The Use of Celloidin for the Study of Leaf Surfaces

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The shapes, sizes, and arrangement of epidermal cells of plant leaves are consistent enough that they can be used in a limited way in classification. However, preparation of paradermal sections of leaves by the paraffin method entails considerable time and difficulty in obtaining a usable section. Transectioning by hand or stripping the epidermis also is laborious and unrewarding in the production of good sections.

A technique, which employs the use of celloidin, has been developed which reveals in detail the size and shape of the epidermal cells. The locations of the stomata are easily observed as well as their position in relation to the other epidermal cells (figs. 1-6). The technique is valuable for quick, easy stomatal counts. Another advantage is that these celloidin peels can be indexed and stored, allowing the examination to be conducted at a later date. Also, the leaves need not be killed, fixed, or embedded in paraffin. Some plants can be used, such as *Zea mays*, without harming the leaves. On other plants, such as soybean, the celloidin penetrates the leaf thereby destroying it, but the plant itself is unharmed.

Leaves to be studied must be clean. Cleaning can be accomplished in one of two ways. The leaf can be brushed with a camel's hair brush, or a thin coat of celloidin can be applied, allowed to dry, then pulled off and discarded. The thick waxy cuticle of some plants, such as banana, may have to be removed by lightly scraping with a razor blade. Care must be taken to prevent injury to the epidermis.

After the leaf is cleaned, a thin coat of celloidin is applied with a camel's hair brush. This celloidin is allowed to dry completely and then is pulled off. Better detail is obtained by using thin sections. The results, for some purposes, are equivalent, if not superior, to paradermal sections prepared by the paraffin technique.

This technique has some disadvantages. Sometimes it is impossible to obtain a microscopic field that is entirely in focus. This is caused either by curvature of the leaf itself or by curvature in the celloidin as it is pulled off. Even though this prevents a photomicrograph from being entirely in focus, (fig. 5), one can observe the shapes and structures of the epidermal cells and stomata counts can be made by adjusting the focus with the fine adjustment knob.

Another disadvantage is encountered when using leaves which have sunken stomata (fig. 2). The depression above the stoma causes holes to form in the celloidin that appear as black spots in the photograph. Even though no details are recorded the number of stomata per unit area can easily be counted and some estimation of size can be made.

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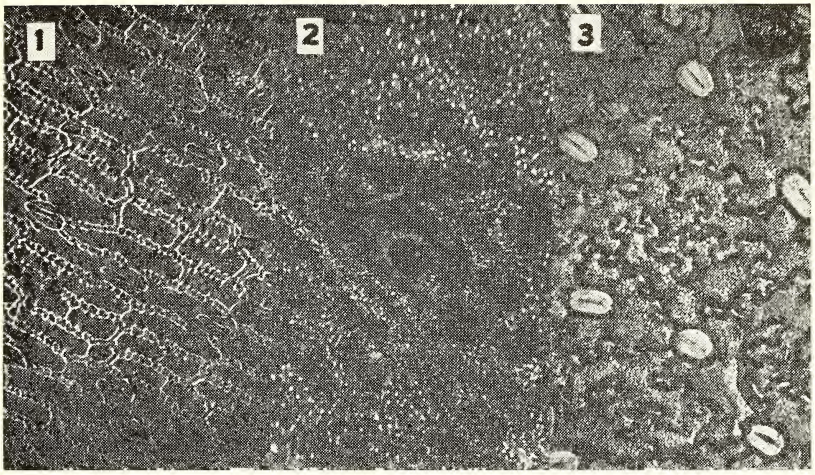

PLATE I

All photomicrographs are of abaxil surfaces unless stated otherwise, and are reproduced at approximately 200 X

1. *Zea mays:* Stomata appear clearly as do the wave-like structure of the cell walls.
2. *Tsuga canadensis:* Black spots are holes in the celloidin caused by the sunken stomata.
3. *Onoclea sensibilius:* Note the irregularity of cell shape.

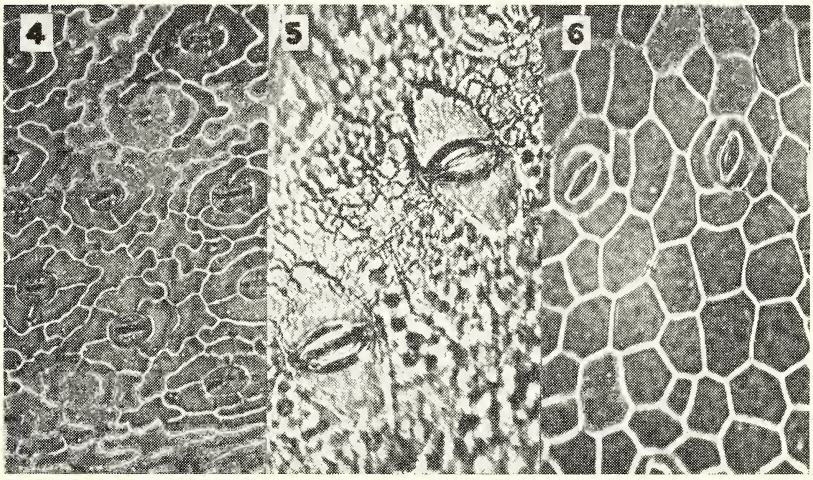

PLATE II

4. *Nephrolepis exaltala:* (var. bostoniensis) The number of subsidiary cells vary from two to four.
5. *Dioscorea:* The extremely large stomata are seen while the other epidermal cells are not clearly distinguishable.
6. *Crassula arborescens:* Adaxil surface. Note regular symmetry of epidermal cells.

The same problem of focus occurs with leaves which have raised stomata. If the focus is made on the epidermal cells, the stomata will appear out of focus. If the focus is made on the stomata, the epidermal cells will appear out of focus. However, this presents no real problem, the solution being to take two photographs, one at each focus.

This technique was developed to determine stomatal numbers in dwarf and normal *Zea mays*. Using this technique a large number of leaf surfaces were counted with a minimum of time and effort. Other information was obtained at the same time, such as the relative size of both the stomata and epidermal cells, and whether or not the stoma were opened or closed.

Studies involving ploidy, leaf ontogeny, classification, and comparison of hybrids may be supplemented by this technique.