

## Observations on the Stomatal Structure of *Ilex Opaca*

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An anatomical study of the leaves of twenty-two shrub-bog species collected in North Carolina pocosins revealed several structural peculiarities in the leaf of the American holly, *Ilex opaca*, Ait. The guard cells of this species were unique among those of the twenty-two species studied in that they were the only guard cells whose walls took such lignin dyes as safranin and crystal violet.

In surveying literature for any previous work on this leaf, nothing was found. Solereder, in his *Systematic Anatomy of the Dicotyledons*, did not mention *Ilex opaca* but made the following statements concerning the holly family as a whole: "Stomata are found only on the lower side of the leaf; special subsidiary cells are wanting. . . . The neighbouring cells of the stomata in some cases bear slight resemblance to subsidiary cells, e. g. *I. Paraguariensis*." These statements are of special interest because the guard cells of *Ilex opaca* appear to have special subsidiary cells.

The purpose of this paper, then, is to present a somewhat detailed study of the stomatal apparatus of this species, pointing out the interesting structural features.

The material examined came from trees in North Carolina, Ohio, and Indiana. Some of these trees were growing in bog water; others were growing in comparatively dry situations. Use was made of leaves that had been put through the paraffin process and various stain combinations. Microtomed paradermal and cross sections from fresh leaves were also studied. Cell wall structure was microanalyzed by various solubility and staining reactions.

The stomata of this leaf when examined on the lower epidermis at a low magnification appear to be nothing different from the ordinary dicot type. Yet, what could be taken on first sight for guard cells and a pore will be found to be raised parts of the cuticular covering. Each stomate is covered by a layer of cutin which extends out over the true pore in the manner of an oval dome with a slit in the ceiling of its long axis. The cuticular margins of the slit are usually jagged. As shown in figure 1, this oval dome is encircled with an irregular cuticular ridge which gives the appearance of a rampart surrounding the dome, a feature occasionally found in the *Ilicineae* according to Solereder.

The guard cells when brought into paradermal focus appear to be the familiar bean-shaped cells. In longitudinal and cross sections their top and bottom walls are quite thick, while the rounded, slightly enlarged ends of the cells have thin walls. The thickening of the bottom wall is not usually extended to the ends but is more confined to the pore length of the cells. Longitudinal views showing this may be seen in figures 4 and 5. In figure 4 is represented one guard cell cut longitudinally through the pore revealing how the cutin from the dome extends inward through the

pore over that part of the guard cells as a lining of the opening. The lumen of the guard cells is greatest at the ends. These cells though covered with a thick cutin layer are not sunken in relation to the other epidermal cells, but are slightly raised being partially supported by a ring of four to six, comparatively large, thin-walled epidermal cells, as seen in figure 2.

The cells of this ring are so different in size, shape, and wall thickness from epidermal cells that they appear to be special subsidiary cells. They may be encircled with one or two rings of narrower cells that appear to be a transition towards the thick-walled epidermal cells. Figure 3 was drawn from a paradermal section and represents the kind of view obtained looking from the spongy layer out through the lower epidermis. The guard cells are hidden in this view by the overlapping thin-walled subsidiary cells. In figure 2 two guard cells, represented by the heavy lines, are seen as they appear in focus just within the cutin layer. The two concentric rings of broken lines outside of the guard cells indicate the position of the cuticular rampart which was referred to before.

The thick-walled epidermal cells are shallow in depth compared with the extent of their lengths and widths. Their sides and ends protrude with curves that give the walls a serpentine appearance in paradermal sections. Especially noticeable in such sections is the pitting of the outer wall. Numerous pits extend into the cutin layer. According to Solereder pitting of this type is rare in leaf structure.

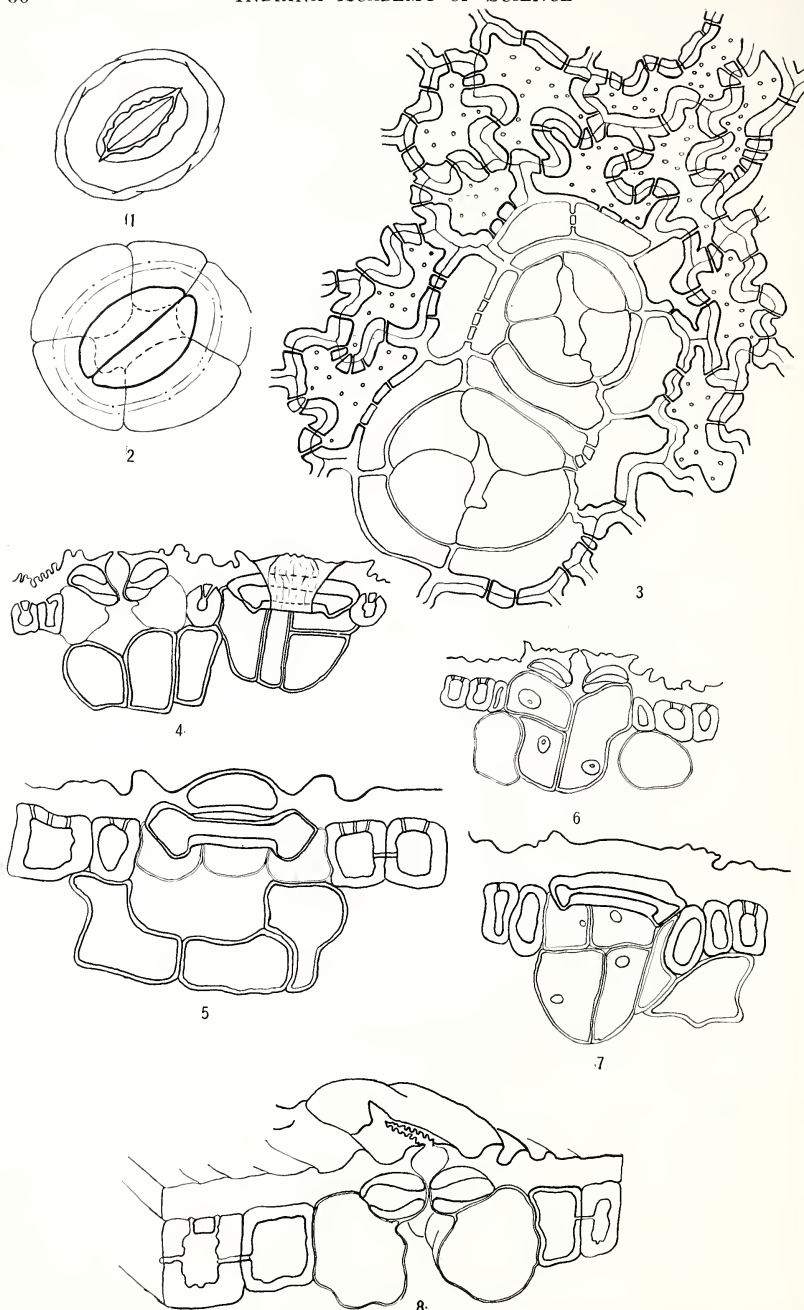
In order to determine something about the chemical nature of the wall structure, of the guard cells especially, staining and solubility tests were made and the following results obtained.

*Lignin:* Fresh sections showed distinctly positive reactions for the Maulé and phloroglucin tests in the thick walls of the guard cells and epidermal cells but negative reactions for the thin walls of the large cells adjacent to the guard cells. Sections were delignified and these two tests again applied. Where positive reactions occurred before, negative results now confirmed the first tests.

*Cellulose:* The chlorozinc-iodide and hydrocellulose tests gave no distinct indication of cellulose until the lignin, pectin, and cutin were removed from the cell walls. These three substances masked the cellulose thoroughly enough to prevent its dissolving in cuprammonia until they themselves were removed.

*Pectin:* Two staining tests, ruthenium red and methylene blue, when applied to delignified sections revealed a comparatively heavy concentration of pectin throughout the thickened wall of the guard cells, but little, if any, in the thin walls of the adjacent cells. These results were checked by applying the same stains to sections from which the pectin had been removed.

*Cutin:* Sudan III was used to bring out any less apparent areas of cutinization. This gave indications of the cutin extending over the areas of guard cell walls forming the pore. The thin walls of the cells adjacent to the guard cells also appeared to be thinly cutinized.



All of the drawings except Fig. 8 were made by camera lucida and represent, here, a magnification of 530 X. Fig. 1. Surface view of a stoma. Fig. 2. Paradermal view of the same stoma just beneath the cutin layer, showing the closed guard cells partly supported by a ring of subsidiary cells. Fig. 3. Paradermal view of epidermal and subsidiary cells as seen from the spongy layer

This wall microanalysis made it clear that the guard cell walls are more like epidermal cell walls in chemical composition and structure than like the thin-walled, large adjacent cells which are so different from both the epidermal and guard cells in form and structure. It seems fitting, then, to refer to these thin-walled cells as subsidiary cells.

It may be expected that these subsidiaries aid in the mechanical functioning of the guard cells. Hinges, which are useful in this respect are noticeable in cross sections. One unusual fact concerning these subsidiaries was evident in sections from older leaves. Not infrequently there was noticed a plugging of the inner stomatal chamber, which must have been affected by one or two divisions of the subsidiary cells. The walls of these plugging cells are usually thicker than the ordinary subsidiary cell wall, and also partly lignified.

Outlines of wall structure in paradermal sections were more distinct when fresh sections were mounted in a salt solution. Plasmolysis of subsidiary and epidermal cell protoplasts was clear. The guard cells, however, revealed no internal changes. This may have been partly because they were hidden by the subsidiaries through which one must focus, or, more likely, because of their small lumen.

It should be pointed out that no new type of stomatal apparatus is presented here, as the stomatal mechanism of *Ilex opaca* would be considered much the same as Schwendener's *Helleborus* type referred to by Copeland<sup>1</sup>. Nevertheless, the cuticular covering, the cutin of the dome

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<sup>1</sup> Copeland, E. B. The mechanism of the stomata. *Ann. Bot.* **16**:327-364, 1902. extending internally through the stomatal pore as a lining even in the thin walls of the subsidiary cells, the partially lignified guard cell walls, and the plugged stomatal chambers, all present an interesting aspect of xeromorphy in the stomatal mechanism.

### Summary

The stomatal structure in the leaves of *Ilex opaca* was studied and the following features pointed out as exhibiting xeromorphy:

- (1) Guard cells with differentially lignified walls.
- (2) Stomatal pores lined with a thin internal extension of the cuticular dome.
- (3) Thin-walled, slightly cutinized cells adjacent to the guard cells, which appear to be subsidiaries considering their location, form, and wall structure.
- (4) Plugged stomatal chambers apparently caused by the division and growth of some subsidiary cells.

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looking out through the lower epidermis. Fig. 4. Sectional views through two adjacent stomata. Fig. 5. Sectional view revealing a longitudinal cut through one guard cell. Figs. 6 and 7. Two sectional views of stomata plugged by apparent division of subsidiary cells. Fig. 8. A three-dimensional concept of a cross section through a stomate and nearby cells.