Art. XII.—*The Palisade Cells of the Seed Coat of Albizzia lophantha.*

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(Communicated by Dr. E. I. McLennan.)

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Introduction.

This paper deals with the sectioning and staining of the seed coat of *Albizzia lophantha,* and the structure of the epidermal palisade cells. The first problem was to soften the seed coat to enable suitable sections to be cut with the microtome. Other problems followed, namely, the question of a suitable stain, the structure of the palisade cells, and the nature of the globule contained in these cells.

The work has been carried out in the Botanical Laboratory of the Melbourne University under the supervision of Professor Ewart, to whom I wish to express my thanks for his assistance and untiring interest, and for making available for me the facilities for the completion of this work. I take the opportunity of thanking Dr. McLennan, of the School of Botany, for her keen interest and valuable assistance.

General Structure of the Seed Coat, and Previous Work on this Subject.

The seed coat of *Albizzia* is composed of the following parts, the character of the cell wall of each part being determined by the chlor-zinc-iodine test:—

1. A cuticle composed of pure cutin.
2. An epidermis of palisade cells—the macrosclerids or Malpighian cells of Pammel (7)—the outer portion of which is cuticularised (the cellulose wall being impregnated with cutin), and the inner portion is composed of unaltered cellulose.
3. A row of hour glass cells—the osteosclerids of Pammel—which are very characteristic of Acacias.
4. Inner layers of integument—the nutrient layer of Pammel—the cell walls of which are composed of hemicellulose.

Rees (8) concluded from an examination of hand sections of the seed coat, that there are two layers of palisade cells instead of one, as is the general rule in Acacias. No reference was made to the globules, but it appears from the diagrams that these were interpreted as intercellular spaces between the two layers of palisade cells (8, pl. lxxxi.).

The Sectioning, Maceration, Staining, and Microscopic Study of the Seed Coat.

The main difficulty in sectioning the seed coat was to soften it, and to retain this softness during fixing and embedding. The first attempts at fixing the material by means of Flemming's fluid and Bouin's formal fixative were failures. The material became very hard and brittle, and difficult to section. Various methods of softening were tried, e.g., soaking the seeds in chloroform, caustic potash, and sulphuric acid, at various temperatures, and for various lengths of time. Then the seed coats were embedded in paraffin in the usual manner, using both the glycerine and spirit methods of dehydration. No improvements on the first methods were noted.

Finally, it was found that by soaking the seeds for at least one week in hydrofluoric acid, and then quickly transferring them through the alcohols and chloroform to paraffin, very satisfactory sections could be obtained. Sections of a thickness of 5μ-10μ were cut, and from these the structure of the seed coat could be clearly defined.

By soaking the seeds in a concentrated solution of caustic potash for 5-6 days, and by teasing out the frayed seed coat resulting from this treatment, single palisade cells were isolated. Single cells were also isolated by boiling the seed coat in a solution of concentrated nitric acid and chromic acid for 2 minutes, and also by boiling in aqua regia for 5 minutes. From an examination of the single cells thus isolated, the relation of the globule to the palisade cell, as determined from sections, was confirmed.

Various stains were tried (Chamberlain (1)). These included Iron alum-haematoxylin and Erythrosin, Ruthenium red, Safranin and Light green, and Gentian violet. Of these, Safranin and Light green used in combination was the most satisfactory, the Safranin staining the cuticularised parts of the seed coat whilst the Light green stained the unaltered cellulose, and thus differentiated the parts of the testa.

From a study of these sections, and of the single cells isolated by maceration, the structure of the testa is seen to differ from the description given by Rees in the following points:—

1. Forming the epidermal layer there is one layer of palisade cells, and not two.
2. A highly transparent globule exists in the cavity of these cells just beneath the limit of cuticularisation.
3. A layer of hour glass cells separates the epidermis from the inner layers of the integument.

Hand sections, where no fine degree of thinness can be obtained, might suggest two layers of palisade cells, and owing to their high transparency, the globules could be overlooked, or might appear as intercellular spaces between the two layers of cells, especially if the sections were not stained.

1. The Cuticle and Epidermal Palisade Layer.

The cuticle, as seen in thin section, is well defined. The outer ends of the palisade cells can be clearly seen. The inner part of the cuticle appears to be laminated, suggesting that cutin has been deposited in successive layers on the outer ends of the palisade cells. (See Fig. 1.)

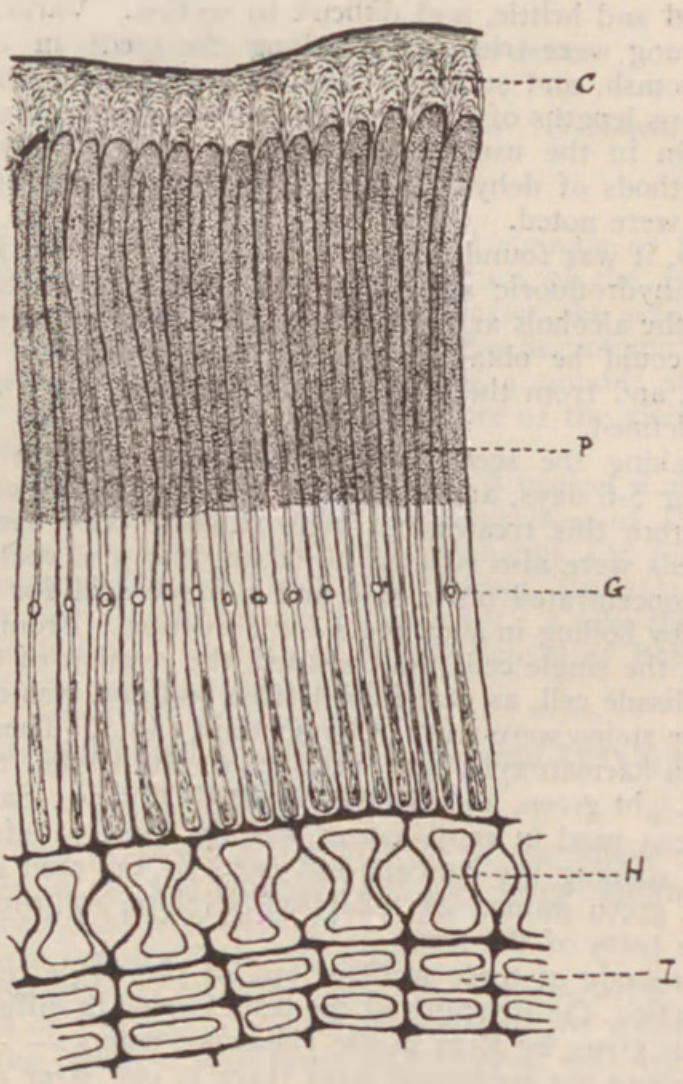

Fig. 1.—Transverse section of portion of the seed coat of *Albizzia lophantha*. C. cuticle; P. palisade cells; G. globules; H. hour glass cells; I. inner layers of the integument. ×300.

The epidermal layer is made up of one layer of long narrow palisade cells. In this respect, *Albizzia* is not an exception to the rule among the Acacias. These cells are packed closely together, and at the inner end they abut on to the hour glass cells. The cell cavity is long, and although it narrows and is difficult to see in the outer portions of the cells, it is quite clearly seen at the inner ends, where it widens out, and is filled with protoplasmic contents,

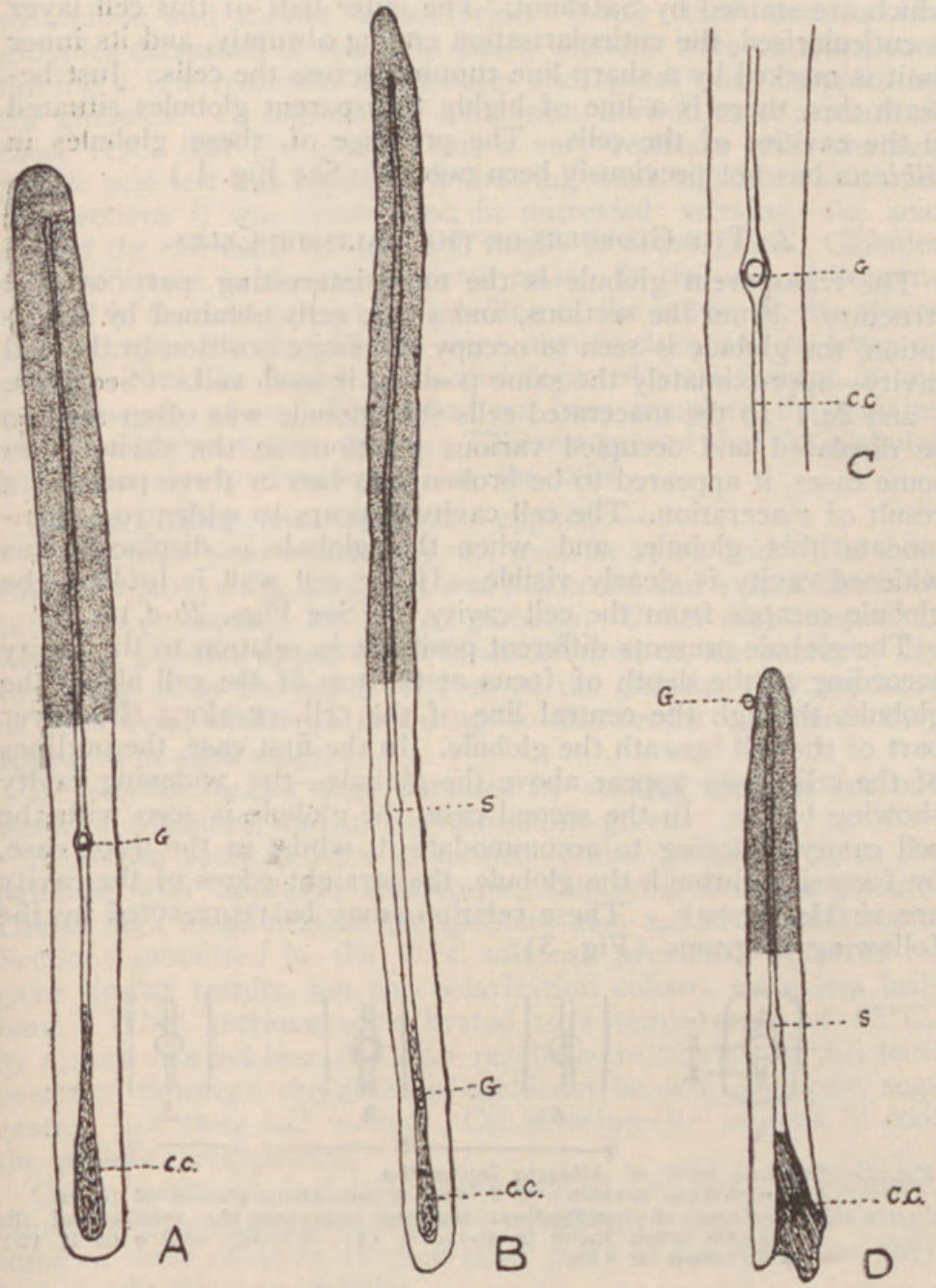

FIG. 2.—Palisade cells of *Albizzia lophantha*, drawn with the aid of a camera lucida. The dotted area represents that part of the palisade cell which is cuticularised.

A. Single palisade cell isolated by maceration. G. globule in its normal position; C.C. cell cavity with protoplasmic contents ×500.

B. Single palisade cell isolated by maceration. S. space in cell cavity previously occupied by globule; G. globule broken into three parts as a result of maceration; C.C. cell cavity with protoplasmic contents. ×500.

C. Portion of a single palisade cell isolated by maceration, and drawn with the aid of an oil immersion lens. G. globule in its normal position in the cell cavity (C.C.). ×720.

D. Single palisade cell isolated by maceration. S. space in cell cavity previously occupied by the globule; G. globule which has escaped from the cell cavity; C.C. cell cavity opening to the exterior owing to a break in the cell wall caused by maceration. ×500.

which are stained by Safranin. The outer half of this cell layer is cuticularised, the cuticularisation ending abruptly, and its inner limit is marked by a sharp line running across the cells. Just beneath this, there is a line of highly transparent globules situated in the cavities of the cells. The presence of these globules in *Albizzia* has not previously been noted. (See Fig. 1.)

2. The Globules of the Palisade Cells.

The transparent globule is the most interesting part of the structure. From the sections, and single cells obtained by maceration, the globule is seen to occupy a definite position in the cell cavity—approximately the same position in each cell. (See Figs. 1 and 2*a*.) In the macerated cells this globule was often seen to be displaced and occupied various positions in the cavity. In some cases, it appeared to be broken into two or three parts as a result of maceration. The cell cavity appears to widen to accommodate this globule, and when the globule is displaced, this widened cavity is clearly visible. If the cell wall is broken, the globule escapes from the cell cavity. (See Figs. 2*b-d*.)

The globule presents different positions in relation to the cavity according to the depth of focus at the top of the cell above the globule, through the central line of the cell, or along the lower part of the cell beneath the globule. In the first case, the outlines of the cell cavity appear above the globule—the widening cavity showing below. In the second case, the globule is seen with the cell cavity widening to accommodate it, whilst in the third case, by focussing through the globule, the straight edges of the cavity are visible beneath. These relations may be represented by the following diagrams (Fig. 3).

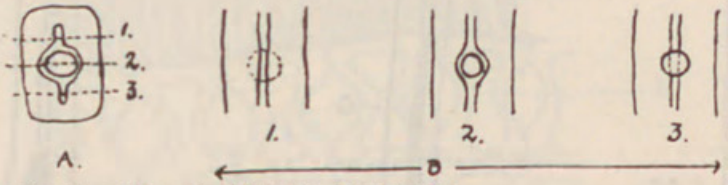

Fig. 3.—Palisade cells of *Albizzia lophantha*.
A. Transverse section; 1, 2, and 3 indicating planes of focus.
B. Portions of longitudinal sections, showing the relation of the globule when focus is above it (1), through centre of it (2), and below it (3).

Following methods set out in Ewart (2), Haas and Hill (3), Lee (4), McLennan (5), and Onslow (6), a large number of microchemical tests were carried out in order to ascertain the chemical nature of the globule. On the results of the following tests it was concluded to be a globule of wax.

Stained sections were placed in the commonly known wax solvents—carbon tetrachloride, carbon disulphide, chloroform, and ether—for a period of five months, and weekly examinations were made of the material. After some time, the globules appeared to have a roughened appearance, suggesting that they were being dissolved. The action was most rapid in the sections in carbon

disulphide and carbon tetrachloride. After four months the globules decreased in size, and finally sections were obtained containing a few remnants of globules and spaces once occupied by the globules. To confirm the conclusion, arrived at as a result of these tests, that the globule was a very resistant wax, the sulphuric acid test was applied. By drawing weak sulphuric acid over the sections it was found that in untreated sections, the acid caused the cell walls to swell and finally to disintegrate. Globules were still seen in the disintegrated mass. In the case of sections treated in the solvents, on the swelling of the cell walls, the spaces once occupied by the globules were seen to close, and no trace of globules could be found in the disintegrated mass, except in one or two cases, where solution had not been complete, the remains of a few globules were found. This test proved that the globules had been slowly dissolved from the cells.

The following tests were also applied:—

1. Sections were soaked for one week in osmic acid. The cuticularised part of the seed coat was blackened and a dark line indicated the position of the line of globules. Under high magnification, the globules appeared to be discoloured on the surface. By focussing on to the top or bottom of the globules they appeared as dark dots, whilst on focussing to the centre of them, they appeared as dark rings.

2. Similar results were obtained by soaking sections for one week in Alkannin, and in Fuchsin-iodine-green.

3. When sections were examined under polarized light, the palisade layer appeared anisotropic, displaying brilliant colours. Under high magnification the globules also appeared anisotropic. Sections submitted to the wax solvents previously referred to, gave similar results, but the polarization colours were less brilliant. When sections were heated to a temperature of 95°C., by means of a hot box, the same results were noted. At this temperature, however, the globules could not be seen distinctly, suggesting that they had melted. On allowing the sections to cool the globules reappeared.

Other microchemical tests were applied for tannins, cellulose, lignin, suberized and cuticularised membranes, etc., and although some of these reagents affected other parts of the seed coat, they had no effect on the globules.

These wax globules probably serve the purpose of plugs to keep open the long palisade cells, and to add strength to the palisade layer. Being of a waxy nature, they form a very effective means of increasing the impermeability of the seed coat to water.

3. The Hour Glass Cells.

The hour glass cells, with their characteristically shaped cell wall and cavity, and with rounded intercellular spaces between them, separate the epidermal palisade layer from the inner layers of the integument. (See Fig. 1.)

Summary.

1. The seed coat of *Albizzia lophantha* can be successfully sectioned after soaking it for at least one week in hydrofluoric acid, and by transferring quickly through the alcohols and chloroform to paraffin.

2. Safranin and Light green used in combination are suitable stains to differentiate the structure of the testa.

3. There is only one layer of palisade cells forming the epidermis.

4. In the cavity of each palisade cell is a highly transparent globule. These globules appear to be arranged in a distinct line across the cells.

5. The globules are of a waxy nature. They dissolve slowly in wax solvents, are stained on the exterior by fat stains, and have a high melting point.

6. A layer of hour glass cells separates the epidermal layer from the inner layers of the integument.

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