The Leaf Glands of Dioscorea macroura, Harms.

BY

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With Plates CXCVII-CXCVIII and Three Figures in the Text.

Dioscorea macroura, the peculiar leaf glands of which have been made the subject of an extended research by the writer, is a West African species, and a native of the tropical rain-forests in the region bordering on the Gulf of Guinea.

First collected by Zenker in the Cameroons in 1891, it was described as a new species by Harms in 1897. In his diagnosis, which appeared in the Notizblatt* at Berlin, Harms lays particular emphasis upon the extraordinary development of the acuminate leaf-apices, which he refers to in the following terms :—"überall tritt an den Blättern eine auffällig lange, schmale, mehr oder minder scharf abgesetzte, verdickte, schwanzartige Spitze hervor." This highly developed acumen presents its most interesting aspect, however, as a study in physiological plant-anatomy, for in its internal construction there appear features that are perhaps unique within the genus. Its chief point of interest lies in the complex glandular system, which traverses its length from base to apex, and which differs markedly from the "sunken glands" of other species, so fully described by Correns † in his work on the extra-nuptial nectaries of *Dioscorea*. Moreover, the mucilaginous secretion which fills the lumen of these glands harbours a species of bacterium, which is always present in great numbers, even in the living leaf.

This constant association of a bacterium with the secretory organs of *Dioscorea macroura* is not without a certain physiological import, and may prove to have a direct bearing on the economy of the plant. In this, and in certain other features, the glands of this species certainly show a striking resemblance to the "bakterienknoten" found in the leaves of other tropical plants, the essence of which, according to continental investigators, is a symbiotic union between the plant and a foliicolous micro-organism. Among the recorded examples of "bakterien-

* See Notizbl. k. bot. Gartens u. Mus. zu Berlin, i. (1897), p. 266.
† C. E. Correns in Sitz. Ber. Math. Nat. Cl. Akad. Wien. xcvii., abt. 1 (1889), p. 651.

knoten," that of *Psychotria alsophila*, described by Boas,* has a particular interest in this respect, since this species was also collected by Zenker in the Cameroons.

The plants of *Dioscorea macroura*, from which the material for this investigation was obtained, have been in cultivation in the hot houses at the Royal Botanic Garden for some years; the tubers, from which the original plants were raised, having been brought from Nigeria by Dr. J. M. Dalziel, and presented by him to the Garden, along with other plants from the same area.

By adopting methods of intensive cultivation, in a moist atmosphere at a temperature ranging from 60 to 80 degrees Fahrenheit, it has been possible to raise, in successive seasons, plants of ever increasing size and vitality. Last year, the climbing stems of this species reached a length of over 18 metres, while the laminae of the largest leaves averaged 15 cms. long by 30 cms. broad, with leaf-tips fully 8 cms. in length. So far, the plants have never flowered, but, each year, they have been singularly prolific in the production of stem bulbils, and these have been made use of to propagate the species. The plant also perennates by means of a "nest" of root-tubers, which are said to be of a poisonous nature.

Reference has already been made to the remarkable length of the leaf-tip of this species, and it will not be out of place, at this stage, to describe more fully the distinctive external features which are so closely correlated with its glandular nature.

In the mature leaf, the acuminate tip is thicker than the lamina, is rigid, and tapers gradually from its base to the pointed apex, which is usually directed downwards. It may reach a length of 8 cms. and is about 5 mm. broad at its junction with the lamina.

The main veins of the leaf, from seven to nine in number, converge towards the apex of the lamina, and are continued outwards into this acumen, but only the inner veins remain visible at the extreme tip. Between the veins, the intervening tissue is slightly swollen, and forms a series of cushion-like ridges, from four to six in number, which are yellow-green in colour and quite opaque, in contra-distinction to the leaf-lamina, which is of a dark green colour and more or less translucent. They are, if anything, more prominent on the under side of the acumen, while, on the upper surface, each ridge is surmounted by a longitudinal slit, which communicates with the glandular interior. Viewed from above, the major portion of the surface of the acumen has the appearance of a series of more or less parallel ridges and shallow furrows, the former

* F. Boas in Ber. d. deutsch. Bot. Gesell. xxix. (1911), p. 416.

enclosing the glands, the latter roofing over the interposing veins.

The innermost pair of these glandular ridges extends practically the whole way from the base of the acumen to its apex, but those more lateral in position, by a repeated process of fusion with their immediate neighbours on the inner side, lose their identity in turn, until all are merged in the two innermost, persistent, glandular tracts.

Near the margins of the acumen, there project, from its upper surface, two thin flanges of tissue, which, in the older leaves, are directed outwards; but, in the younger leaves, the entire marginal portions of the acumen, from which the flanges arise, bend inwards, and completely enclose the upper surface and its longitudinal slits. As the leaf matures, the involuted margin gradually unfolds, and the slits become exposed.

The disparity in size between the young leaf and its acumen is very marked, for example, an undeveloped lamina, 7 mm. long, may be surmounted by a tail-like tip of 40 mm. in length. It does not follow, however, in spite of this disproportion in size, that the acumen necessarily becomes physiologically functional before the lamina reaches maturity, for it can be shown that, at this stage, the glands of the acumen are still in process of formation, and they only reach their highest state of development when the leaf-blade has expanded to more nearly its adult proportions.

Before proceeding to a consideration of the anatomy of this specialised organ, it might be well, at this point, to refer briefly to some of the technical methods employed.

The fluids used for the fixation of the parts to be examined were acetic-alcohol and chromo-acetic solution, and the material was embedded in paraffin, and sectioned by means of a microtome. By piecing together the serial sections obtained by cutting the material in different directions, it was possible to secure a mental picture of the convolutions of the glandular system, which was helpful in elucidating its construction. Various stains were employed, but perhaps the best results were obtained by using safranin, iron-alum haematoxylin with a contrasting stain, or a combination of gentian-violet and eosin. For the examination of the micro-organism present in the glands, the usual bacteriological methods of staining were followed.

The general anatomy of the leaf-tip can best be illustrated by reference to Plate CXCVII, which is a photograph of the transverse section of a medium-sized acumen, cut from near the apex. In this, the position of the glands, their relationship to the other tissues, and the general configuration of the organ are clearly demonstrated.

The glands are the dark-coloured areas, the contrast being produced by the deeply-staining secretion, which fills the glandular "pockets." It will be observed that only two of these glandular areas appear in the section, in place of the four or six referred to in the description of the external features of the acumen. This reduction in number is accounted for by the process of fusion which has been shown to take place between the outer and inner glandular ridges, resulting in the formation of the two combined glands, which alone persist in the distal portion of the acumen. Each gland is an elongated "pocket" in the mesophyll, which, in transverse section, has an extremely irregular outline, due to the frequent infoldings of the wall-layers. Its truly convoluted character, however, can only be fully appreciated by following its course through a series of longitudinal sections.

This "pocket" communicates with the upper surface of the acumen by way of the longitudinal slit, referred to above, the epithelium of which is epidermoid in character, and is strongly cuticularised, judging by its reaction to microchemical tests, and this property is shared, but to a less degree, by the cells which form the lining of the gland cavity itself. In the apical region of the acumen, from which this section was taken, the undulating character of the upper surface is not so pronounced as it is nearer the base, and the ducts, which, in the section, appear as narrow, winding canals, open here into a shallow channel formed by the two marginal flanges of parenchyma.

Internally, the opposing epithelial layers of the duct diverge abruptly to form the roof of the gland cavity, turning upwards in their course until the upper limits of the gland are reached, and thus partially enclosing a patch of mesophyll, roughly semi-circular in outline, with a much-indented circumference due to frequent infolding of the limiting layer. From the extremities of the gland, the epithelium is continued downwards to form the curved floor of the crypta, from which the secretory tissue of the gland is developed.

The cells which compose this superficial layer, with the possible exception of those which form the basal attachments of the secretory elements, have not only their outer, but also their lateral walls cuticularised, somewhat after the manner of an endodermis, while their inner walls remain unaltered. Something of a similar nature was observed by Correns in the extra-nuptial nectaries of those species of *Dioscorea* that he investigated. This distribution of the cutin in the cell-walls was particularly obvious in sections stained with safranin or victoria blue.

Underlying this cutinised epithelium are two or three layers of smaller, thin-walled cells, which form a compact sheath, partially enclosing the gland, and separating it from the larger, chlorophyll-containing cells of the mesophyll. This sheath does not extend beyond the upper extremity of the gland.

The actual secretory tissue of the gland consists of a large number of vermiform, filamentous outgrowths, each the product of a single epithelial cell. These are produced solely from the basal epithelium, from which they project into the lumen of the gland, there becoming intertwined to form a tangled plexus, the interstices of which are filled with a tenaceous fluid, possessing a strong affinity for such stains as corallin, Hoffman's and methylene blue. In the living state of the plant, this fluid forms a medium, which appears to be eminently suited to the growth and development of the bacterium which abounds in it.

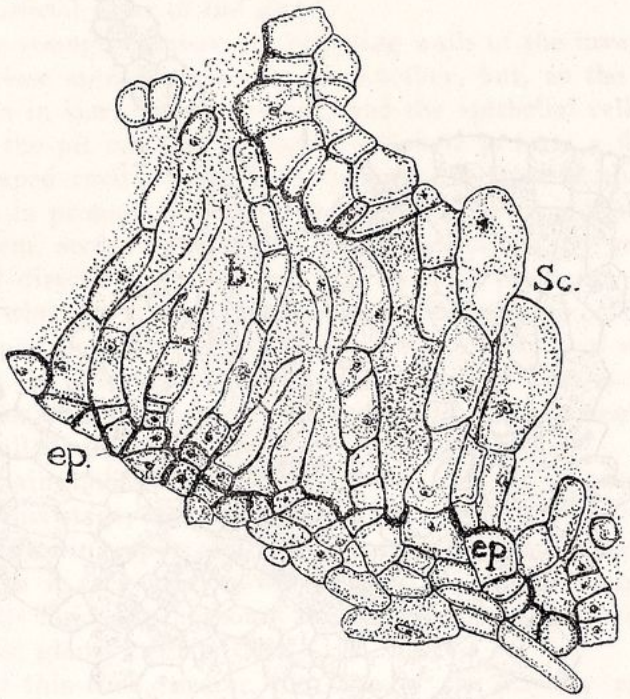


FIG. 1.—A portion of the secretory tissue of a mature gland. sc., vermiform secretory elements; b., fluid content, with bacteria; ep., cuticularised epithelium. x 250.

In the mature gland, these filamentous outgrowths are subdivided by cross septa into as many as six elongated cells, which are nearly circular in section, but, unlike the epithelial cells, they have thin walls, although the basal cell of each filament may show a certain amount of cuticularisation in its lateral walls. The nuclei of these cells are particularly prominent, and usually there are one or more large vacuoles in the cytoplasm. An enlargement of some of these features of the glandular tissue will be found in fig. 1.

The mode of origin of these cell-filaments will be touched upon when dealing with the development of the gland, after the remaining features of the mature acumen have been disposed of.

In the parenchyma, immediately adjoining the glandular tissue on its upper side, there are numerous air-channels, which follow the convolutions of the gland in a longitudinal direction, thus forming a complex aeration system. Each of these channels is enclosed by a definite sheath of parenchymatous cells.

Likewise intimately associated with the glands are the vascular bundles, which lie parallel to them, and, at frequent intervals, send out smaller, lateral veins, which encircle the lower rim of each gland, being separated from the actual glandular tissue only by the parenchyma sheath. In these small vascular strands, there is no apparent inequality in the amount of xylem and phloem developed to meet the requirements of the gland.

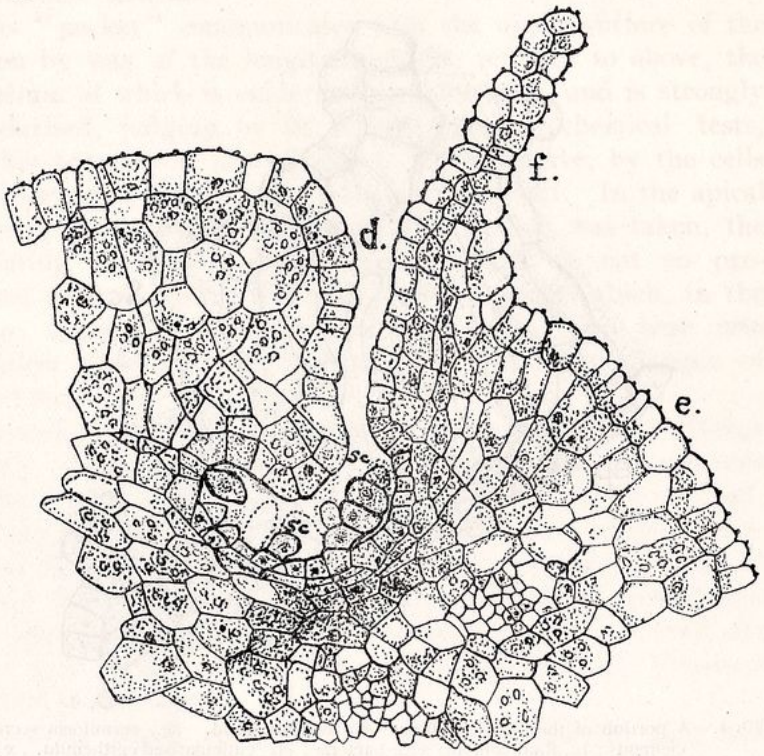


FIG. 2.—Transverse section of the acumen of a young leaf, with developing gland. sc., secretory tissue, in process of formation; d., duct; e., "corrugated" epidermis; f., "flange" of tissue. x 350.

Here and there in the surrounding tissue, are to be seen large oval cells, each containing a bundle of raphides, invested with a mucilaginous envelope. Other cells containing tannin occur in the mesophyll, just below the glands, and are particularly conspicuous in material fixed in chromic acid. Stomata are

singularly infrequent in the epidermis of the acumen, and, when present, are confined, more or less, to the margins.

To investigate the origin of the glands, it was found necessary to select very young leaves from the region just behind the growing-point of the shoot, for the initial stages of development are passed through almost before the young leaf unfolds. The comparatively advanced stage represented by fig. 2 was obtained by cutting sections of an acumen, less than 4 mm. in length, and only .7 mm. broad.

The first indication of the glands is the appearance, in the upper epidermis, of a simple invagination, interposed between the two flanges which, at this stage, are very minute and close together. The cells which form the walls of this pit resemble those of the epidermis, except that the corrugations, so obvious on the outer surface of the latter tissue, are absent entirely from the superficial layer of the gland.

In the youngest leaves, the opposing walls of the invagination are in close approximation to one another, but, as the acumen increases in size, the slit widens, and the epithelial cells at the base of the pit increase by radial divisions to form a flattened, flask-shaped cavity. The cells forming the floor of the cavity are rich in protoplasmic contents, and become the initials of the vermiform, secretory elements. Each initial cell, by growth in a radial direction, increases in size until it protrudes into the lumen, when division of the protoplast takes place. The projecting portion of the cell is then cut off by a periclinal wall, and becomes the first cell of the filament. This process is repeated in a series of intercalary cell-divisions until each filament attains to its full dimensions.

A certain amount of fluid is present in the developing gland, even at this stage, and, in fact, it appears almost at its inception, for the growing apex of the stem, and the leaf primordia, are bathed in it, a condition which has a particular significance, since this fluid also harbours the bacterium found in the fully-developed gland. (Plate CXCVIII., fig. 1.)

About this time, nearer the base of the rapidly enlarging acumen, there are indications of the formation of additional gland-cavities by a process of subdivision; the new "pockets" being formed directly from the pre-existing ones, not by invagination, but by an inverse process. This is brought about by active growth in the band of tissue immediately underlying the long axis of each concavity, resulting in the elevation of the central portion of the floor to the level of the epidermis, and thus forming a ridge of tissue, several cells across, which bisects the original pocket longitudinally, and divides the now greatly distended duct into two. With the growth of the acumen, these

"twin" glands diverge, and the process is repeated until all the glands found in the fully-developed organ have come into existence. At a later period of development, the epithelium forming the roof of the gland becomes extensively folded, owing to the excessive multiplication of the cells composing it, which has the effect of increasing the internal area of the gland-cavity, while, externally, the hollows between the folds are filled in by the unequal growth of the adjoining ground tissue.

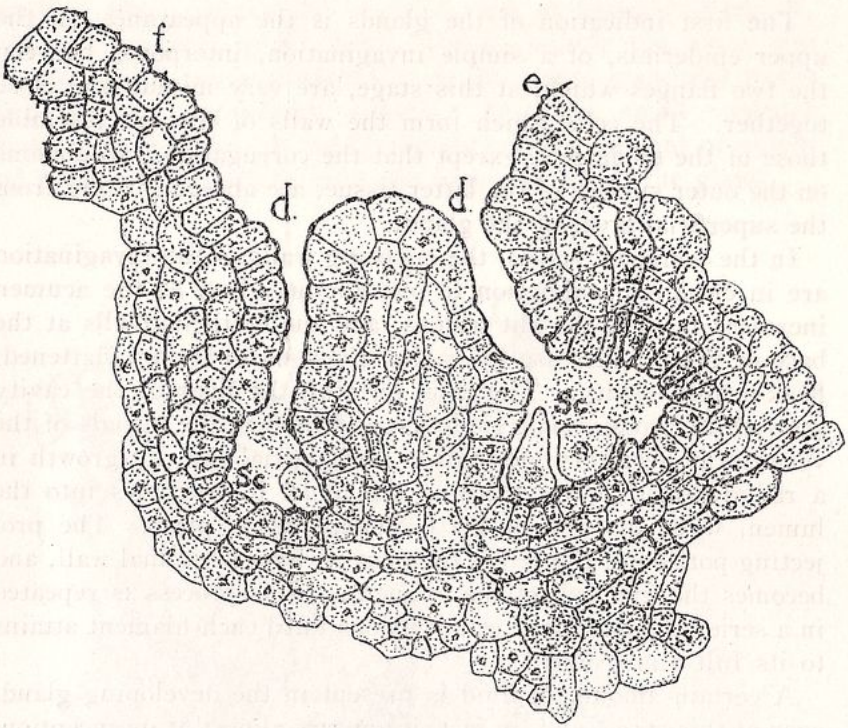


FIG. 3.—Transverse section of a portion of the glandular region at the base of a developing acumen, showing two immature glands, which have been formed by the subdivision of the original gland cavity. sc., secretory tissue; d.d., ducts; e., epidermis; f., base of "flange." x 350.

Meanwhile, other cells of the peripheral layer encircling the lower half of the gland undergo tangential division to form the compact, parenchyma sheath, which, together with the epithelial layer, separates the lumen of the gland, on its under side, from the associated vascular tissues, and the adjacent less-compacted mesophyll.

The very young leaf, and its acumen, are covered with minute, multicellular scales, which are most obvious in sections of the bud, where they appear immersed in the deeply-staining fluid. They are notably evanescent, and are wanting in the fully-developed leaf. These epidermal outgrowths bear a striking resemblance to the immature secretory elements, which are,

themselves, the products of a tissue that is epidermal in origin, the chief point of difference between the two being, that while the former are temporary structures, the latter become permanent constituents of the glandular tissue of the acumen. It is not unreasonable to assume, therefore, that the secretory filaments of the gland are merely persistent epidermal scales, which have been retained, and modified, for a definite physiological purpose.

Attempts to determine the chemical composition of the fluid content of the gland cavity were rendered difficult by the presence of the micro-organism, and to its influence, no doubt, may be attributed some of the unexpected results obtained.

At no time was there any visible exudation of fluid from the external orifice of the duct, and chemical tests had therefore to be applied directly to sections of the acumen, by immersing them in a small quantity of the reagent, on a slide, or in a test tube. In this way, a study of the biochemistry of the secretory organs, and associated tissues, was accomplished, the results obtained being briefly collated below.

Fairly thick sections were treated with Fehling's solution, alone, and with the addition of an acid, and were then heated, the process being conducted on a slide in the manner recommended by Schimper, but with purely negative results. Barfoed's solution likewise produced no visible reaction in the tissue of the gland, or its secretion. This failure to detect the presence of sugar, in some form or other, was unexpected, although Correns remarks that he was unable to find either sugar or starch in the developing extra-nuptial nectaries of those species of *Dioscorea,* that he investigated. In the present case, starch was located in the cells of the mesophyll, but it was entirely absent from the glands which were coloured a bright reddish-brown by iodine, suggesting the presence of proteids.

The application of the standard tests for the recognition of proteids produced somewhat surprising results. When sections were placed in a drop of Millon's reagent on a slide, and heated, a dense brick-red precipitate appeared within the glands. Treated in the same way with hot nitric acid and ammonia, the glands responded at once to the xanthoproteic reaction. They were coloured red by the so-called Raspail's reagent, and were turned to a violet colour by the more delicate biuret reaction.

The glands were the only parts of the acumen affected by these reagents, and, while it is recognised that the successful application of any one of these tests does not in itself afford conclusive proof of the presence of proteids, yet, taken together, the combined results are undoubtedly indicative of the gland content being of an albuminoid nature.

In this particular, the glands of *Dioscorea macroura* resemble the "proteid-containing glands" of *Ardisia*, first described by Hohnel.* These glands are likewise the home of a micro-organism, whose relationship to the plant, particularly in the case of *Ardisia crispa*, has been subjected to a critical investigation by Miehe.† Other analogies exist, as exemplified by the parallel features of the glands in certain Rubiaceae, described by Zimmerman‡ and Boas. This aspect of the glands will be discussed at a later stage.

Standard tests for the detection of tannins were applied to thick sections of the acumen, but no trace of any such astringents could be found within the glands, which is in accordance with the findings of Correns, who failed to discover any tannins in the extra-nuptial nectaries. On the other hand, certain large elements of the ground tissue gave characteristic tannin reactions with iron salts, potassium bichromate, ammonium molybdate, cupric acetate and osmic acid. With Schultze's solution, the whole of the glandular tissue was coloured dark-yellow, and the cuticularisation of the epithelial cell-walls became apparent, but neither with this reagent, nor with iodine and sulphuric acid, could a definite cellulose reaction be obtained in the thin cell walls of the secretory trichomes. No trace of oil could be found in any part of the acumen.

The pronounced proteid character of the gland content, as disclosed by these microchemical tests, was further emphasised by a comparison of the nitrogen content of the acumen with that of the lamina. This was ascertained by the Kjeldahl method of analysis, applied to leaves taken from the living plant at intervals during the day. In every case, the tissue of the acumen was found to contain a much higher percentage of nitrogen than the tissues of the lamina. The following table, in which the results of two of these nitrogen estimations are compared, will serve to illustrate this point.

Nitrogen Content in 100 grams of Leaf Tissue.

Time.	Lamina.	Acumen.	Excess of N_2 Content in Acumen.
10.50 a.m.	246 mgs.	560 mgs.	314 mgs.
4.30 p.m.	173 mgs.	486 mgs.	313 mgs.

The remarkable difference between the nitrogen content of the acumen and that of the lamina, as shown by this process, could only be accounted for by the existence within the former, and

* F. X. R. von Hohnel in Sitz. Ber. Wiener Akad., lxxxiv., abt. 1 (1882), pp. 574, 583.
† H. Miehe in Ber. d. deutsch. Bot. Gesell. xxix. (1911), p. 156 and in Jahrb. für wiss. Bot. liii. (1914), p. 1, and lviii. (1917), p. 29.
‡ A. Zimmerman in Jahrb. für wiss. Bot. xxxvii. (1902), p. 1.

conversely by its absence from the latter, of some factor capable of bringing about this increase in the amount of combined nitrogen in the tissues of the acumen. Bearing in mind the proved proteid character of the glands, an explanation for the increase was sought for in the activities of the bacterium associated with them.

A necessary preliminary to a study of the organism and its vital processes was its isolation from the plant, and this was accomplished by adopting, with some modifications, the methods employed by Harrison and Barlow,* and others, to isolate the bacterium from root-nodules. The acumen of a living leaf was first sterilised by dipping it into a fluid containing small proportions of hydrochloric acid and mercuric chloride. After washing with distilled water, it was cut across with a sterilised scalpel, and by means of a platinum loop, a small quantity of the gland content, with the included bacteria, was then transferred from the cut surface to a test tube containing a liquid culture medium. From this, other tubes were inoculated, and pure cultures of the bacterium were obtained. The usual precautionary measures, to guard against contamination, were taken at each step in the process, and controls were set up for all subsequent cultures, and these remained perfectly sterile.

Various culture media, both liquid and solid, were used for the cultivation of the bacterium, but it was found that liquid media gave the best results in the first transference from living leaf to artificial conditions. The following culture fluid, which has been used in the investigation of root-nodule bacteria, proved very successful in the initial stages of isolation.

saccharose,	1.0 grm.
acid potassium phosphate,	0.5 grm.
magnesium sulphate,	0.02 grm.
distilled water,	100 cc.

Substitution of other sugars for saccharose did not produce the same amount of growth, and glucose was particularly unsatisfactory, but excellent results were obtained by using a sterilised decoction of the leaf tissue. Only neutral, or slightly alkaline solutions, were employed throughout the experiments.

After incubation for a period of 36 hours, at a temperature of 25° C, the inoculated fluids become distinctly turbid, and at the end of 3 days, the cloudiness took the form of a dirty-white growth of a somewhat ropy nature, which collected at the bottom of the tube. Microscopic examination of an air-dried drop of this milky liquid, after staining with carbol-fuchsin, or gentian-

* Harrison and Barlow in Proc. Roy. Soc. Canada, 1906.

violet (Gram's method), showed that it consisted of a pure culture of rod-shaped organisms, from 1.6μ to 2.4μ in length, and from .6μ to .8μ in breadth. They proved to be Gram positive, and it is interesting to note further, that the gentian-violet stain remained in the organism, after dehydration with amyl alcohol, but not after ethyl alcohol, resembling in this particular the similar reaction obtained by Harrison and Barlow on *Pseudomonas radicicola*. About 24 hours after inoculation, the organisms were actively motile in liquid media, and by careful staining, using the van Ermengen method* in a slightly modified form, it was possible to determine the presence of a single polar flagellum on the bacterium cell.

Stab, smear and plate cultures were also made on agar and gelatin, by inoculation from liquid media. The agar was prepared by adding 2 per cent. of the substance to the nutrient solution containing saccharose; in the case of gelatin, the percentage was a little higher. The agar cultures were incubated at 25° C, but those on gelatin, were, of necessity, developed at a lower temperature. It was found, however, that within certain limits, the difference in temperature did not appear to have much influence on the rate of growth.

On saccharose agar, the colonies were round or oval, from 3–5 mm. across. Raised above the substratum, they were hemispherical in form, sabaceous, faintly reticulated, and had a slightly fimbriated margin. On nutrient gelatin, the colonies were ochraceous, but otherwise they resembled those produced on agar. Smear cultures, on the other hand, rapidly became agglomerated to form nodulose masses (Plate CXCVIII., fig. 2). In stab cultures, the growth was strongest at the point of entrance, and relatively weak internally, along the edges of the stab.

On agar and gelatin, the colonies made their appearance in from 2–4 days after inoculation, the time taken varying with the composition of the medium used as a source of infection. For example, cultures on nutrient gelatin, initiated by inoculation from solid media, became apparent in 24 hours, while, under the same conditions, cultures made from liquid media required from 4–5 days to reach a similar state of development.

The culture of the organism, under artificial conditions, was maintained over a period of three months, at a mean temperature of 15° C, and during that time, cross infections between different media were carried out, the cultures, in all cases, remaining pure.

That the organism is aerobic was suspected from its behaviour in stab cultures, and this was confirmed by the impossibility of

* See Centr. für Bakt. xv. (1894), p. 969.

inducing it to grow in a Buchner's tube, over strong pyrogallic acid in caustic potash. Liquefaction of gelatin was brought about after a comparatively long period of growth, ranging from 18 to 28 days, the liquefied gelatin subsequently becoming cloudy. Sterilised milk, to which a culture of the bacterium had been added, ultimately became clear and translucent, and gave a slightly acid reaction with litmus. The transference of a drop of this fluid to nutrient gelatin resulted again in the production of a pure culture of the organism.

In suitably stained sections of the glands of *Dioscorea macroura,* relatively large, irregularly shaped bodies, resembling the bacteroids of leguminous nodules, had been observed, and these also appeared in the cultures on artificial media. Similar involution forms were found by Faber* in the bacterial leaf-glands of *Pavetta* and *Psychotria*. This author carried out an extended research on the physiological attributes of the organism inhabiting these "bakterienknoten," and, as a result of his experiments, he came to the conclusion that the "mycobacterium," isolated from the glands, possessed the power of utilising free nitrogen, and that the inter-relationship of plant and micro-organism was therefore of the nature of a "bakteriensymbiose."

A similar view of the intimate relationship existing between *Ardisia crispa* and its associated bacterium was expressed by Miehe, after a lengthy series of observations on the biology of the micro-organism and its effect on the growth of the plant.

In the light of these researches, and having regard to the several points of resemblance between the glands of *Dioscorea macroura* and those of other tropical plants investigated by Faber and Miehe, it was essential that the *Dioscorea* organism should be subjected to similar tests, in order to determine whether, or not, it was endowed with like properties, and capable of bringing free nitrogen into combination. Kjeldahl determinations of the nitrogen content of cultures of the bacterium, isolated from the glands, were therefore compared with similar estimations of the amount of nitrogen present in the culture medium in sterile controls. In every case, there was evidence of an appreciable gain in the nitrogen content, presumably due to the activities of the organism.

The percentage increase in nitrogen varied according to the nature and composition of the medium employed, and seemed to be inversely proportional to the amount of combined nitrogen present in the nutrient medium at the time of inoculation, e.g., a nutrient medium with a low initial nitrogen content, three weeks after inoculation, showed a percentage increase in nitrogen

* F. C. von Faber in Jahrb. für wiss. Bot. li. (1912), p. 283, and liv. (1914), p. 243.

of just over 100, while a medium of a different composition, and a higher initial nitrogen content, showed a gain of only 67 per cent., although, in the latter experiment, the culture had been maintained over a much longer period of time.

The power of nitrogen fixation possessed by the *Dioscorea* organism was tested up to fully 80 days after inoculation, but its effect on the nitrogen content of artificial media is typified by the following result, obtained by the Kjeldahl analysis of a 23 days culture growth.

Nitrogen Content in 100 grams of Culture Medium.	
In the control,	23.18 mg.
In the culture medium at conclusion of experiment,	48.61 mg.
Gain,	25.43 mg.

The increase in the nitrogen content may appear small when contrasted with the results obtained from similar experiments carried out with nitrogen bacteria from root-tubercles. It compares not unfavourably, however, with some of Faber's estimations of the nitrogen increase in culture solutions, brought about by the organism isolated from *Pavetta Zimmermanniana*. He found that, with a nitrogen content of 12.256 mg. in the control, the gain in three separate experiments was, respectively, 9.694 mg., 19.592 mg. and 12.750 mg. of nitrogen per 200 cc. of culture solution. The duration of each experiment was 20 days. Using an almost nitrogen-free culture solution, Faber found that the increase in nitrogen, for the same length of time, was much greater than the amounts quoted above, while with a higher percentage of nitrogen in the control, the increase was correspondingly less.

That the bacterium found in the glands of *Dioscorea macroura* stands in the same relationship to the plant, as do those organisms, described by Faber and Miehe, to the plants with which they are allied, is an interesting hypothesis, which gains support from the behaviour of the isolated bacterium in culture media. But whatever powers of nitrogen-fixation may be possessed by the organism, when divorced from the plant, it is quite another matter to state categorically, that, within the plant, the bacterium plays the rôle of nitrogen provider, in the same way as do the bacteria in root-nodules, although the high nitrogen content of the acumen does seem to postulate a certain activity.

It may be merely a case of contingent symbiosis, or a fortuitous association, such as that mentioned by Koorders* in his description of the "wasserkelche" of tropical plants.

* S. H. Koorders in Ann. Jard. Bot. Buitenzorg, xiv. (1897), p. 451.

On the other hand, this association of a bacterium with *Dioscorea macroura* has every appearance of being of the nature of a more permanent alliance, for, as it has been pointed out already, the organism is present in the secretion enveloping the bud which originates from the tuber, by which avenue it is apparently able to reach the developing glands of the new plant.

All attempts to grow the plant from a sterilised tuber, under perfectly sterile conditions, have so far proved abortive, and it is therefore impossible to say what effect the disassociation of bacterium and plant would have upon the growth of the latter.

Although dissimiiar in structure and mode of origin to those of *Ardisia, Pavetta* and *Psychotria,* the leaf-glands of *Dioscorea macroura* undoubtedly possess some of their peculiar physiological properties. They have been shown to differ in many ways from the extra-nuptial nectaries of other species of *Dioscorea,* and in one particular—their bacterial nature—they are possibly unique within the genus.

In conclusion, the writer desires to record his indebtedness to Dr J. M. Dalziel, for much helpful information regarding the plant in its native habitat, and to Mr I. H. Burkill, Director of the Botanic Gardens, Singapore, for his invaluable assistance in the matter of identification. An expression of thanks is also due to Mr R. M. Adam, for the photograph of the culture, reproduced on Plate CXCVIII., and to Mr J. J. Campbell, for the care expended on the cultivation of the plants, which furnished the material for this investigation.

SUMMARY.

1. The leaf-glands of *Dioscorea macroura* form a series of elongated "pockets" in the mesophyll of the prominent acumen.

2. The "pockets" are formed by a process of invagination followed by subdivision, and when mature communicate with the upper epidermis by way of narrow slits or ducts.

3. Each glandular "pocket" is lined, in part, with a secretory tissue composed of vermiform, multicellular trichomes, and is filled with a mucilaginous fluid which gives a strong proteid reaction.

4. The fluid content of the gland cavity is the home of a bacterium which is always present in great numbers in the glands of the living leaf.

5. The bacterium, when isolated in pure cultures on artificial media, is found to possess the power of nitrogen-fixation to an appreciable degree.

6. The nitrogen-content of the glandular acumen is greatly in excess of that of the leaf-lamina, postulating a similar process of nitrogen-fixation taking place within the glands.

7. The presence of nitrogen-fixing bacteria in the glands of *Dioscorea macroura* would appear to constitute a parallel case to that of such a species as *Psychotria bacteriophila*, in which the foliicolous micro-organism is a source of nitrogen supply to the plant.

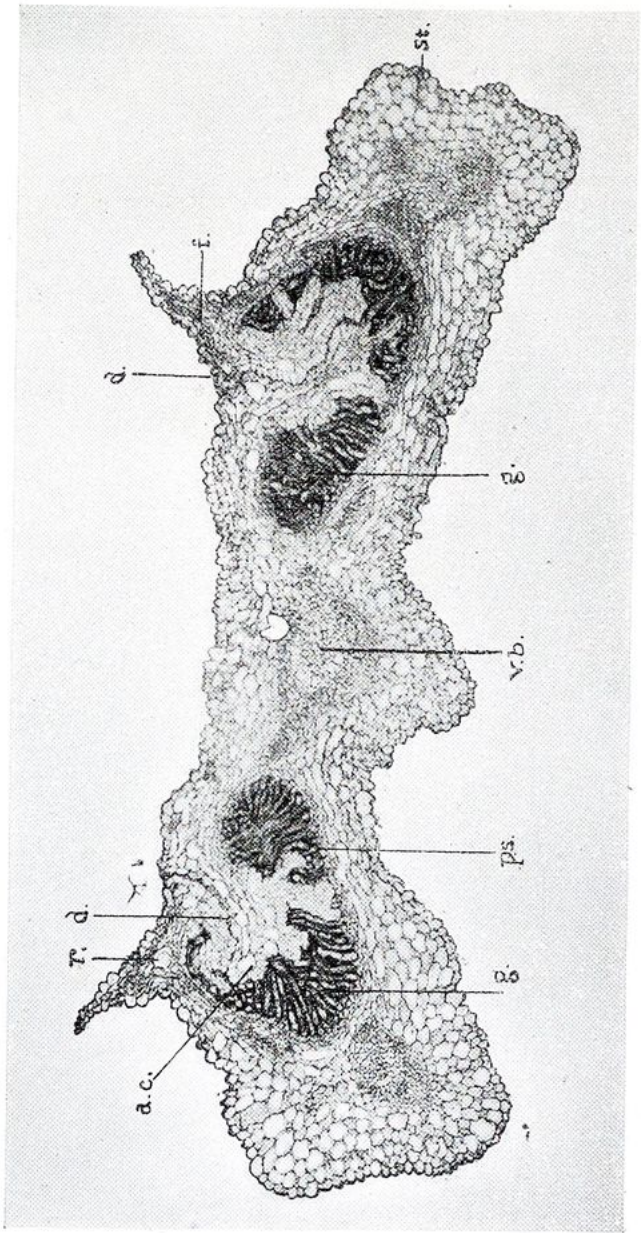
EXPLANATION OF PLATES CXCVII-CXCVIII.

Illustrating Mr M. Y. Orr's paper on Dioscorea macroura.

PLATE CXCVII. Leaf-acumen of *Dioscorea macroura*, in transverse section. g.g., glands, with vermiform secretory elements and darkly-stained fluid content; d.d., ducts, leading to the upper surface; p.s., parenchyma sheath, encircling the gland; a.c., air channel; r., raphides; v.b., vascular bundle; s.t., stoma; f., "flange" of tissue.

PLATE CXCVIII. Fig. 1.—Apical region of a shoot of *Dioscorea macroura*, in longitudinal section.
a., apical meristem; s., mucilage, containing bacteria; t., epidermal scale; r., raphides.

Fig. 2.—Gelatin culture of the bacterium isolated from the glands of *Dioscorea macroura*, at the point of liquefaction.



Orr—Leaf glands of *Dioscorea macroura*.

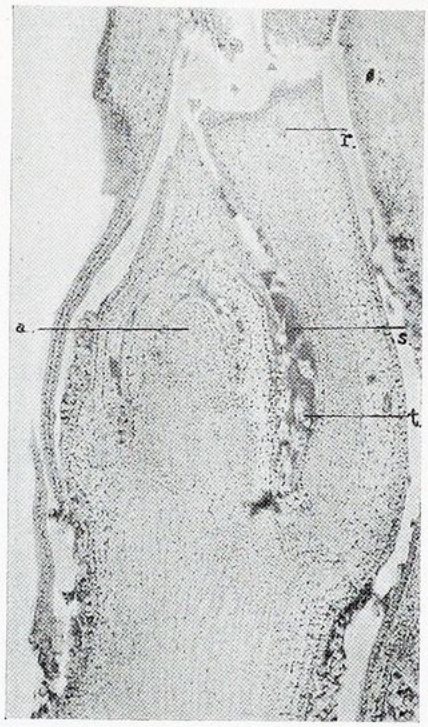


FIG. 1.

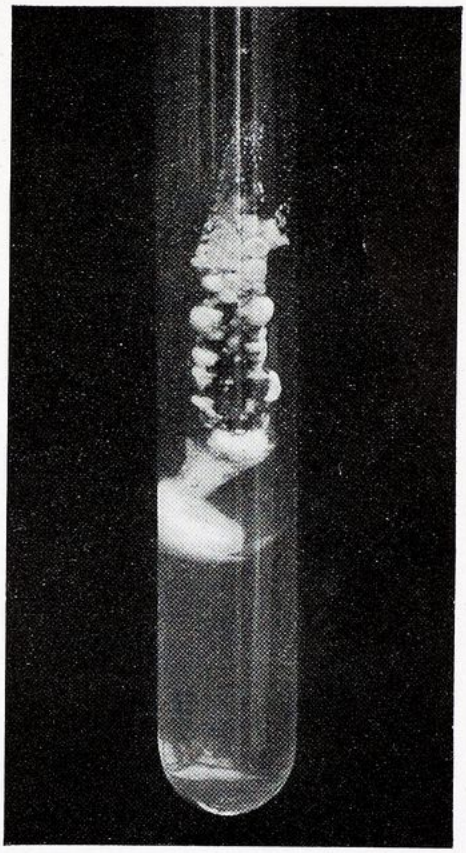


FIG. 2.

Orr—Leaf glands of *Dioscorea macroura.*