A MICROCHEMICAL EXAMINATION OF McINTOSH APPLE LEAVES SHOWING RELATIONSHIP OF CELL WALL CONSTITUENTS TO PENETRATION OF SPRAY SOLUTIONS

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(WITH THREE FIGURES)

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Spray solutions containing minor elements, nitrogen, "hormones" and organic fungicides have been applied as foliage sprays to apple trees to reduce nutritional deficiencies, prevent fruit drop or increase resistance to disease. The fact that such applications have given results showed that they had penetrated the leaves and raised the question of how penetration occurred.

The quality, quantity and location of wall substances throughout the apple leaf and the part these play in the entrance of materials applied as sprays, either as fungicides or nutrient elements, has not been reported other than the preliminary reports of the authors (**5**) and (**6**).

WYLIE (**7**) found that the epidermis is important for water conduction in areas between veins. In experimenting with dilute solutions of potassium ferrocyanide, he showed that it was quickly conducted throughout the epidermal layers of leaves and that the "vein extensions" and epidermal layers "constitute in some degree a supplementary conductive system." MACDANIELS and COWART (**3**) claim that "the outer cell walls" of the epidermis of apple leaves "are covered with a continuous cuticle." While this would not prevent the lateral movement of materials after once having entered the leaf, it would not account for the entrance of foliage sprays as is indicated by HAMILTON, PALMITER and ANDERSON (**2**) and PALMITER (**4**).

In this investigation, a determination of the quantity and location of cutin, cellulose and pectinaceous substances of the cell walls was made by the following methods: Sections were cut from fresh McIntosh apple leaf material, using a freezing microtome. All photomicrographs were made by the use of a Leica Camera attached to a Leitz microscope by means of an Ibso attachment with correction lens giving an initial magnification of $\frac{1}{3}\times$ Periplan $10\times$ eyepiece and a water immersion or other objective. Kodachrome K-135A artificial light film was used. Methods of microchemical determination of the cutin, cellulose and pectinaceous substances were those given in Eckerson's "Microchemistry" (**1**).

The structure of the untreated walls of the epidermal cells (fig. 1) is particulate in character. It shows clearly that these particles are in layers in the outer and vertical walls.

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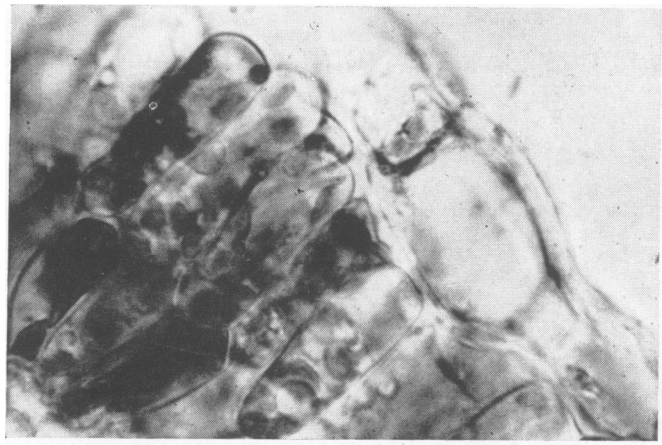

FIG. 1. Cross section of epidermal cells of leaf showing its particulate nature.

In both a surface view of the outer wall of the epidermal cells (fig. 2) and in the cross section, the particulate character and layering of the particles in the walls are shown. The inner portions of the walls, which are the more recently formed, show much irregularity in the layering. This layering shows that the wall is by no means a uniform, solid structure.

The location of cutin, a substance which is known to prevent the absorption of water, was determined by the use of Sudan III, which gives a red color reaction with this substance. A cross-section of a leaf treated with Sudan III shows that cutin exists in lamellae parallel to the outer epidermal walls, but it by no means forms a continuous covering (fig. 3A). Sections parallel to the surface showed the walls with their cutinized areas in discontinuous lines.

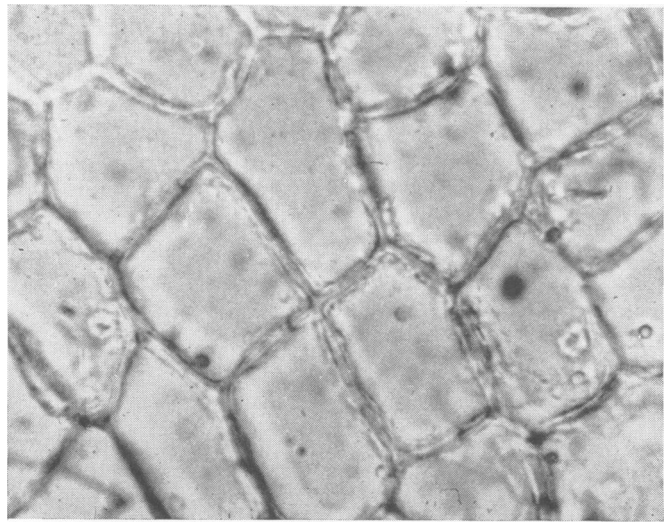

FIG. 2. Surface view of outer wall of epidermal cells showing layering of particles.

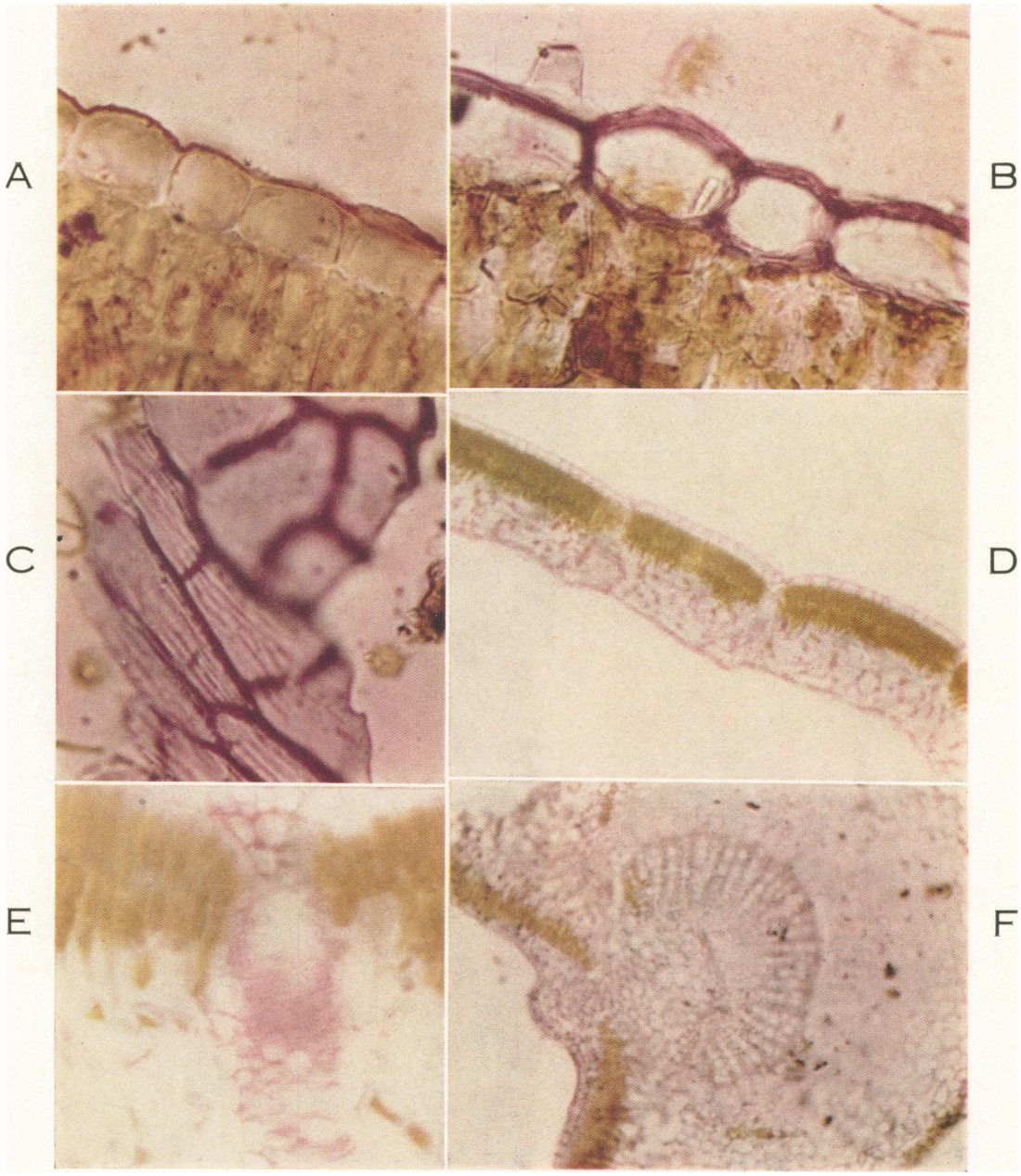

FIG. 3. Tissue sections. A. Cross section showing cutin present in lamellae parallel to outer walls of epidermal cells. B. Cross section of epidermal cell treated with ruthenium red showing location of pectinaceous substances. C. Wall treated as in B showing pectinaceous substance in intermittent strands. D. Cross section treated with ruthenium red, showing pectinaceous substances forming continuous path from outside to walls of "vein extensions." E. Showing amount of pectinaceous substances in walls of vein extension cells. F. Shows large amount of pectinaceous substances in walls of cells of bundle sheath of large vein.

Since the pectinaceous substances are known to have great water absorption power, tests for these substances in sections corresponding to figures 1 and 2 were made. In the presence of Ruthenium red, the pectinaceous substances give a reddish pink color. B and C of figure 3 are cross and parallel sections respectively of the epidermal cells, which show clearly the presence of the pectinaceous substances in all the walls of the epidermal cells. There is much of these substances in intermittent parallel layers in the outer walls of the epidermal cells interspersed with cutinized areas. Layers of these substances extend vertically, whereas the cutinized areas were only parallel. The pectinaceous substances form a continuous path reaching from the outside of the leaf and extending to the walls of the "vein extensions" (fig. 3D).

The walls of the vein extension cells (fig. 3E) contain a large amount of pectinaceous materials as well as do the walls of the cells of the bundle sheath of a large vein (fig. 3F). These are composed largely of pectinaceous substances.

The epidermal cell walls of the McIntosh apple leaf, therefore, can no longer be considered as covered with a continuous cuticle which prevents the absorption of water. The amount and location of pectinaceous substances present in the leaves account for the entrance of water soluble materials such as minor elements, nitrogen, hormones, and organic fungicides sprayed upon apple trees.

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