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ANATOMICAL CHANGES IN CLADODES OF PHYLLOCACTUS HYBR. IN RELATION TO FLOWERING

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INTRODUCTION

Phyllocactus plants coming into flower exhibit thickened strands, often coloured a purple tone by anthocyanins, which run from the "midrib" of the leaf-like stem (cladode) to the budding areoles (Plate 1*a*). During the progress of flower-bud growth the strands increase in thickness. Resting areoles do not show this phenomenon. It was first described by VÖCHTING (1873–'74) for species of *Rhipsalis* and related genera and by SCHUMANN (1895) for *Phyllocactus*. Anatomical data, however, are, as far as the present authors are aware, not available. The purpose of the present paper is to give some information as to the anatomical background of the observed phenomenon.

MATERIAL AND METHODS

The plant used in our investigation is grown in large quantities in the Netherlands flower centre of Aalsmeer, under the name of *Phyllocactus ackermannii* (Haw.) Salm–Dyck. This is certainly not the correct name, since the plant in question is not a true species but a highly hybridized cultivar. The colour of the plant is bright green. The basal part of the leaf-like stems (cladodes) is cylindrical. The flowers measure about five inches in diameter and are of a bright orange-toned red colour. Plants well-set with flower buds in various stages of development were obtained from the local market and kept in the greenhouses of the Botanical Garden.

To obtain information on the gross anatomy of the shoots after decolouration and dehydration in a series of concentrations of aethanol, they were made transparent by means of methylbenzoate. In preparing slides, great care had to be taken with the fixation and the embedding of the material. As a matter of fact, this type of material may cause difficulties in connection with the presence of huge amounts of mucilage and with the marked differences in firmness of the cell walls of the tissues involved.

Fragments of stems with resting areoles and with areoles bearing flowerbuds in different stages of development, from very small buds to fully expanded flowers, were fixed for 48 hours in vacuo in a mixture of 12.5 cc glacial acetic acid, 12.5 cc propionic acid, 25 cc formaline and 450 cc distilled water. After fixation the material was washed for 4 hours in running tap water, after which the fragments were rinsed with distilled water and dehydrated according to JOHANSEN's

method (1940) with tertiary butanol. Impregnation with paraffin wax was carried out very carefully. Small pieces of aerated paraffin wax (melting point 50° C) were added hourly to the tertiary butanol until saturation was reached. After evaporation of the butanol the paraffin wax was replaced first by one with a melting point of 54° C and later by one with a melting point of 64° C. After renewing this last wax once or twice, embedding proceeded in paraffin wax of the same melting point.

Cut into 10 μ thick sections, the material was deprived of paraffin in a range of xylene-tertiary butanol mixtures. Via 70 % and 50 % aethanol the sections were placed in a 1 % saffranin solution in 50 % aethanol in which they remained for 16 hours. Washed successively in 50, 70 and 95 % aethanol, the sections were counterstained for 1 minute in a 1 % solution of fast green in 96 % aethanol. Via a tertiary butanol-xylene range, the sections were finally enclosed in Caedax. Notwithstanding careful treatment, deformation of parenchymatous tissue as well as some rupture of the very tender-walled cambial zone was unavoidable.

Morphology and gross anatomy

The stems of *Phyllocactus* species have a more or less leaf-like character. They are green in colour, are flattened, and show a distinct "mid-rib" with "lateral veins" interconnected by many very thin secondary veins, resulting in a sort of network. Such stems have been termed cladodes. The "midrib" is the central cylinder of the stem, while the major "lateral veins" are formed by leaf and branch traces running through the cortex of the stem towards the very small scale-like rudimentary leaves, the so-called areoles being the axillary buds belonging to these leaves. From such areoles flower-buds or new stems may develop. Moreover, minor "lateral veins" originate from the central cylinder. Together with the leaf traces, the minor lateral veins and the secondary veins form the cortical vascular system.

By using the clearing method described in the preceding section, the whole vascular system can be demonstrated. It appears that leaf and branch traces anastomose to a varying degree. Moreover, it can be seen that the development of flower-buds and of the correponding vascular system is simultaneous (Plate 2).

Anatomy

General survey

The major object of the present publication is to describe the changes which take place in branch traces in relation to the development of flowers. Since the changes observed are the direct cause of certain alterations in the cortical region, a short description of the anatomy of the whole shoot will be given.

Under the epidermis, which is provided with a well-developed cuticle the peripheral layer of the cortex consists of small cells forming a distinct hypodermis (Plate 1*b*). The remaining part of the well-

developed cortex which gives the plant its succulent character is of a parenchymatous nature. A special feature is the presence of numerous mucilage idioblasts which have already drawn the attention of early plant anatomists (*see* STEWART, 1919). This type of cell is characterized by large dimensions and the mucilaginous substance which it contains.

Embedded in the cortical tissue are the branch and leaf traces and the minor veins. These are composed of collateral vascular bundles, whose number varies with the thickness of the various strands. It is striking that during the development of flower-buds from resting areoles not only the branch traces but also the leaf traces and their anastomoses gain in importance (Plate 2).

Vascularization of the resting areole

Close to its insertion, the branch trace consists of three small vascular bundles separated by parenchyma. The number of bundles increases considerably towards the areole. This is due in part to division of the original three bundles and in part to the contribution to the configuration of anastomoses from the leaf traces. Finally, very close to the areole minor bundles from the cortical system may enter the ring of vascular bundles. The amount of vascular tissue increases towards the areole not only by the increase of the number of vascular bundles but also, because of the occurence of some secondary growth (Plate 4*a*). As a result, localized dilatation of cortical tissue takes place. Details will be given in a separate paragraph.

Vascularization of the flowering areole

The most conspicuous phenomenon is the very extensive secondary growth that occurs during the period between initiation of flower-buds and anthesis (Plate 2). This growth takes place over the whole distance between insertion site of the traces and the areole, with maximum development close to the areole (Plate 4*b*). Secondary phloem and xylem are formed in considerable quantities by the action of fascicular cambium. True interfascicular cambium is not formed. The cells of the interfascicular regions divide several times, giving rise to irregular radial rows of cells which clearly show their origin (Plate 5*a*). Although it is not absolutely absent in traces leading to resting areoles, those which end in flowering areoles exhibit the following feature: corresponding to and localized at the periphery of the phloem bundles, more or less reticulated sclerenchyma cells are present (Plate 5*a* and *b*). It is not clear whether these cells belong to the cortex or to the central cylinder, and, in the latter case, whether they are part of the pericambium or of the phloem. The circumstance that the cells in question are situated exclusively at the periphery of the phloem bundles argues for the latter possibility. Close to the main central cylinder, lignification of pith cells takes place. This is also the case for those cells of the interfascicular regions which lie on the secondary xylem side.

Details of changes observed in the cortical region will be discussed in the next paragraph.

Secondary changes in the cortex

An increase in diameter of the central cylinder by the formation of secondary tissues will of course cause changes in the cortical as well as in the epidermal regions of the cladodes. Furthermore, these changes will be conspicuous where the formation of secondary xylem and phloem is at its highest. It must be stressed that this type of change is not restricted to flowering areoles but also takes place, to a more moderate extent to be sure, in the case of resting areoles.

There appeared, however, to be a second type of secondary change restricted to flowering areoles and taking place prior to the other type. Originally, the two types of cortex cells, i.e. chlorenchyma cells and mucilage cells, are both isodiametric in shape. The changes run along two lines: regular expansion in the case of flowering areoles only, on the one hand, tangential deformation and dilation caused by cambial activity in both flowering and resting areoles on the other. The mucilage cells especially exhibit considerable expansion (compare Plates 3*a* and 3*b*). Later on, in those areas where the formation of secondary vascular tissues takes place to a higher degree, the mucilage cells become deformed tangentially. True dilatation by cell division, however, is only rarely encountered (Plate 4*b*).

Expansion of chlorenchyma cells occurs to a lesser degree. Deformation in a tangential sense and dilatation may take place to a very considerable extent. As a matter of fact, even in those areas where no secondary growth occurs tangential deformation of some cells and a few cell-divisions can already be observed. (Plate 3*a*). These phenomena occur on a large scale where considerable amounts of secondary xylem and phloem have been formed. (Plate 4*b*). In such cases practically the whole cortex is subject to the transformations described.

Quantitative data

From cladode fragments containing vascular strands leading to resting and flowering areoles, several series of sections were prepared in such a way that branch and leaf traces were cut transversely. By means of a camera lucida, drawings were made on squared paper of sections of branch traces from certain measured distances from the midrib. Outline drawings were prepared of phloem and xylem, and of the sclerenchymatous tissue situated at the periphery of the phloem. The numbers of squares (mm^2) of the areas covered by these tissues were calculated and plotted graphically (Fig. 1).

From the data presented it is clear that branch traces of resting and flowering areoles differ greatly as to their rate of development. On the other hand, no fundamental differences could be observed. In the case of resting areoles, branch traces show more or less gradual increase in the amounts of phloem and xylem along the line from midrib to areole. In the case of flowering areoles, however, the amount

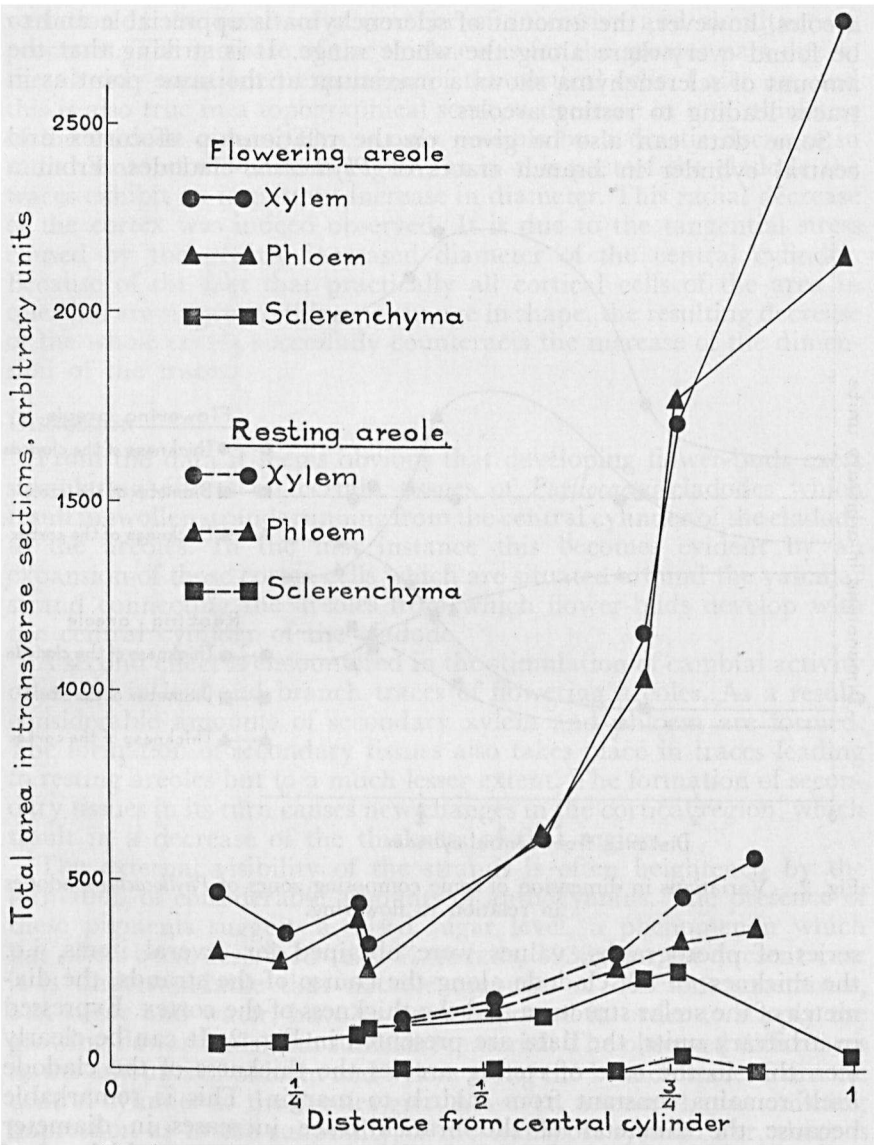

Fig. 1. Total areas of xylem, phloem, and sclerenchyma in transverse sections of branch traces of resting and flowering areoles of *Phyllocactus* at different distances from the central cylinder of the cladode.

of secondary phloem and xylem is very dramatically increased towards the areole.

As to the sclerenchyma, in the case of resting areoles only a very slight amount was encountered at a point about three quarters of the distance between midrib and areole. For the rest, no such sclerenchyma could be observed in this category. In the case of flowering

areoles, however, the amount of sclerenchyma is appreciable and to be found everywhere along the whole range. It is striking that the amount of sclerenchyma shows a maximum at the same point as in traces leading to resting areoles.

Some data can also be given on the relationship of cortex and central cylinder in branch traces of *Phyllocactus*-cladodes. From a

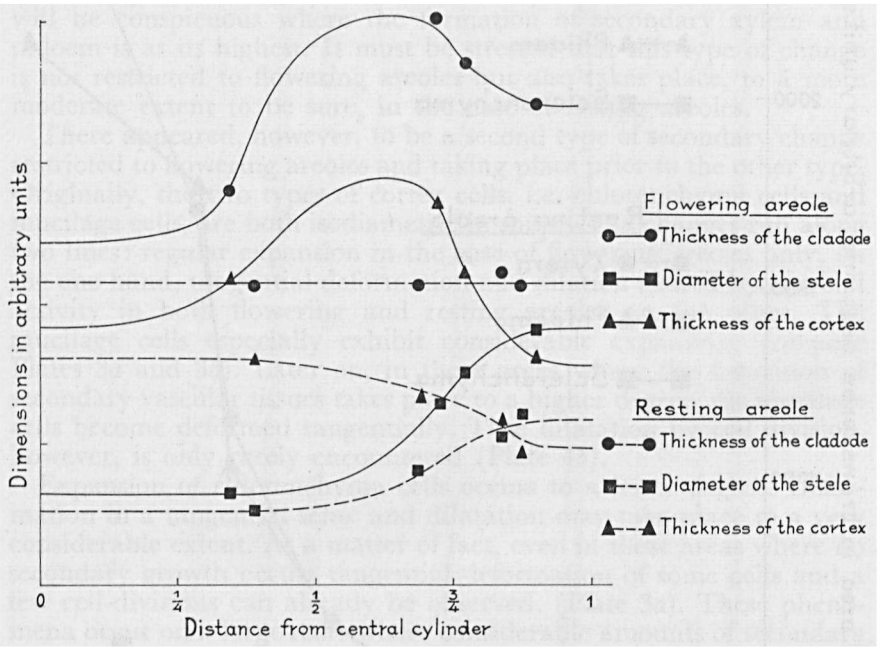

Fig. 2. Variations in dimension of some composing zones of *Phyllocactus* cladodes in relation to flowering.

series of photographs, values were obtained for several items, i.e. the thickness of the cladode along the course of the strands, the diameter of the stelar structure and the thickness of the cortex. Expressed in arbitrary units, the data are presented in Fig. 2. It can be clearly seen that in the case of resting areoles the thickness of the cladode itself remains constant from midrib to margin. This is remarkable because the diameter of the branch trace increases in diameter towards the margin. The only way by which a constant thickness of the cladode can be effected is obviously by a decrease of the dimensions of the cortical portions. This is indeed the case, as the radial dimensions of the cortical cells become reduced by the tangential stress roused by the expansion of the stelar structure. In the case of flowering areoles, the situation is fundamentally different which was to be expected from the previously described expansion of cortical cells in such cases. At a point about two thirds of the distance from midrib to margin, the cladode is at its thickest, while from this point toward the areole a slight decrease in thicknes can be observed. This

variation is the result of a number of successive and simultaneous processes. The increase of the thickness is for the greater part due to the already-mentioned expansion of the cortical cells. Later on, and this is also true in a topographical sense, a decrease in total thickness of the cladode occurs. This is only possible by a drastic decrease in radial sense of the cortical region, as in this part of the cladode the traces exhibit an important increase in diameter. This radial decrease of the cortex was indeed observed. It is due to the tangential stress caused by the greatly increased diameter of the central cylinder. Because of the fact that practically all cortical cells of the area in question are subject to this new change in shape, the resulting decrease of the whole cortex succesfully counteracts the increase of the dimension of the traces.

Discussion

From the data it seems obvious that developing flower-buds exert stimulating effects on certain tissues of *Phyllocactus*-cladodes which result in swollen strands running from the central cylinder of the cladode to the areoles. In the first instance this becomes evident by an expansion of those cortex cells which are situated around the vascular strand connecting the areoles from which flower-buds develop with the central cylinder of the cladode.

A second effect is encountered in the stimulation of cambial activity observed in leaf and branch traces of flowering areoles. As a result, considerable amounts of secondary xylem and phloem are formed. The formation of secondary tissues also takes place in traces leading to resting areoles but to a much lesser extent. The formation of secondary tissues in its turn causes new changes in the cortical region, which result in a decrease of the thickness of that region.

The external visibility of the strands is often heightened by the formation of considerable amounts of anthocyanins. The presence of these pigments suggests a raised sugar level, a phenomenon which has often been described (e.g. Karstens, 1938). In the present case it is understandable that notable amounts of different substances, a.o. sugar and water, have to be transported in order to supply the growing flower-buds with sufficient material. The observed differences in the amounts of phloem and xylem along the branch traces from the central cylinder to the areoles give, however, a somewhat astonishing impression as to the suitability of the transport system. For, there is a considerable discrepancy between the amounts of phloem and xylem in the vascular bundles just below the areole and those close to the main vascular cylinder, i.e. 7:1. This forms a bottleneck close to the main cylinder of the cladode. It seems, therefore, rather doubtful whether it is justified to consider the formation of vascular tissue as a provision to promote transport to the growing flower-buds.

With the results of Snow (1935), Gouwentak (1936, 1941) and Gouwentak and Maas (1940) in mind, the possibility of an activation of cytokinetic processes of the cambium by the developing flower-bud was considered. This is the more likely because of the fact that

leaf traces and anastomosing bundles of flowering areoles also exhibit secondary growth. Some experiments using lanolin paste containing growth substances to replace excised resting areoles did not lead to any stimulation of cytokinetic activities of the cambium. The thickening obtained adjacent to the application of the hormone paste proved to be caused only by a localized multiplication of cortical cells. Many of these newly formed cells, arranged in neat rows, are very much enlarged. Since they contain appreciable amounts of mucilage they greatly resemble the slime idioblasts described above.

As to the failure to induce cambial activity, the possibility remains that we have to do with a case comparable to that described by GOUWENTAK and MAAS (1940) and GOUWENTAK (1941). According to these authors cambium must be roused from dormancy before growth hormones can stimulate cytokinesis. It is our intention to continue our experiments with this point in mind.

SUMMARY

Phyllocactus plants coming into flower exhibit thickened, often purplish coloured strands running from the central vascular system of the cladode to the budding areoles. The anatomical background of this phenomenon forms the major part of the present paper. It appears that the growing flower-bud stimulates certain tissues of *Phyllocactus* cladodes, resulting in the formation of swollen strands. This stimulation successively affects two types of tissue, i.e. the parenchymatous cortex surrounding the vascular traces connecting the central cylinder of the cladode with the areole and, secondly, the cambial zone of the vascular bundles present in such traces. The cortex parenchyma as a result exhibits a general expansion of the cells, and the cambial zone is stimulated to form considerable amounts of secondary tissue. The production of these tissues causes tangential stretching of the surrounding cortical cells which results in a decrease in their radial dimension. In the proximal part of the strands, cell expansion proved to be the major component of the swelling; in the distal part this is caused preponderantly by the formation of secondary vascular tissues.

There is a marked difference in the quantities of vascular tissue formed along the strand. A ratio of 7:1 was observed on comparison of distal and proximal portions of branch traces.

Some introductory experiments with lanolin paste containing growth substances to replace excised resting areoles produced negative results. These investigations will be continued.

ACKNOWLEDGEMENTS

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Plate 1*a*. *Phyllocactus*. Cladode with a number of flower-buds in various stages of development. The "midrib" and the thickened strands towards the areoles are clearly visible. ± nat. size.

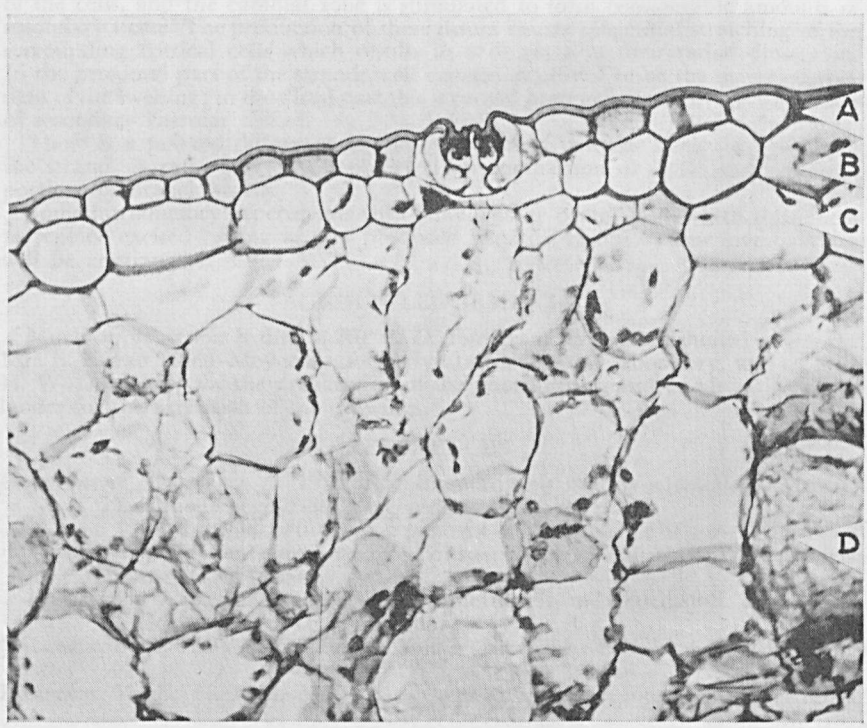

Plate 1*b*. Epidermis and cortex of *Phyllocactus* cladode. A, epidermis with cuticle and stoma; B, hypodermis; C, chlorenchyma cell; D, mucilage idioblast. 200 ×.

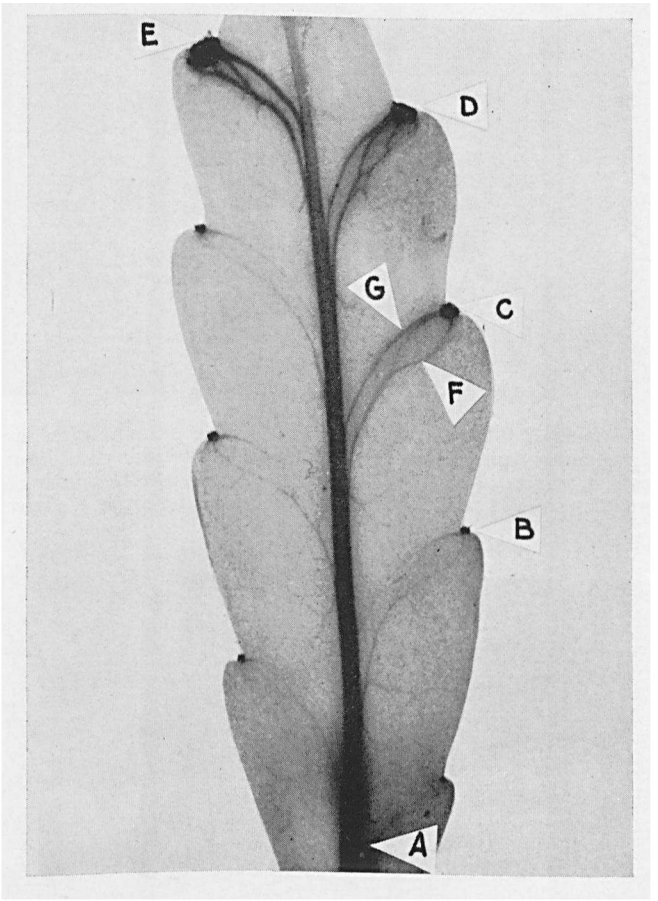

Plate 2. Vascular system of *Phyllocactus* cladode, decolourized and made transparent. Flowers and flower-buds removed. A, central cylinder ("midrib") of the cladode; B, resting areole; C, place of insertion of a one-inch long flower-bud; D, ditto of flower-bud 4.5 inches long; E, ditto of fully expanded flower; F, leaf trace; G, branch trace. Anastomoses between leaf and branch traces are clearly visible. Somewhat larger than natural size.

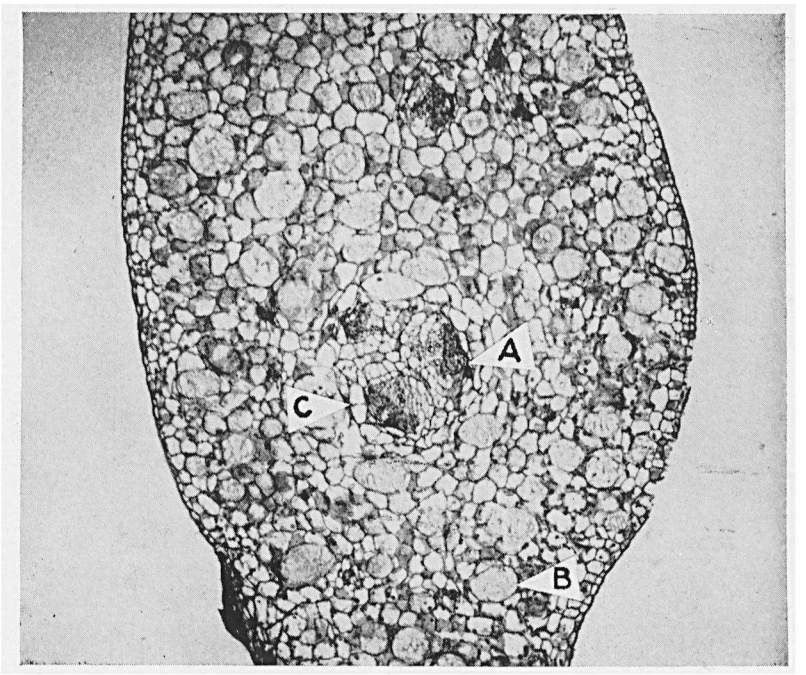

Plate 3*a*. *Phyllocactus*. Resting areole. Section perpendicular to the long axis of a branch trace close to the central cylinder of the cladode. A, branch trace; B, mucilage idioblast; C, first indication of dilatation in the cortical region. 35 ×.

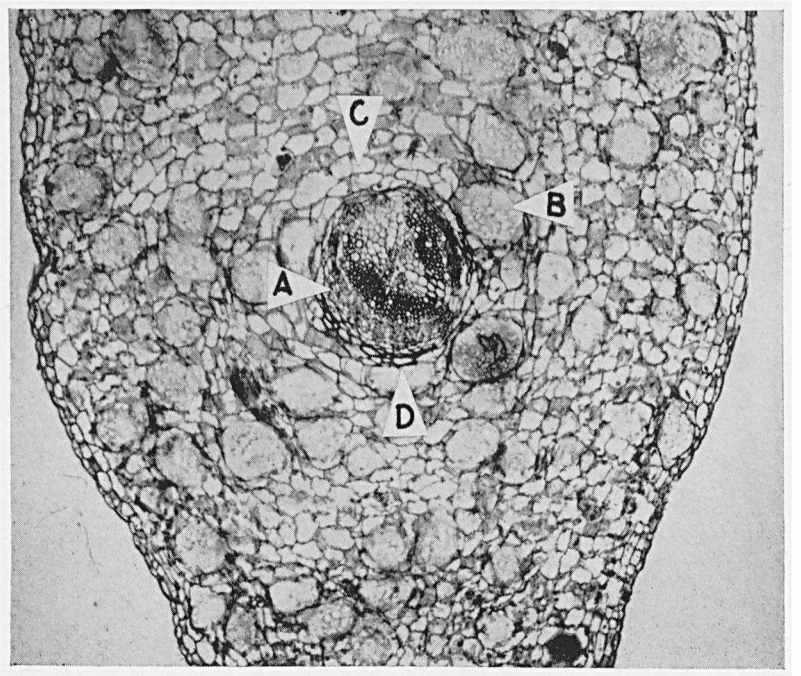

Plate 3*b*. *Phyllocactus*. Flowering areole. Section as in Plate 3*a*. A, branch trace; B, mucilage idioblast; C, dilatation of cortex cells; D, sclerenchyma. 35 ×.

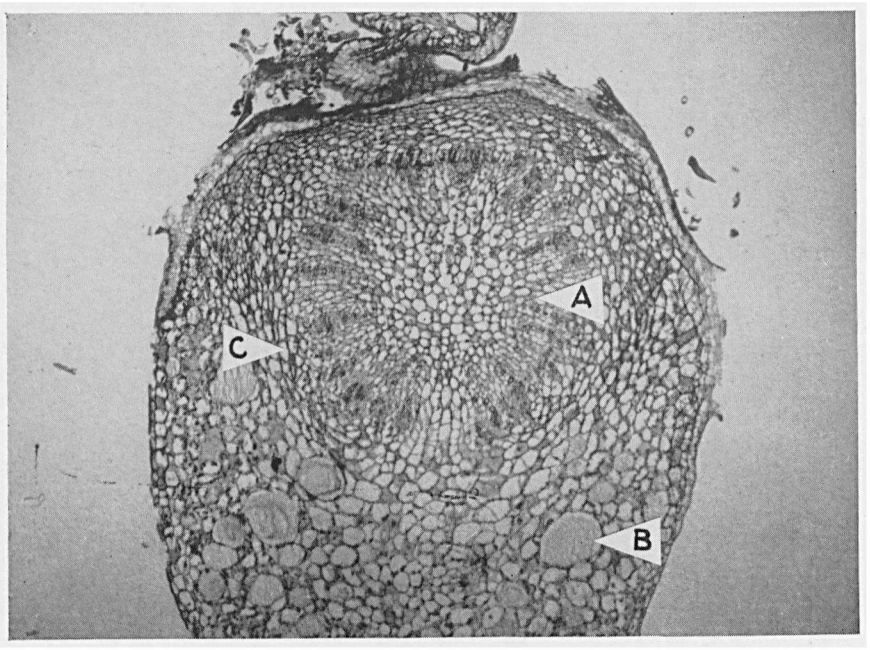

Plate 4*a*. *Phyllocactus*. Resting areole. Section perpendicular to the long axis of a branch trace close to the areole. Several cortical layers show dilitation. A, branch trace; B, mucilage idioblast; C, dilatation of the cortex. 35 ×.

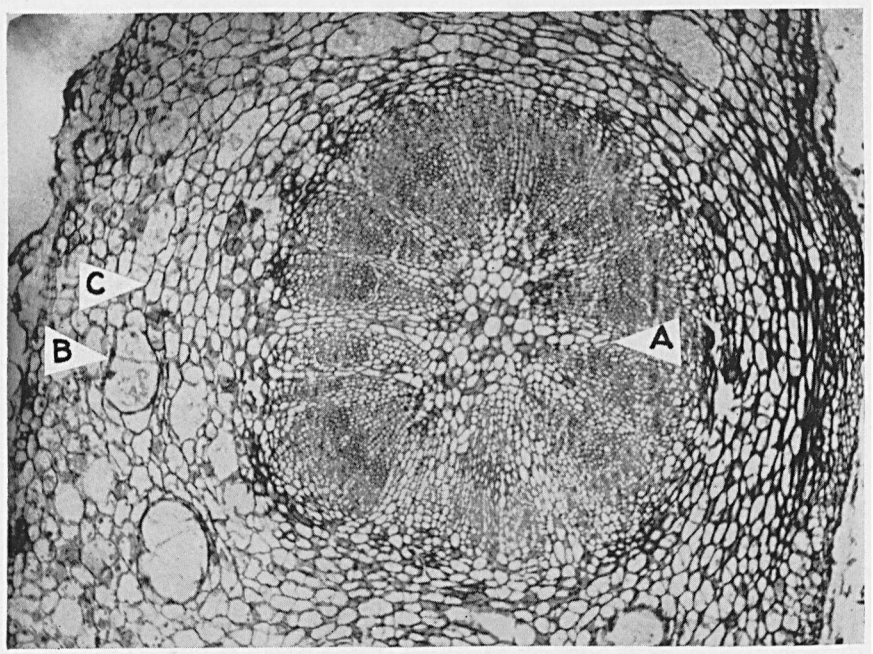

Plate 4*b*. *Phyllocactus*. Flowering areole. Section as in Plate 4*a*. Many cortical layers show dilatation. A, branch trace; B, dilatating mucilage idioblast; C, dilatation of the cortex. 35 ×.

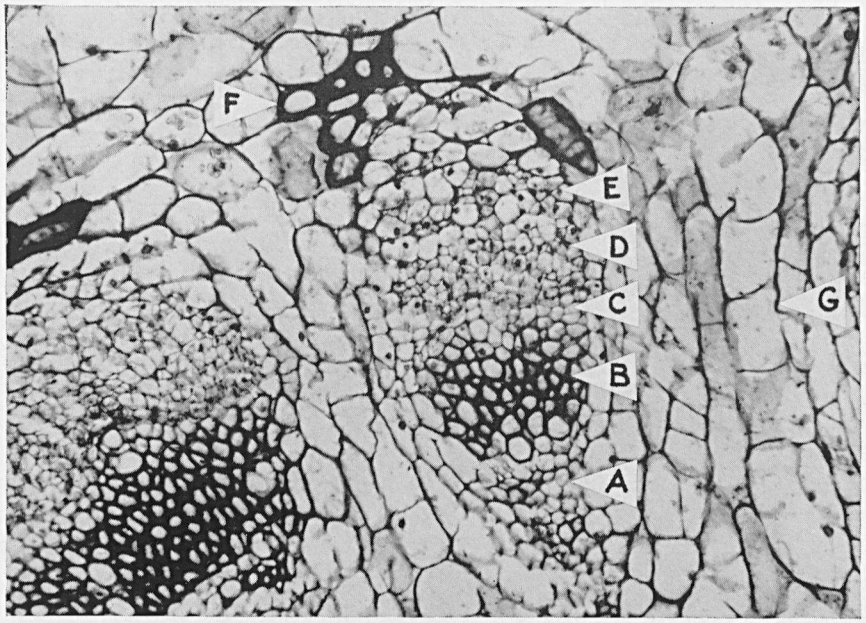

Plate 5*a*. *Phyllocactus*. Flowering areole. Detail of a branch trace in transverse section. A, primary xylem; B, secondary xylem; C, fascicular cambium; D, secondary phloem; E, primary phloem; F, sclerenchyma; G, interfascicular region composed of irregular rows of radially orientated cells. At the top left, many dilatating cortical cells. 150 ×.

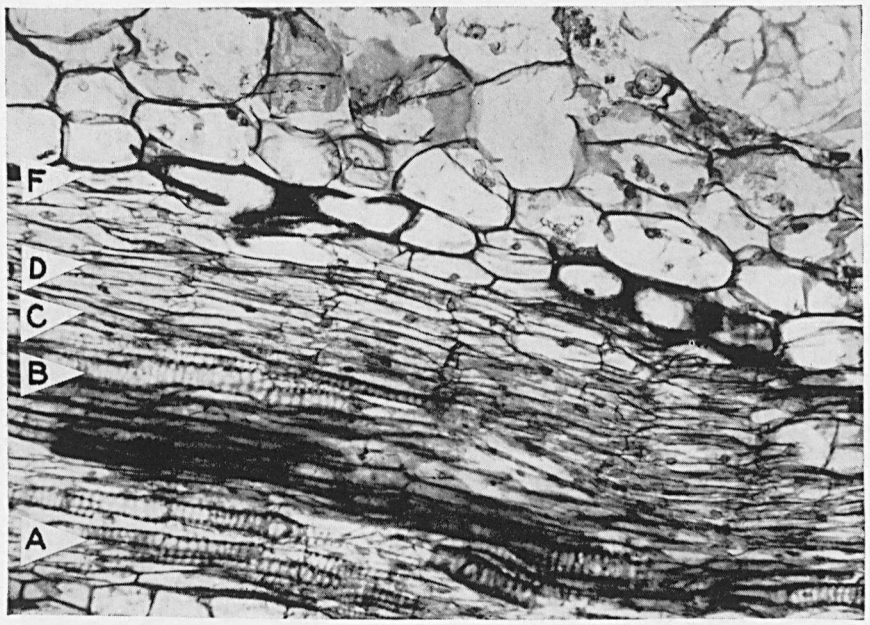

Plate 5*b*. *Phyllocactus*. Flowering areole. Detail of a branch trace in longitudinal section. A, primary xylem; B, secondary xylem; C, cambial zone; D, secondary phloem; F, sclerenchyma. Primary phloem indistinct. At the top right, part of a mucilage idioblast is visible. 150 ×.