorption and respiration will affect the subsequent germination percentage. The application of these facts is involved in the vacuum method of seed testing, used by the authors to detect sensitive seeds in the laboratory (see footnote 5, p. 14).

When cottonseed are immersed in water and subjected to greatly reduced atmospheric pressure for several minutes, air is withdrawn and water is absorbed rapidly. Fuzzy seeds subjected to this treatment become water-soaked, and a large percentage remain in a dormant condition if germination is attempted immediately. If the seeds are redried before germination is attempted, no apparent injury is caused by vacuum treatment. With slick or delinted seeds, the effects of the vacuum treatment are less drastic, as the seeds are not sealed by the water-soaked fuzz; in general, nonsensitive seeds are unimpaired by vacuum treatment, but weak or sensitive seeds fail to germinate after such treatment.

Comparisons of regressions of standard and vacuum laboratory-germination percentages on field-germination percentage are shown in figure 10. Standard error of estimate for these regressions and correlation coefficients derived from the data used in figure 10 are given in table 5. In all four germination tests, lower standard errors of estimate were obtained for the regressions with vacuum-laboratory-germination percentage than with those of standard-laboratory-germination percentage. In all tests the correlation between vacuum-germination

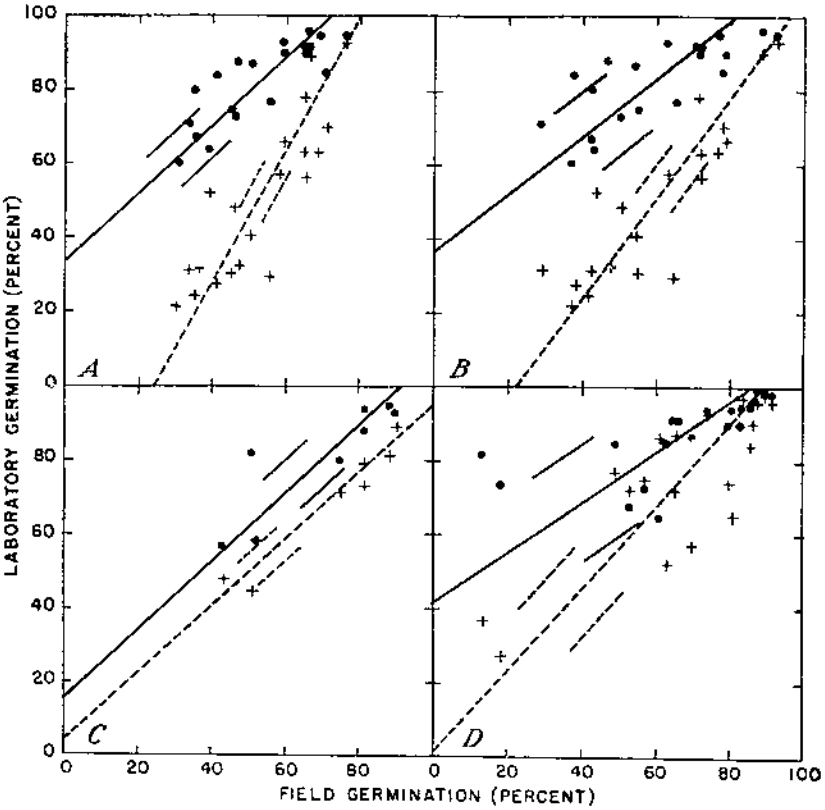

FIGURE 10.— Regressions of standard and vacuum laboratory-germination percentages on field-germination percentage of cottonseed: *A*, Early planted germination test in 1937; *B*, second planting of germination test in 1937; *C*, seed-treatment test in 1937; *D*, germination test in 1938.

percentage and field-germination percentage was higher than between standard and field. All correlation coefficients in table 5 are highly significant, exceeding odds of 99:1. Although correlation of the vacuum method with field stand is better than the standard method, the regression lines for the vacuum method show that the values for the poorer samples are depressed abnormally.

These data indicate that more accurate estimates of field germination may be obtained in the laboratory by the vacuum method of germination than by the standard method now in general use. The vacuum method is particularly useful in detecting seed lots that are

highly sensitive to adverse germinating conditions. Neither method is adequate for predicting the possible effect of seed-borne or soil microorganisms on field germination.

TABLE 5.—*Correlation coefficients and standard errors of estimate for regressions from the data used in figure 10*

Regression (method of germination)	Germination test (fig. 10)							
	A		*B*		*C*		*D*	
	[1]$S_{y.x}$	[2]r	$S_{y.x}$	r	$S_{y.x}$	r	$S_{y.x}$	r
Standard × field	8.17	0.8280	12.38	0.7664	9.73	0.8778	16.44	0.6904
Vacuum × field	7.47	.8585	9.25	.8773	5.60	.9613	13.11	.8155

[1] $S_{y.x}$=standard error of estimate. [2] r=correlation coefficient.

SUMMARY

In these investigations the anatomical structure of the cottonseed coat has been studied in relation to the germination of the seeds. The semihard covering or coat of the mature cottonseed may be divided roughly into five layers; epidermal, outer pigment, colorless, palisade, and inner pigment. The epidermal and outer pigment layers are continuous over the seed and around the funiculus. These layers are readily permeable to moisture and are highly absorptive. Vascular bundles radiating from the chalazal region occur in the outer pigment layer. The colorless layer is usually of one-cell thickness.

The palisade layer is composed of a single row of long, narrow cells, highly lignified. In the side-wall area these cells comprise approximately 50 percent of the total thickness of the seed coat. The palisade layer is not readily penetrated by moisture. The palisade layer is discontinuous at the micropyle end of the seed, the micropyle being lined with incurved rows of palisade cells, the outer walls of which come into contact and completely close the micropyle. At the chalazal end of the seed, the palisade layer terminates at the tip of the chalazal cap, leaving a definite opening through which the outer absorptive pigment tissue is in direct contact with the chalazal cap, a part of the inner pigment layer. The inner-pigment layer is continuous over the inner surface of the seed coat.

In the mature cottonseed the membranous covering of the embryo is attached to the seed coat at the base of the chalazal cap, but otherwise is free from the seed coat. In general, the structure of the seed coat is similar in all species and varieties of cotton upon which studies have been made. Differences are apparent in the thickness of the palisade layer, especially around the chalazal opening and in the proportion of cell length that is lignified.

Modification in the seed-coat structure can be effected by sulfuric acid delinting. Delinting by this method dissolves the lint and fuzz fibers of the seed coat and breaks and distorts the epidermal and outer pigment layer. Water absorption is more rapid in delinted seeds than in fuzzy seeds, but the difference is primarily due to the removal of the fuzz rather than to the disintegration of the outer layer of the seed coat. The disintegration of the outer pigment fre-

quently leaves the chalaza uncovered, and in many cases the palisade layer splits and curls back from over the chalazal cap. The opening of the chalaza facilitates the rapid intake of water.

The principal requirements for the initiation of germination in cottonseed include a supply of water, a supply of oxygen, and a favorable temperature. Unless these factors are present and are held within proper relation to each other, germination will not take place. The seed coat appears to play an important part in the regulation of the water absorption and gas exchange, which are necessary in seed germination.

The principal point of entrance of water to the embryo is through the chalazal opening in the seed coat. Entrance may occur through the micropyle but at a slower rate. Water does not readily pass directly through the side wall. Water may enter the outer pigment layer at any point and move through the pigment layer to the chalazal or micropyle opening. Movement is more rapid toward the chalazal end of the seed, possibly due to the vascular bundles in the pigment layer, which converge at the chalazal opening. Differences in rate of water absorption are apparent between seed lots, and rapidity of germination and rate of absorption are correlated.

Certain seed lots are sensitive to prewetting or oversoaking. Such samples when placed in a too moist substrata remain in a dormant condition or germinate very slowly. Too much water hinders the passage of air into the seed and interferes with germination. Respiration is high during the period of germination. In recent tests, germinating seeds at 30° C. during a period of 72 hours gave off 120 mg. of carbon dioxide per gram of seed.

Immaturity or underdevelopment of the seed coat may contribute to susceptibility of the seeds to the attack of micro-organisms. The softer seed coat of an immature seed would be more easily penetrated and would likely furnish nutrients more suitable for the growth of micro-organisms than would the semihard coat of a mature seed.

Seeds of different varieties, grown under identical conditions, differed in rapidity of germination and degree of dormancy. Seeds from these same lots with seed coats removed showed no tendency to dormancy whatsoever. Field experiments have shown that modification of the seed-coat structure by scarification or mechanical delinting may result in lowered germination percentage under adverse conditions. Apparently, in the germination of cottonseed, any condition of the seed coat or of the substratum that induces an oversoaked or waterlogged condition and excludes oxygen will probably result in lowered germination percentage.

Seeds that germinate well in the laboratory but that are highly sensitive to adverse conditions in the field frequently are encountered in germination studies of cottonseed. A vacuum method of detecting sensitive seeds in the laboratory has been used by the authors with better results than have been possible with the standard method of germination. Essentially, the method consists in subjecting acid-delinted seeds to reduced atmospheric pressure while immersed in water, after which germination tests are made as in the standard method.

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