On the Leaf-glands of *Ipomoea paniculata*.

BY

MARY FRANCES EWART, B.Sc.,

University College, London.

—♦—

With Plate IX.

—♦—

IN fully developed leaves of *Ipomoea paniculata*, at the top of the petiole immediately below the blade of the leaf, are two small depressions, one on either side. Each of these is covered with a thin colourless fluid secreted by a gland hidden within the tissues of the leaf. If the depressed region be examined more closely with a hand-lens, a small papilla is seen projecting from the centre; at the apex of the papilla is a wide mouth, from which the secretion of the gland beneath is poured over the surrounding concave surface.

These glands, originally discovered by Mr. Walter Gardiner, F.R.S., are altogether peculiar and belong to a type but rarely encountered amongst plants. Till the appearance of Macfarlane's observations upon the pitcher-plants (to be alluded to hereafter), there was, so far as I have been able to ascertain, no description of glands of the present type in the literature of Botany. Adequate living material for an investigation into their nature and development was accessible to me in the hot-house attached to the Botanical Department of University College, and upon this the results here recorded were obtained;

[Annals of Botany, Vol. IX. No. XXXIV. June, 1895.]

most of them, however, have been confirmed on material from other sources.

I made use of young vigorous shoots of *Ipomoea paniculata*. The younger leaves were detached and the glandular region was cut away from the rest of the blade and petiole and put at once into absolute alcohol. The axillary buds which had been carefully left untouched on the stem were removed in succession at various stages.

Description of Gland. The under side of the leaf-blade is traversed by five prominent veins which diverge from the top of the petiole, the midrib passing up the centre of the leaf, with two smaller veins on either side. The tissue covering these smaller veins expands at their point of union on the under surface of the leaf into a projecting cushion (*c.* Fig. 1) which overhangs the glandular region. Below the cushion is the slight concavity holding the fluid secretion, and from its centre, in the plane passing midway between the two veins, rises the papilla with its crater-like mouth. The papilla has a darker colour than the rest of the leaf, owing to a purple pigment in its sub-epidermal cells; it is elongated more or less horizontally, i.e. in a direction at right angles to the long axis of the petiole (*p.* Fig. 1). The duct which traverses it is short and straight, and passes horizontally inwards (perpendicular to the surface of the petiole), to widen out in the gland below. A tangential section of the papilla presents the appearance shown in Fig. 4. Occasionally, instead of forming a long straight narrow slit (*d.*), it has the appearance of a Y in transverse section. Its lining-membrane is strongly cuticularized, and the cells immediately surrounding it are marked off from the neighbouring parenchyma by their smaller size and more compact setting (Fig. 4 *p.*). It is the multiplication of these smaller cells which gives rise to the papilla on the surface, i.e. to the tiny perforated cone. The cushion of tissue overhanging the gland serves to deepen the depression in which it lies, and so allows a considerable amount of secretion to accumulate there before falling.

The internal orifice of the duct widens out into the lumen

of the gland (see Fig. 5). The gland itself can be plainly seen with the naked eye if the petiole is cut through horizontally, being distinguished in a fresh leaf from the surrounding green tissue by its lighter colour and greater opacity. It has very much the form of a flask rather flattened on the side opposite the insertion of the duct. This flattened side is parallel with the vascular cylinder of the petiole, and in large glands is only separated by four or five rows of cells from the common starch-sheath of the bundles.

The inner walls of the gland are folded into deep crypts lined by secretory epithelium. This is supported on a mass of small-celled parenchyma, from which it is cut off by a basal layer of cuticularized cells which appear to wall it in (Fig. 5, *bas*¹). The roof of the gland (*r*), round the exit of the duct, is non-glandular and composed of the small-celled tissue which forms the papilla and walls of the duct; the cell-walls which border the lumen of the gland (like their continuation, the lining of the duct itself) in this region are thick and cuticularized.

The whole gland is about as long as broad, and Fig. 5, which represents a radial section through the gland, might serve equally well for one taken horizontally, except that the duct in this case would be rather wider.

In examining a number of leaves I occasionally found one in which two glands were present on one side, the other side of the petiole containing but one. In these cases there were two distinct papillae, rather smaller than the normal single one on the opposite side, sunk in a common depression (Fig. 2), and usually the ducts were flattened in somewhat different directions. The glands into which the ducts led were wholly distinct and quite normal.

Structure of the Secretory Epithelium. The secretory epithelium in an active mature gland (taken from a leaf whose blade measured 10 cm. in length) is arranged in compact bundles of narrow prismatic cells set on end, the number of cells in each bundle varying from ten to twenty (see Fig. 16). Each entire bundle is supported on a single flattened polygonal cell, the upper wall of which is slightly convex.

These basal polygonal cells fit closely one to another, and their side-walls are thickened and slightly cuticularized, staining a deep orange-brown with iodine and with chlor-zinc-iodide.

Fig. 17 represents a tangential section (parallel to the secreting surface) across a bundle of secretory cells, and Fig. 18 a similar section at a lower level, across the polygonal cells.

Immediately below each polygonal cell is another cell (Fig. 16, *bas*²), having much thinner walls of cellulose, not flattened, but nearly as deep as broad; and adjoining this deeper layer (*bas*²) is the small-celled parenchyma forming the 'packing' of the gland, which gradually merges, after two or three layers, into the large loose ground-tissue of the leaf. The walls of all the parenchymatous cells are marked by numerous large simple pits.

The cells of the secretory epithelium are partly filled with granular protoplasm, which contains large vacuoles; in the fresh leaf they have a swollen appearance (*s.e.*, Fig. 16). The nuclei are large and fairly distinct, often showing a nucleolus. The outer walls of the cells are very thin. Each bundle of secretory cells is conical, the narrow base resting on the single basal cell, while the wider free extremity fits closely with its neighbours over the rounded curves of the much-folded gland. Transverse septa occur here and there in some of the older epithelial cells, but the majority appear to be undivided.

The two basal layers of cells (*bas*¹, *bas*², Fig. 16) present a great contrast to the bundles of cells above them; they contain comparatively little protoplasm, and very large vacuoles, and a distinct nucleus. They have a clear transparent appearance, especially those of the upper row (*bas*¹), whose walls are so much cuticularized that they seemed in section to form a firm hard wall shutting off the secretory epithelium from the cells beneath. The lower cell-walls of the second, deeper row are thin and cellulose in character, and communicate by pits with the small-celled parenchyma below.

Since examining these glands of *Ipomoea*, my attention was called to Macfarlane's description of the secreting glands of

Nepenthes. In some of these, more especially the 'alluring stem- and leaf-glands,' the analogy of the general structure is very noticeable; and as the author points out, it closely resembles that of animal glands. Speaking of the alluring leaf-glands he says¹, 'when placed on the outer surface of the lid or pitcher they much resemble attractive lid-glands, with the addition that a covering flap of epidermal tissue varying in extent grows over them, or more commonly even, like certain attractive lid-glands (*N. Lowii*, *N. laevis*, *N. Pervillei*), they are so encircled and closed in by the epidermal covering that the gland becomes "perithecioid," and the sugary secretion exudes from a small circular orifice of the epidermis. On tendrils and on the under surface of the lamina the perithecioid form is characteristic, and it attains its most gigantic proportions on the tendril of *N. bicalcarata*, where, owing to rapid growth of the tendril, the gland-orifice becomes slit-like, and the gland-tissue may be one-eighth of an inch in length and one-sixteenth of an inch in width.' In the glands of the stem, 'the three layers of gland-cells all show clear finely-granular protoplasm, and a vascular diverticulum ends beneath. But in all cases the diverticulum is separated from the gland-tissue by two layers of bead-like cells, which in position and function seems to correspond to the *membrana propria* of animal glands. The similarity of this to a simple animal gland in shape, structure, excretion, and vascular supply is obvious, and need not further be dwelt on. The resemblance, however, is even more striking in the pedicel-gland of *N. bicalcarata*, ... where a tendency to branching of the gland-tissue occurs.' Here, then, we have the two layers of clear cells which Macfarlane compares to the *membrana propria*, the vascular diverticulum just below the gland, the covering epidermal flap and perithecioid arrangement, and the branching of the gland-tissue, just as in the gland of *Ipomoea*.

Development of the Gland. In order to investigate the origin of the gland and its subsequent development, sections

¹ Observations on Pitchered Insectivorous Plants (Part II). *Annals of Botany*, Vol. vii. p. 429.

were cut from leaves of various ages. But the gland was already considerably advanced in leaves whose blade measured only 6 mm. in length; and it was found necessary to imbed the minute leaves of the axillary buds in paraffin, after staining in bulk with haematoxylin. In this way a complete series of the successive stages was obtained. I frequently found that the lowest (oldest) leaf of a bud exhibited no trace of a gland, and in cases where the bud was allowed to develop, this leaf often remained stunted, and more rounded in outline, partaking probably of the nature of a bud-scale.

In its earliest stages the gland commences as a simple invagination or pit of the epidermis. It appears at about the same level on the two sides of the base of a leaf, at the junction of the lamina with the very short petiole. The lamina of so young a leaf is only about 1 mm. long. A diagrammatic representation of a transverse section through the glandular region is given in Fig. 6, the gland itself in Fig. 7. The simple tube, which subsequently becomes the duct of the gland, dilates into a spherical cavity at its inner end, which may be traced through five or six successive sections.

In the next leaf of the bud, whose lamina had attained a length of 2.5 mm. from base to apex, the dilatation at the end of the duct had become wider, and the epidermal cells which line it were sharply marked off from those which formed the walls of the duct (see Fig. 8 *s.e.*). The epidermal cells at the mouth of the duct were also dividing and proliferating by septa parallel to the surface as well as perpendicular to it. Fig. 9 represents a longitudinal section through the other gland of the same leaf. The lumen of the gland can at this stage be traced through ten or eleven successive sections, either longitudinal or transverse.

The lamina of the third youngest leaf of the bud had attained a length of about 5 mm.; and the gland had reached a stage practically the same as that in one of the youngest leaves on the main shoot; a transverse section through the gland of the latter is shown in Fig. 10. The duct has become wider, and the epidermal cells lining it have undergone

tangential and radial divisions, especially in the region of the internal orifice. This results in a thick cuticular rim or cushion of tissue, which projects into the lumen of the gland (*r*). The tissue so formed by the proliferation of the epidermis can easily be distinguished from the underlying parenchymatous ground-tissue by the layer of thickened membrane which separates them, and is continuous at the mouth of the duct with that dividing the epidermis of the leaf-surface from the sub-epidermal tissue.

The inner wall of the glandular dilatation is no longer concave; the floor is raised into a rounded projection in the centre in consequence of the multiplication of the cells beneath; and the surrounding concavity is filled up from above by the projecting cushion of tissue (*r*) derived from the epidermis at the insertion of the duct. The epidermal cells lining the gland have also increased by radial divisions, so that the lumen, although flattened, is now wider than before and measures about $\frac{1}{5}$ mm. from side to side.

The gland has now attained its characteristic form, and in successively older leaves, whose blades measure 1.1, 1.5, 3.8, 6.0, and 8.0 cms. respectively, the main changes consist, (1) in the formation of numerous folds and pits in the walls of the gland, caused by the repeated radial divisions of the lining (secretory) epithelium and the unequal proliferation of the small-celled parenchyma below; (2) in the increased thickening of the tissue derived from the epidermal lining of the duct, giving rise to the roof of the gland, on the one hand, at the inner extremity, and on the other hand to the projecting papilla at whose apex the duct opens on the surface of the leaf; and (3) changes in the nature of the secretory epithelium itself, which will be described below. Fig. 5 represents a vertical section through a fairly advanced gland, whose walls, however, have not yet become very much folded. The lamina of the leaf from which this section was taken measured 8 cm., and the gland itself measured $\frac{3}{4}$ mm. from side to side, and $\frac{5}{8}$ mm. deep, including the duct.

In the development of the gland of *Ipomoea* we have again a

close similarity with the glands of *Nepenthes*. Macfarlane states¹ that 'in the formation of the alluring perithecioid glands of leaves, and in similarly shaped glands of some lids, an evident epidermal depression in the region of the future gland appears about the time that division in its cells is beginning. Owing partly to rapid division and growth of the marginal gland-cells, but specially to similar activity in the surrounding epidermal cells, these last rise up round the central gland-mass and cover it in until only a small circular or elliptic aperture is left in the middle of the covering-in cells.' And again, 'there is a decided tendency in many species to sinking and infolding of the gland with restriction of the exposed surface, but in *N. Pervillei* this is carried to such an extent that each gland opens by a very narrow elongated orifice.'

Development of the Secretory Epithelium. In the earliest stages of the gland (Fig. 6) the lumen is lined with ordinary merismatic epidermal cells, which, however, are crowded closely together and have conspicuous nuclei. Fig. 7 represents the left-hand gland and duct of Fig. 6 more highly magnified; and Fig. 11 a small portion of the epithelium at this stage. Its cells are full of granular protoplasm which stains darkly with haematoxylin, yellow with iodine, and brown with chlor.-zinc-iod. There are no vacuoles and the nuclei, which show distinct nucleoli, are near the basal cell-walls.

In an older gland, but before the walls have become at all folded, the cells present the appearance shown in Fig. 12. They are more elongated perpendicularly to the surface, and the protoplasm, still granular, is collected near the free walls, carrying with it the nuclei, which are now very conspicuous. The basal half of the cell is occupied by a large clear vacuole.

In the next stage (see Figs. 10 and 13) some of the cells near the centre of the gland-wall have divided tangentially by a single transverse septum. Most of the protoplasm is cut off in the upper cell. The formation of the transverse

¹ Observations on Pitchered Insectivorous Plants (Part II). Ann. Bot., Vol. vii. p. 435.

wall spreads centrifugally through the gland, and then the central cells undergo a further tangential division, a second cell being cut off from the upper one which borders the lumen. This cell usually has a large vacuole and a clear appearance, while the upper one contains very granular protoplasm and a large nucleus and nucleolus about the middle of the cell.

Finally, two or three of the surface-cells in the very centre of the gland undergo radial divisions (perpendicular to the surface), so that we now have four or five elongated cells (*s.e.*), supported on one basal polygonal cell (*bas*¹), and below this another cell (*bas*²), which has thin walls,—all these being derived from one original epidermal cell. A single section through a young gland would show all these stages, since the development does not take place simultaneously throughout. (See Fig. 13.)

The two lower cells derived from the epidermis remain clear and contain vacuoles; the lateral walls of the uppermost one (*bas*¹), adjoining the true secretory cells, become thickened and cuticularized, and the cell itself becomes one of the polygonal layers already mentioned (see Fig. 18).

In the next oldest leaf on the shoot, whose blade was 11 mm. in length, the multiplication of the surface-cells of the gland by vertical divisions had continued, and now we have as many as ten or twelve secretory cells supported on one basal polygonal cell (Fig. 14). These secretory cells are full of protoplasm, which, however, is not quite so granular in appearance as before, and the large nuclei are usually in the upper third of the cell.

The next oldest leaf (blade 15 mm. long) had a gland whose secretory epithelium exhibited the same general structure as the previous one (see Fig. 15). The secretory cells have become longer and narrower, and are crowded closely together; and it is not easy to distinguish the various groups of cells from one another. The protoplasm is not so granular, and small vacuoles are beginning to appear.

When the secretory epithelium has become fully developed it presents the appearance shown in Fig. 16. This drawing

was taken from a section of a fresh leaf which had not been in alcohol, but had been simply cut and mounted in water. The whole gland was very similar to that shown in Fig. 5. Some transverse walls had appeared at intervals in the secretory cells, but the majority of them were undivided, and very long and narrow. The nuclei, still large but not so conspicuous, occupied various positions in the cells, but for the most part were situated in the basal or middle portions.

The protoplasm had become much vacuolated, and this gave the cells a lighter, somewhat swollen, appearance. The two supporting cells below each group of secreting cells contained little protoplasm, and the lateral walls were thickened and cuticularized to a considerable extent, especially in the upper (*bas*¹). Fig. 17 represents a section taken across a group of epithelium-cells.

While cutting some of the smaller leaves, I noticed a large number of small apparently sessile glands scattered over the surface both of petiole and blade (*gl.* Fig. 6). Examination under a higher power showed that each was in reality shortly stipitate, being supported on a single cell. The epidermal cell below this stalk was slightly depressed, so that the cluster of four or five cells constituting the gland proper appeared to rest on the epidermis (Fig. 19).

In rather older leaves the glands were separated by wider intervals, and the depression of the epidermis round each gland was more marked (Fig. 20).

The secretory cells had multiplied by divisions vertical to the surface, but the stalk-cell had undergone very little alteration. The wall dividing it from the epidermal cell immediately below had become thickened and cuticularized, and now the resemblance to an early stage of a single bundle of secretory epithelial cells from the large glands already described, with its two basal cells, became obvious. Even in the youngest leaves, before there was any trace of the invagination to form the future large gland, these small surface-glands were partially formed, and consisted usually of epidermal cell (*bas*¹), stalk-cell (*bas*²), and group of two

or four secretory cells on the surface. Here and there, however, was an epidermal cell projecting, which had only undergone transverse division, and would probably have proceeded to form a gland later.

It would seem that the secretory epithelium lining the large gland of the leaf is homologous with an aggregation of these small surface-glands, the only difference being that the development of the epidermal cells which form the invagination in the earliest stages of the gland has been retarded, and that when they do become differentiated, *each* cell gives rise to a small gland; these are consequently crowded thickly together in a curved surface, and so give rise to the complicated secretory epithelium above described (compare Fig. 20 with Fig. 15). This view obtains additional support from the reactions of the glands with certain staining reagents. The secretions of the large gland and of the small superficial glands appear to be identical also.

Nature of the Secretion. In mature leaves the secretion of the glands accumulates in a large drop projecting from the depression in which the mouth of the gland lies. I was able to get a considerable quantity of it by cutting off some large leaves and putting the cut ends of the petioles in water. They were covered over with a bell-jar to prevent evaporation, and in this way I was able to remove the secretion at intervals as it was renewed. It consists of a thin, clear, transparent fluid, having a taste like dilute saccharine. After placing some on a slide with Fehling's solution and heating, an abundant reduction of the copper occurred, demonstrating the presence of a sugar. Tannin was also present in considerable quantity.

Thick sections were cut in the hope of finding some of the secretion in the duct, but the result was always disappointing because of its extreme solubility. Nor were the results afforded by the secretory cells themselves much more satisfactory. At an early stage many granules were present in the protoplasm which stained in dilute haematoxylin, were coloured brown by iodine, and were presumably proteid in

nature. But at no stage could I find starch-granules in the cells; and therefore I conclude, as sugar in some form is present in the secretion, it is, as Acton says¹, 'not formed by hydrolysis of a carbohydrate.' He compares its formation with that of cellulose from microsomata, and starch from amyloplasts in its general nature, but mentions that this is not capable of direct proof, on account of the solubility of the sugar.

Treatment with ferrous sulphate, &c., gave an abundant tannin-reaction, and with one per cent. osmic acid solution the cells became a dark blue colour. But since the cells were also darkened with osmic acid after remaining some time in ether, it would appear that the coloration is due to the presence of tannin, and not of oil. This was confirmed by negative results with alkannin. With an ammoniacal solution of Cu SO_4 the whole of the secretory cells became bright lemon-yellow, and the same result was obtained with cold Fehling's solution. On heating the slide, or dropping boiling Fehling's solution on to the sections, no alteration could be obtained in the colour; and since tannins will also reduce Cu SO_4 and Fehling's solution, I was unable to conclusively prove the presence of sugar *in the cells*. With chlor.-zinc-iod. the whole of the gland-cells became a bright yellow, the lateral walls of the polygonal cells below the secretory epithelium staining a dark orange, and the outer cell-walls of the epidermal lips of the duct also showing a marked cuticularization. The free cell-walls of the secretory epithelium were very thin, but appeared to have a yellow, not a violet reaction.

I could find no rupture of the outer cell-walls bordering the gland-cavity, and no breaking down into mucilage, and therefore conclude that the secretion is forced through the cell-wall in some way by the protoplasm of the cell itself. The parenchymatous cells surrounding the gland are furnished with very large pits in their cellulose walls, and only five

¹ On the formation of sugars in the septal glands of *Narcissus*. Annals of Botany, Vol. ii. No. V. 1888.

or six layers of cells in the narrowest part separate the glandular epithelium from the starch-sheath of the vascular cylinder; but no starch could be detected in the intervening cells. When the leaf shows signs of decay and begins to turn yellow, sections of the gland show that the epithelial cells undergo degenerative changes, and become filled with oil-globules. Oil is also found then in the surrounding parenchyma.

EXPLANATION OF FIGURES IN PLATE IX.

Illustrating Miss Ewart's paper on the Leaf-glands of *Ipomoea*.

Fig. 1. View of glandular region of mature leaf whose lamina measured 10 cm. from base to apex. Drawn from lower surface, right-hand side. *m*, midrib; *e*, margin of leaf-lamina; *c*, projecting cushion of tissue; *p*, papilla; *d*, aperture of duct; *s*, petiole.

Fig. 2. Do. left-hand side, to show two papillae, *p*.

Fig. 3. Left-hand glandular region of younger leaf, whose blade measured 8 cm. from base to apex.

Fig. 4. Tangential section of papilla, near surface, from leaf whose lamina measured 1.5 cm. *d*, duct; *p*, small cells forming papilla and walls of duct, derived from epidermis; *t*, surrounding leaf-parenchyma, derived from ground-tissue.

Fig. 5. Longitudinal section of mature gland whose external surface is represented in Fig. 3.

In this and the following figures the lettering has the same significance. *d*, mouth of duct; *p*, tissue forming papilla, derived from epidermis; *s. e*, secretory epithelium; *bas*¹, supporting cell on which the secretory epithelium rests, derived from epidermis; *bas*², second supporting cell, below *bas*¹, derived from epidermis; *t*, small-celled parenchyma, forming the 'packing' of the gland, derived from ground-tissue; *r*, roof of gland, formed by proliferation of epidermal cells lining duct; *l*, lumen of gland; *n*, nucleus.

Fig. 6. Diagrammatic transverse section of very young leaf of axillary bud, whose blade was about 1 mm. in length, through glandular region. *gl*, small surface-glands; *vb*, vascular bundles.

Fig. 7. Do. left glandular invagination more highly magnified.

Fig. 8. Transverse section through gland of older leaf of bud (blade equals 2.5 mm. long).

Fig. 9. Longitudinal section through the other gland of same leaf.

288 *Ewart.—On Leaf-glands of Ipomoea paniculata.*

Fig. 10. Transverse section through leaf whose lamina measured 6 mm. from base to apex.

Fig. 11. Secretory epithelium at stage represented in Fig. 6.

Fig. 12. Do. in leaf whose lamina was 5 mm. long.

Fig. 13. Same section as shown in Fig. 10, under higher power, to show various stages of development in the secretory epithelium. The cells included by the lines which meet at *x* show the line of development.

Fig. 14. Secretory epithelium at older stage, from leaf whose lamina was 11 mm. long.

Fig. 15. Do. from leaf whose lamina was 15 mm. long.

Fig. 16. Do. from fully formed gland.

Fig. 17. Tangential section across surface of glandular epithelium to show grouping of cells.

Fig. 18. Do. at lower level, to show the lateral walls of the supporting cells, *bas*¹.

Fig. 19 Transverse section of very young leaf to show early stage of small surface-gland (*gl*, in Fig. 6).

Fig. 20. Do. from mature leaf.

14.
x 80.

Macdonald, M. F. 1895. "On the leaf-glands of *Ipomoea paniculata*." *Annals of botany* 9, 275–288. <https://doi.org/10.1093/oxfordjournals.aob.a090738>.

View This Item Online: <https://www.biodiversitylibrary.org/item/233478>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a090738>

Permalink: <https://www.biodiversitylibrary.org/partpdf/318378>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.