A Study of Tissues about a Large Leaf Mine

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The role of the epidermis in leaf organization has not been extensively explored. As a surface covering it affords some protection, but this is due chiefly to the cuticular film which protects both the epidermal layer and underlying cells. The importance of the epidermis, as a tissue of living cells, is probably much greater than has been generally realized. For this reason observations and experiments providing further information about the epidermis are of special interest. The present paper summarizes a study of a blotch-type leaf mine in the upper side of a leaf (*Eupatorium purpureum*), with special reference to its epidermal cover. It seemed of importance, also, to record for general reference the detailed structure of this leaf from an herbaceous dicotyledon plant growing in partial shade.

These plants were collected on Aug. 18, 1950, by Mr. Marcus J. Fay, in Section 10, Sugar Creek Township, Cedar County, Iowa. They were then in full bloom, growing on a dry, west slope, in a scattered upland forest of *Quercus alba* and *Q. rubra*. Leaves on these *Eupatorium* plants had several mines and all seemed deserted at the time of collection. One mine in the upper side of a normal upper leaf was selected for study because of its considerable size and symmetrical outline. The seemingly imperforate upper epidermis was somewhat convex, because of enclosed gases, and had a soft flexible texture. This inflated mine which was whitish in color with a faint green tinge, was about 20 x 60 mm. and its area, measured by planimeter, was 1020 sq. mm. There were no islands within its borders and the upper epidermis was attached only along the margins of the mine.

A portion of this leaf, including the mine and adjacent tissue, was killed in FAA. First, the upper epidermis was cut free along its margins, and, as a thin translucent film, placed in fixitive. Next the area of the mine was excised, and cut into small rectangles before fixation, as were contiguous areas of the blade. Portions of these three areas were then imbedded separately in paraffin and sectioned in both transverse and paradermal planes. Pieces of the upper epidermis were also subsequently mounted for microscopic study.

La Rue (1) noted the natural separation of the lower epidermis, and its contiguous layer of spongy mesophyll, from the body of the
leaf in *Mitchella repens*. The entire area of the lower epidermis from margin to midrib was detached, but in all observed cases this layer continued to live without change in appearance. While he found no other leaf having a natural separation of either covering layer, he discovered a number of species with leaves in which the epidermis could readily be separated, either by manipulation or operation, and in all of these any isolated epidermis, if unbroken, remained alive for several weeks. He also noted the apparently living epidermis over leaf mines; some of these he sectioned to verify their normal condition.

The general structure of this leaf of *Eupatorium purpureum*, about the mine, was typical of shaded herbaceous dicotyledons. (Fig. 1) The blade was thin, with some taper between midrib and margin, and averaged about 80 μ in thickness. This depth of lamina was distributed as follows: upper epidermis, 16 μ; the single palisade layer, 19 μ; the three layers of spongy mesophyll, combined, 33 μ. and the lower epidermis, 12 μ. Chloroplasts were found in all living tissues outside the vascular bundles; in addition to the mesophyll they were fairly abundant in both epidermal layers and in cells of the bundle sheath.

Cells of the upper epidermis, except over veins, have long lateral lobes on all sides and these cells have an average overall spread of about 92 x 77 μ (Fig. 3). The main body of these cells was about 16 μ in diameter, while its lobes tapered to a thickness of 5-6 μ in the plane of cell junctions. Many of these lobes were broader at the tip and some were bluntly branched at the apex. These cells were thus peculiarly integrated and had an extensive area of lateral contact between them. Many of these cells were nearly radial in symmetry but those over smaller veins were long and slender averaging about 130 μ in length. Cells over the larger veins were much shorter and their strongly oblique cross walls left them with pointed ends. Due to the wide separation of veins in this blade most of the epidermal cells were of the large, lobed type. A conspicuous nucleus was located near the center of mass in each cell and a number of chloroplasts were scattered over the floor of the cell. Counts for a number of cells showed sixteen per cell as the most common num-

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**DESCRIPTION OF FIGURES**

Fig. 1. Transection of leaf of *Eupatorium purpureum* in area adjacent to the leaf mine.
Fig. 2. Transection through the margin of a leaf mine. The larvae had destroyed the single layer of palisade mesophyll.
Fig. 3. Paradermal section through the isolated upper epidermis forming the roof of the leaf mine.
Fig. 4. Paradermal section through the lower epidermis under the leaf mine.
Fig. 5. Transection through a portion of the upper epidermis over the leaf mine. This layer has been isolated by the destruction of the palisade mesophyll.
Cells of the lower epidermis were somewhat smaller and had shorter lobes, but were less regular in form. The body of these cells often consisted of two or three major branches and these frequently in contact with widely separated stomata (Fig. 4). Walls were thinner than in the upper layer and the number of chloroplasts per cell fewer.

The single layer of palisade parenchyma consisted of relatively short cells which were closely united in the plane of the junction with the upper epidermis. At lower levels they were separated, and, seen in paradermal sections, were often slightly meshed into circular groups. This grouping may have been due to their anchorage to the spongy mesophyll cells below them which were definitely meshed. Expansion of spongy mesophyll cells often shifts the lower end of the contiguous tissue. All mesophyll cell walls were very thin.

The spongy mesophyll consisted usually of three layers and cells all excepting the uppermost were widely meshed. When portions of these meshes were superimposed the cells appeared as short columns in transections of the blade. All the mesophyll tissue included much intercellular space.

Brief reference should be made to the wide spacing of both the minor veins and the bundle sheath extensions of this leaf. One of the desirable features of this leaf, from the view point of the infesting larvae, is the wide separation of veins and the soft tissue above them, permitting larvae to cut or push their way freely over the veins. The mean vein separation, or intervascular interval, was 346 µ, thus leaving relatively broad areas of uninterrupted mesophyll. The bundle sheath extensions formerly called "vein extensions" (Wylie 2), were farther apart because not all veins carry extensions; their mean separation, in the mine area, was 1070 µ. This particular mine extended lengthwise along a lateral half of a smaller leaf and crossed a number of major veins. On another leaf of about the same size the larvae had crossed over the midvein for a length of 30 mm., thus uniting two large mines and resulting in a bilobed mine having an area of over 2000 sq. mm.

Transverse sections through a smaller vein on the same leaf showed that the single palisade layer was the only blade tissue destroyed by the larvae (Fig. 2). Cells of this layer had been cleanly cut from the epidermis above and the spongy mesophyll below. Its isolation was then completed by the destruction of the thin walled layers of cells over the vascular bundles. This permitted the upper epidermal layer to be pushed up from margin to margin and
enclosed gases gave the mine a blister like appearance. Sections through the detached epidermis, prepared from imbedded material, showed no significant change in its cells after detachment, (Fig. 5). The spongy mesophyll of this smaller mine seemed to be intact, but corresponding sections through the floor of the larger mine showed some slightly compressed or flattened portions of this tissue. In general, however, the lower portion of the blade seems to have continued to function as a part of the leaf.

**DISCUSSION**

Leaf mines are of general biological interest both with respect to the insect invader and the host plant. The larvae, once established, seem to enjoy a carefree life; they are provided with food, water, shelter and a degree of protection from their enemies without necessary movement except as they eat their way around. In the present instance the larvae showed good workmanship in trimming the palisade layer so neatly from the upper epidermis, and they used good judgment in avoiding injury to this covering layer which would have brought speedy disaster upon them. Instead they released the upper epidermis from under contacts by eating through the softer cells over the vascular bundles; thus detached, except along margins, the epidermis was readily lifted up. This accommodated the increasing size of the larvae which presently had a length fifty times greater than the thickness of the leaf blade. Their selective taste, however, is puzzling; why consume only the thin (16 \( \mu \)) layer of palisade when the contiguous spongy mesophyll, 33 \( \mu \) in thickness, of like texture and rich in the same food products, was used only as a substrate? But one's admiration of their skills is tempered somewhat when it is recalled that this is only another modification of an old and worldwide formula, with an animal as destroyer and a plant the victim.

Sections through this large leaf mine showed no marked fungal invasion; this was surprising because the mine had evidently been abandoned for some time. It also suggests a quick closing of epidermal openings through which the larvae escaped. Other, and possibly older mines, showed brown spots about 1 cm. in diameter about a short scar near the center of each, which probably marked the place of larval exit. Sections showed no cicatrization of blade tissues along the border of the mine. This was not unusual because it has long been recognized that high humidity is retardative of this type of wound response. In this particular mine no intumescence was found though many enlarged cells were noted in sections through another mine on the same plant. Perhaps the most striking
feature of this leaf mine was the maintenance of a living upper epidermis stretched across a cavity 20 x 60 mm. This situation was complicated by its position on the upper side of the leaf and because of the normal transpiration loss from this area of leaf.

The large marginal lobes of these upper epidermal cells are probably an asset. They provide an extensive area of lateral contact between cells which encounter stresses in all directions in the plane of the blade. The tubular to globular form of the main body of each of these cells provides an elastic rigidity in the vertical plane. The wide spread of single cells reduced the number of cells through which translocation would move materials for a given distance. The mean spread of these epidermal cells, including the lobes, was over five times that for the corresponding epidermal cells of the white oak leaf on an over arching tree.

In connection with consideration of water supply to the isolated epidermis over the leaf mine it should be recalled that this type of epidermis makes no special provision for water storage, and that need for water supply to its cells ranges from zero during some hours of darkness, at least on certain nights, to an insistent need on hot afternoons. Alternatives with respect to sources of water supply to this layer are limited, and La Rue (1) summarized them briefly. He noted the possibility of at least occasional absorption of water through the cutinized outer cells walls. Recalling the location and the average weather during early August it seems that this possible source could be of little help in the present instance. Similarly any intake of water by the epidermal cells from the humid air within the mine is probably not significant. This mine chamber is connected through the spongy mesophyll with the intercellular space system of the blade. Even under normal conditions the greatest transpiration loss from such leaves is via the internal space system of the leaf. There remains the probability that the main supply of water to the epidermal layer of this mine was by translocation through its cells from marginal contacts with the blade. Also, except as the epidermal cells are able to carry on photosynthesis, their nutrition was likewise transported through these cells from the body of the leaf.

Certain related observation were provided by sections through a smaller mine on the same leaf, over which the upper epidermis appeared dry, broken and partly gone when collected. These sections showed that though the upper epidermis over this mine had been exposed to water loss from both the upper and under sides, a marginal zone about 650 µ wide had remained alive. This strip, ad-
jacent to the blade, had been able to transmit through its cells sufficient water and food to remain alive and normal in appearance.

In the normal leaf of this plant, *Eupatorium purpureum*, the non-cutinized lower surface of the upper epidermis is in contact with closely arranged palisade cells and between them limited areas are exposed upon the intercellular spaces of the blade. At intervals of about 1000 μ it is serviced by the network of bundle sheath extensions which are in contact with its cells. At closer intervals (345 μ) are the minor veins, which lie but one or two cell layers beneath the upper epidermis. All of these tissue contacts were cancelled by the larvae, leaving the thin (16 μ) epidermis isolated except for marginal junction with the blade. The considerable capacity for translocation through this epidermal layer, evidenced by its survival, while revealed by hazards brought on by injury, were not induced by these wounds. This capacity for translocation through its cells seems rather to be a normal function of this epidermal tissue.

**Summary**

This paper describes a large leaf mine on the upper side of a leaf of *Eupatorium purpureum*. The plant grew in the partial shade of a grove of oak trees in Eastern Iowa. The structure of its normal blade is described and measurements given for all tissues involved, as well as for the spacing of bundle sheath extensions and of the minor veins. The upper epidermis over this mine (20 x 60 mm.) remained alive throughout its area, though cut off from tissue contact except along the margin of the mine. Some photosynthesis was possible because chloroplast occur in all epidermal cells, but the main supply of both food and water came from the adjacent leaf area by translocation through cells of the epidermal layer. This capacity for translocation through the epidermis, while revealed in this instance by the wounds, is probably a normal function of this tissue.

**Bibliography**