On the influence of wound stimuli on the formation of adventitious buds in the leaves of Gnetum Gnemon L.

by

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It had been observed for a long time already that adventitious sprouts were formed on the leaves of a specimen of *Gnetum Gnemon* L., cultivated in the Botanic Garden at Utrecht.

In January 1906 my attention was drawn to this circumstance by Prof. Went, who advised me to study the development of these adventitious sprouts and to try to discover the origin of their formation.

The results of this investigation will be concisely communicated here.

The adventitious buds appeared on the tip of the leaves, while these were still attached to the plant.

As far as I have been able to find out, the formation of these adventitious buds has never before been observed with Gnetum Gnemon, neither in its natural sites, nor in botanic gardens [1]) except at Utrecht. The Utrecht Garden

1) On this point I gained information from the other botanic gardens in our country, from that at Munich and also from that at Buitenzorg.

possesses three specimens of Gnetum Gnemon. One of these has been continually cultivated in a hothouse where in winter the temperature was kept at about 25° C. and the air was very damp. The other two were, when I began my investigation, in an other hothouse where the temperature was lower (in winter on an average 15° C.) and the humidity less [1]. Whereas of the former I have always obtained leaves in differents stages of bud-formation, the other two showed the phenomenon only after they had been conveyed to the warmer and damper hothouse.

Although all three plants, paying no attention to the formation of adventitious buds, evidently were healthy and did not make a morbid impression at all, they flowered very rarely. Personally I only observed it with one of the plants from the cooler hothouse. This latter plant produced one single ♂ inflorescence, which enabled me to check the accuracy of the determination.

The first external change, noticed with a leave which will form adventitious buds, is that on the tip extremely small yellow dots appear which are seen best when light is falling through the leaf. They remind us in this respect of the oil dots in the leaves of the *Rutaceae* or *Hypericum*, but as a rule they are bigger and less densely spread than these.

With the bigger ones a hand-magnifier will show that where the dots are, the epiderm of the upper or lower side or of both together is slightly bulged, so that we have to do with small vesicles.

It will be shown presently that these vesicles are caused by the sting of a scale-insect *Aspidiotus dictyospermi* Morg., and as such not restricted at all to the tip of the leaf.

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1) These two hothouses have been demolished during the recent rebuildings in the Garden.

Normally, however, it is only the tip which can form adventitious buds. The remaining part of the leafblade can only form adventitious buds when the organic relation with the tip has been disturbed in some way or other. But even then they arise apically in this part. Hence only the vesicles which have originated on the apical part of a leaf, form the introduction to the process of bud-formation.

For the sake of simplicity we shall in what follows, only mention the tip of the leaf, since the statements referring to the tip also apply to the other cases.

After some time also the region, surrounding the vesicles, becomes discoloured; as a rule the tip of the affected leaf soon becomes distinctly yellow, although in some cases it long keeps a more or less greenish tint.

At the same time with this discoloration the tip of the leaf becomes thicker. This thickening is at first not easy to observe macroscopically; gradually, however, it becomes stronger and at last generally advances so far that the tip becomes stiff and difficult to bend.

Of the yellow vesicles nothing can then be seen any longer.

The extent of this region of discoloration and thickening varies much in a basal direction; along the edge it generally extends farther basipetally than in the middle; always, however, the phenomenon is restricted to the apical part of the leaf. A new stage sets in, when the surface of the thickened leaf-tip which until now had remained smooth, on account of the swelling being even, becomes uneven: as well on the lower as on the upper surface this may as a rule be observed; on the upper surface it is generally more pronounced.

During the first weeks generally no striking changes are observed until after about a month a varying number of local elevations, yellow like the leaf-tip that produces them, becomes prominent and reveals the differentiation of special proliferating centres.

As a rule we see these grow to real knobs, especially in the direction perpendicular to the surface of the leaf (Pl. II, fig. 4).

While the knobs are still relatively small, brownish grey streaks begin to appear on their tops, which gradually extend, so that finally the whole knobbed surface becomes brown.

For some time such a knob shows nothing particular, except that it becomes larger and thicker. Next on a certain day a small opening is formed in its top through which a small green point projects which will grow out into a leafed sprout (Pl. II, fig. 1, fig. 5 *a* and a^1).

As well on the lower as on the upper surface of the leaf-tip knobs may form. Mostly they form on the upper surface, though. With some leaves I have observed knobs on both sides at the same time.

The observations on the time, needed bij a leaf in order to form „ripe" knobs, after the yellow vesicles have appeared, have led to somewhat diverging results. The shortest period was observed with leaves on the upper branches or in the periphery of the crown, which consequently occupied the most favorable position with regard to light. On these good-sized, brown knobs had generally formed half a year after the appearance of the yellow vesicles.

Also for the question, how old and how large a knob must be in order to open and give the adventitious bud an opportunity for sprouting, no rule can be fixed. I saw one sprout five weeks after the knob had first been observed as a special elevation, while others were still closed after five to seven months.

About the size of the knobs we may state that some knobs, scarcely rising more than a millimetre above the surface of the leaf-tip, opened, while others of double and even treble height remained obstinately closed. Yet these latter contain as well an adventitious bud and not seldom even more than one.

The microscopical investigation was for the greater part carried out on microtome preparations. For fixing the material I used the mixture: zincchloride-glacial acetic acid-alcohol, (2 grams of zincchloride and 2 ccm. of glacial acetic to 100 ccm. of alcohol of 45—50 pCt), recommended by Juel [1]. The particular hardness of the leaf tissue made it necessary to treat the material, before being embedded in paraffin, during 3 to 4 × 24 hours with a 40 pCt. aqueous solution of hydrofluoric acid. After this treatment it was then washed for 8 to 10 hours in streaming water, dehydrated by the usual method and after treatment with chloroform embedded in paraffin (melting point 62° C.).

For staining the sections I used ad first Haematoxylin-Delafield and saffranin, according to the prescriptions given in Chamberlain's „Methods in Plant Histology" [2]; but this method proved unsatisfactory for differentiating the very thin-walled meristem cells. Therefore I afterwards always stained with methyl green and acid fuchsin [3], by which very good results were obtained.

A consequence of the treatment with hydrofluoric acid was that the microtome preparations were not suitable for all observations. In these cases I used hand-cut preparations, if necessary stained with Haematoxylin-Delafield.

The anatomy of the normal leaf, on which something

1) H. O. Juel, Ueber den Pollenschlauch von Cupressus. (Flora. Bd. 93. 1904. pag. 56—62).

2) C. J. Chamberlain, Methods in Plant Histology 2nd ed. Chicago. 1905. pag. 30, 38 and 54.

3) Chamberlain. l. c. pag. 40, 44 and 68.

may be found in literature with Bertrand[1]), De Bary[2]), Scheit[3]) and Haberlandt[4]), is as follows.

The epiderm of the lower and upper surface consists of relatively small, cubical cells, the outher wall of which is strongly thickened and provided with a strong cuticle and from which capriciously shaped and canaliculate outgrowths project into the lumen of the cell. (cf. Pl. II, figs. 8 a. o. and Bertrand, l. c. Pl. II fig. 6, 7, 8.). In the epiderm of the lower side numerous, irregularly placed stomata are found. (Pl. II, fig. 8 *st*).

Under the upper epiderm lies the palissade parenchyma (Pl. II fig. 8 *p*,) formed by one continuous row of cells, slightly elongated in the direction perpendicular to the leaf surface. (dimensions 13—21 μ by 21—30 μ). Between the palissade cells and the lower epiderm lies the spongy parenchyma (Pl. II, fig. 8 *sch.*) consisting of tubular cells, the diameter of which is on the average 18 μ, as a rule is not more than 9 μ at a partition wall between two tubes and does not reach more than 28 μ. Between the cells of the spongy parenchyma remains a system of large intercellular spaces. In the spongy parenchyma numerous thick-walled sclerenchyma fibres (Pl. II, fig. 8 *skl.*) are found, which are generally ramified and often have an enormous length.

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1) C. E. Bertrand, Anatomie comparée des tiges et des feuilles chez les Gnétacées et les Conifères. (Annales d. Sc. nat. Botanique 5ième série Tome XX. 1874).

2) A. De Bary. Vergl. Anatomie der Vegetationsorgane der Phanerogamen und Farne. (Handb. der Physiol. Botanik von W. Hofmeister. Leipzig. 1877.)

3) M. Scheit, Die Tracheïden-Säume der Blattbündel der Coniferen etc. (Jenaische Zeitschr. f. Naturw. Bd. XVI. Neue Folge Bd. IX. 1883.)

4) G. Haberlandt, Physiologische Pflanzenanatomie 2te Aufl. Leipzig. 1896.

A section through a leaf-tip on which yellow vesicles are found (Pl. II figs. 9 and 10.), shows that these vesicles are caused by hypertrophy of cells of the spongy parenchyma which have there entirely lost their tubular shape and among which specimens are found, measuring 91 by 109 μ, 100 by 73 μ, 100 by 113 μ etc. Among the cells, constituting the vesicle, some are always found which in unstained preparations are conspicuous by their wall being more or less swollen and brown (with x in fig. 9, the somewhat darker drawn walls in the middle part of fig. 10). In preparations, treated with acid fuchsin and methyl green, the wall of these cells is blue, those of the other cells red. Applying the usual reactions we find that these walls have become suberized. Also of the palissade parenchyma some cells may have become larger, but always in a small degree. (cf. fig. 10).

While in the vesicles themselves the process goes a little further still on account of partition walls forming in some of the enlarged cells, whose walls have not become suberized, also the region, surrounding the vesicles, evidently answering to a stimulus, proceeding from them, begins to undergo similar changes. Macroscopically we detect this by the more or less yellow tint, assumed by the vicinity of the vesicles. A microscopical examination of the section (Pl. II, fig. 11) shows that now also outside the vesicles the cells of the spongy parenchyma are hypertrophical. As with the formation of the visicles the chlorophyl is disorganised in the hypertrophical cells.

At this stage no function of importance may be ascribed yet to the cells belonging to the palissade parenchyma.

In most preparations now already the peculiar behaviour is noticed of those spongy parenchyma cells which border immediately on the palissade parenchyma. While the other cells of the spongy parenchyma swell as evenly as possible in all directions, those which lie immediately below the palissade parenchyma become enlarged especially in a radial direction, thus making the impression of a second layer of palissade cells (Pl. II, fig. 11). Since also in later stages they will repeatedly draw our attention, I shall in what follows call these cells *subpalissade cells* instead of using the cumbrous longer definition,

This extension, especially in a radial direction, of the subpalissade cells, is illustrated by the following two tables.

A	Subpaliss. cells of a normal leaf-tip	Height μ: 16	16	14.5	18	14.5	16	14	14.5	14.5	22
		Breadth μ: 31	18	16	25.5	27	27	18	25.5	31	31

B	Subpaliss. cells of a yellow leaf-tip	Height μ: 33	26	38	44	47	42
		Breadth μ: 31	29	18	31	33	33

In a leaf-tip which macroscopically is distinguished, besides by the yellow colour, by a distinct thickening (Pl. III, fig. 13), the intercellular cavities of the spongy parenchyma are found to have entirely disappeared, excepting a small corner here and there. The mutual pressure which the cells consequently begin to exert on each other, causes them to assume a more polygonal shape. The cells, bordering on the subpalissade cells and often also the rows, turned towards the lower epiderm, show a tendency to stretch themselves in a direction perpendicular to the surface of the leaf. In many spongy parenchyma cells partition walls have formed.

The part, played by the subpalissade cells in the process of thickening, is generally a very important one. So I found in a leaf-tip in a place, where it was 332 μ thick (the section of a normal tip is on the average 170 μ), cell rows, formed of subpalissade cells, partitioned by two or three walls and measuring 90, 110, 115 and 127 μ in height. — The palissade rows were in these places not sensibly enlarged (Pl. III, fig. 13).

That the different tissues also in the same leaf-tip do not every-where play the same part in the process of thickening, appears from the following figures, from measurements in two different places of the same leaf-tip;

Height of the palissade cells.	36 to 45 μ. } most cells with 2 or 3 partitions
Height of the subpaliss. cells.	73 to 82 μ. (mostly partitioned)
Height of the spongy parench.	273 μ.
Height of the palissade cells.	27 μ. non-partitioned.
Height of the subpaliss. cells.	146 μ. } with numerous partition walls.
Height of the spongy parench.	273 μ.

The special thickened outgrowths, mentioned on page 152, are caused by the same processes of proliferation which cause the thickening of the whole leaf-tip and which in some places go on with particular activity, while the surrounding region seems to come to rest.

On the upper surface they are formed by locally strong proliferation of the subpalissade cells. Sometimes also the underlying cells, originating from the spongy parenchyma contribute to them and then it cannot be ascertained as a rule what part is derived from the subpalissade cells and what from the original spongy parenchyma. In most cases the contribution of the palissade parenchyma to the formation of the special elevations is rather unimportant.

The special thickenings on the lower surface of the leaf are entirely formed by cells which genetically belong to the spongy parenchyma but for the rest in exactly the same way as those on the upper surface.

As it is shown clearly by a hand-cut preparation of living material, the above-mentioned discoloration of the leaf-tips is caused by a destruction of the chloroplasts in the affected cells. In fig. 12 part of a section of a yellow and distinctly thickened leaf-tip has been drawn 400 times magnified. It may be seen, that the destruction of the chloroplasts has gone furthest in the cells of the spongy parenchyma in which only slight, yellow fragments of the chloroplasts are to be found; such remainders find themselves also in the sub-palissade cells although the destruction is less there than in the spongy parenchyma. Best preserved the chloroplasts remain in the palissade cells, in which one finds besides yellow fragments and chloroplasts that are more or less swollen and discoloured such as have yet their normal color and shape at the time the other cells contain only those yellow shapeless rests.

Since the cells from which they are built up, divide parallely to the surface of the leaf and the so-formed division-cells stretch themselves again, these special thickenings, which at first appear as small unevennesses, grow out into the knobs, already mentioned on p. 152.

How has the epiderm been able to follow the increase of surface, accompanying these thickening processes?

In a normal leaf-tip we find for the dimensions of the epiderm cells 9 to 18 μ height and 9 to 29 μ breadth, while in the epiderm, covering a special thickening, amidst cells of normal dimensions others are found which measured:

Height μ:	9	11	13	13	13	9	11	11	9	9
Breadth μ:	31	36	45.5	36	49	45.5	45.5	42	54.6	45.5

Hence some epiderm cells seem really to broaden; whether this is only an extension or active growth, I dare not decide.

Besides, the epiderm soon gives way and is rent. Like the part of the epiderm which gives way to the pressure, some cells of the tissue underneath die off, the cell-walls turning brown (Pl. III, fig. 16 *a*, at *k*). In this way arise the brown streaks on the surface of the knobs which finally by extension in tangential direction of this suberizing process becomes entirely brown. A special suberizing meristem, a phellogen, is not formed.

The regular structure of these cell-hills is lost as soon as the differentiation of a meristem commences. Some cells, assembled in a small group, then enter a new stage of strong growth, which makes them conspicuous in the preparations by a more rounded form amidst the adjoining cubical cells (Pl. III, fig. 14, *m.a*). A number of the surrounding cells are compressed by the pressure which these primordial cells cause by their growth and die. (fig. 14 *pr. c.*)

Soon the primordial cells divide into a number of small filial cells with extremely thin walls and dense contents, after which the primordium has become meristem (Pl. III, fig. 15 *m.*).

For answering the question in what place in a knob the meristem is formed and which is the descent of the initial cells, we have the following data. An otherwise 415 to 450 μ thick leaf-tip had by local swelling, to about 840 μ, formed a knob, which by a small depression in the middle was, so to speak, divided into two halves, each of which contained a primordium of a meristem. One of these primordia is the one figured in fig. 14. The surface of the knob was entirely suberized to a fairly considerable depth. In one half the primordium lay 220 μ below the top of the knob and its cells in all probability descended from the subpalissade cells, in the other half the primordium lay 180 μ below the surface and was of the same origin as in the former case.

While in another case a primordium was noticed which genetically belonged to the original spongy parenchyma I found in a small knob which was still covered by an intact epiderm and did not rise more than 85 μ above its surroundings and in this case had been formed by special proliferation of the palissade parenchyma, a distinct young meristem immediately below the epiderm. *The epiderm cells themselves however, did not take part in the formation of the meristem.*

In a word, meristem formation may take place as well by cells, descending from the spongy parenchyma and the subpalissade cells, as from such as have been formed by hyperplasia of the palissade parenchyma, the epiderm, however, plays no part. In other words: *the adventitious buds on the leaves of Gnetum Gnemon are endogenic formations.*

In the beginning the young meristem increases in size by its own active growth as well as by new cells from the immediate vicinity becoming meristematic.

When the meristem has reached certain dimensions, it partly becomes loose from the surrounding tissue. This is brought about by some of the cells, forming the transition between the meristem and the surrounding tissue, being dissolved and resorbed. (Pl. III, fig. 16*a*, *s*).

This dissolution process proceeds along the whole upper side of the meristem, so that the growing point of the adventive bud comes to be placed in a slit-shaped space (Pl. III, fig. 17, *s*).

The greater the depth at which the meristem was originally formed inside the knob, the thicker is the layer of tissue which ultimately separates the bud from the outer world and the further the development within the enclosure proceeds (cf. Pl. IV, fig. 18 and Pl. III, fig. 19). This explains how it is possible that knobs, no larger than 1 millimetre, open, while much larger ones remain persistenly closed.

The appearance of two meristems within the same knob is a very common occurrence; once I found as many as four meristems in one knob.

The buds assume a green colour while they are still entirely enclosed within the knob and hence I think it probable that they have the power, like the germinating plants of Ephedra and the Coniferae, to form chlorophyl independent of light.

The sprouts growing out of the adventitious buds always remain short and tender (cf. Pl. II, figs. 2, 3 and 6). The biggest I observed reached a height of about 3,5 centimetres and consisted of a stem with 5 internodes (including the basal part) of which the upper one reached the greatest length (almost 2 centimetres), while the leaflets on the last node became largest (about 3 cms. long). The position of the leaves is alternating, the innervation of the leaf the typical one for Gnetum Gnemon [1]). The leaflets on the first node as a rule remain scale-shaped; in some cases, however, they develop to leaflets, differentiated into stalk and blade.

Although in the axils of the leaflets axillary buds are certainly formed, (figs. 19—22), I never saw the adventitious sprouts ramify themselves, except in a single case, when, as I surmise, of the basal piece of an adventitious sprout the terminal bud did not develop for some reason or other and instead the buds in the axils of the scale-leaves sprouted (Pl. II, fig. 3).

On a differentiation of histogens at the vegetative cone I have not been able to form a definite opinion from my preparations of adventitious sprouts.

The numerous attempts which I made, in order to induce the adventious sprouts to produce roots, have all failed. This agrees with the circumstance that in my pre-

1) Viz. Nr. 3 of the leaf nervations, distinguished by Karsten for the species of Gnetum [G. Karsten, Untersuchungen über die Gattung Gnetum. I. (Ann. du Jardin Bot. de Buitenzorg Volume XI. 1893. pag 195—281)].

parations I have never been able tot discover anything that resembled root-formation. The sprout-carrying leaves which had been planted in wet sand did not form roots either. As far as I know formation of adventitious roots does not occur at all with *Gnetum Gnemon.*

A connection between the vascular system of an adventitious sprout and the nerve system of the mother leaf is established by procambial bundles, formed by cells of the tissue situated between the meristem and a leaf bundle.

As a rule we find as the first indication of this vascular bundle connection in the immediate vicinity of very young meristems even, some tracheids and cells, changing into them (Pl. III. fig. 15). The degree of development, reached at a certain moment by this vascular bundle connection, is not directly dependent on the degree of development of the adventitious bud in question, but seems to me to stand in close relation to the distance between meristem and leaf bundle and to the diameter of this latter (cf. figs. 17, 18, 19, 20). When a complete connection has been established we see the vascular bundles of the adventitious sprout within the knob in which the bud has formed, bend towards each other and unite with a more or less cylindrical group of of locally formed vessels and tracheids, the ramifications of which are connected with the vascular bundles of the mother leaf (Pl. IV, fig. 22).

When describing the changes, macroscopically observed with a leaf in which adventitious buds are forming, it has already been briefly stated that the yellow vesicles initiating the process of budformation, are caused by a scale-insect, *Aspidiotus dictyospermi* Morg..

By mediation of Professor Cockerell of the *University of Colorado* in Boulder, U. S. A., the insect has been determined for me at the U. S. Department of Agriculture, Bureau of Entomology, in Washington. Hence I got the following information: „The scale insect proves to be *Aspidiotus dictyospermi* Morg., a species often found on a considerable variety of greenhouse plants, notably on palms. It is also common in tropical countries, and has a worldwide distribution, out of doors in the tropics, and in hothouses in temperate countries."

On this place I wish to express my thankfulness to Professor Cockerell for his friendly mediation and to the entomologists of the U. S. Department of Agriculture in Washington for their determining of the Coccid.

That suspicion fell on this *Aspidiotus* had a very simple reason.

Although not nearly all the leaves, showing yellow vesicles, still carried scale-insects, yet the reverse was generally true and it soon became apparent that the leaves, carrying scale-insects generally also had some yellow vesicles.

But unexpected difficulties were experienced when it was attempted by means of microtome preparations to obtain certainty and a clearer insight in what had been rendered probable by macroscopical observation. A great difficulty was that the majority of the insects refused to stick to the bits of leaf from which the microtome preparations were going to be made. While a great part already let loose during the treatment, preceding the embedding itself, their example was followed by most others when they were put into the melted paraffin. It was supposed that perhaps the reason of this was that the scale-insects, when coming into the fixing solution, withdrew their suction organ from the tissue of the leaf, possibly on account of a pre-mortal reactional movement. After this unfavourable result the leaves carrying the insects were always treated before fixation with an anaesthetic, namely aether. This precaution, however, did not materially improve the results.

Among the microtome preparations which I obtained in spite of these difficulties, there is not a single one in which a scale-insect may be seen in a sucking position. But always in the places were an insect was on the leaf, in the tissue the changes were found which we described as characteristic for the yellow vesicles.

Here also hypertrophy, accompanied by disorganisation of the cholorophyl, of cells, belonging to the mesophyl; some of these cells have brown walls. Also in the epiderm on which the scale-insect is found, some cells are found, the walls of which are suberized and which besides are sometimes slightly swollen.

I was more fortunate with hand-made preparations, some of which show the suction apparatus of the scale-insect inside the leaf tissue. From these we see that in the yellow vesicles those cells, the wall of which has become suberized, have been in direct contact with the suction apparatus of the scale-insect and that the other cells, that become hypertrophical, only react to a stimulus, exerted by the wounded cells. On the character of this stimulant action we shall speak presently.

That here the enlargement of the cell should take place at the expense of its own contents, as is stated for many similar hypertrophical processes, is not the impression I received. Although in many of the very strongly hypertrophical cells a large central vacuole may be observed, yet I saw nowhere reduction of the protoplasm to a very thin wall-lining. The nucleus does not show any deviation and the cell-wall does not become perceptibly thinner.

That the sting of the scale-insect not only causes the formation of the yellow vesicles but through them also all further changes, including the formation of the adventitious buds, has become clear to me by:

1. the microscopical examination of a very large number of preparations, relating to these stages;

2. the continued observation of a number of leaves on

the tree, showing that those leaves on which scale-insects or the yellow vesicles, caused by them, were seen, underwent the above described changes, while the control leaves remained free from them. On Sept. 13, 1906, the top of that plant which forms adventitious buds most strongly and one of its branches were each surrounded by a muslin balloon, after they had first been carefully inspected and cleaned. These balloons were supported by skeletons of galvanised iron wire and closed below by pulling them on to a pad of cotton wool, placed round the sprout. At the top of the plant were then only young leaves, on the branch full-grown ones, all of them free from scale-insects and vesicles. The balloon, surrounding the top of the plant had repeatedly to be replaced by a bigger one as the top grew.

On January 22, 1907, the balloon was removed from the branch and the leaves were examined. Of two of these leaves the extreme part of the top had turned yellow. A microscopical examination of these leaf-tips showed, however, that here was no initial stage of bud-formation. Hypertrophical cells, such as we ought to have found in this case in the mesophyl, were not present. The yellow colour was caused by the dying of the tissue, the cell-contents then discolouring.

On May 10 the top of the plant was liberated. A number of full-grown leaves which at the beginning of the experiment were still young and young leaves at lateral sprouts which during the isolation had been formed by sprouting of the axillary buds, were now seen. All these leaves were perfectly normal, healthy and strong with a normal green colour; on no one of them anything could be detected of yellow vesicles or spots, of no one the top showed any discoloration or thickening. The isolation by means of the muslin balloon had not hindered these leaves at all in their normal development. Only scale-insects and other animals had been prevented from settling on the leaves with the formerly described result.

Similar tumours as the yellow, thickened leaf-tips of *Gnetum Gnemon* really are, have also been repeatedly observed with other plants and described under the name of „yellow specks" (Gelbfleckigkeit), oedemata or intumescences. The word „Intumescentia" was introduced into phytopathological nomenclature by Sorauer with the definition [1]: „Intumescentia" sind „diejenigen Erscheinungen, die das gemeinsame Merkmal haben, als kleine knötchenförmige oder drüsige Auftreibungen der Blätter aufzutreten, die meist an diesen Stellen gelb verfärbt erscheinen und eine aussergewöhnliche Zellstreckung ohne wesentliche Zellvermehrung zeigen". That the thickened leaf-tips of *Gnetum Gnemon* are not indeed „kleine Auftreibungen" and do present „wesentliche Zellvermehrung" need not necessarily prevent us from counting them among the intumescences, since as well very large [2] or mutually coalescent [3] as typically hyperplastic [4] intumescences have been described for other plants.

We cannot now deal with the very divergent views of different investigators about the cause of the formation of intumescences; we will only mention that in most cases

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1) P. Sorauer, Die symptomatische Bedeutung der Intumescenzen (Bot. Zeitg. 48 Jahrg. 1890. pag. 241).

2) H. v. Schrenk, Intumescences formed as a result of chemical stimulation (Missouri botan. garden. 16th ann. report. 1905. pag. 125).

3) Miss. G. E. Douglas, The formation of intumescences in potatoplants. (Bot. Gazette Vol. XLIII. 1907. pag. 233).

4) E. Küster, Ueber experimentell erzeugte Intumescenzen. (Ber. deutsch, bot. Ges. Bd. XXI. 1903. pag. 452). P. Sorauer, Ueber Gelbfleckigkeit. (Forsch. a. d. Geb. d. Agrik. Phys. h. v. Dr. E. Wollny. Bd. IX. 1886. pag. 387) and Intumescenz bei Solanum floribundum. (Zeitschr. f. Pflanzenkrankh. Bd. VII. 1897. pag. 122).

it has been stated that a high temperature and great humidity of the air are essential factors.

Experiments enabled me to form an idea about the character of the stimulus exerted by the scale-insects on the tissue of the leaf of *Gnetum Gnemon*, on which the formation of the intumescences is the reaction. After I had tried artificially to produce intumescences in leaves by mechanically wounding them in all sorts of ways and treating them with poisons, without obtaining the desired result, I arrived at the conclusion that either my method of wounding, compared with that of the scale-insects, was too coarse or that the insect injected some stimulating substance into the leaf. In order to settle these points the following experiments were carried out: 1). In the leaf-tips extremely small wounds were made by means of the sterilised, very fine point of an injection syringe. 2). The same was done after the point had first been stuck into yellow vesicles, caused by the scale-insects. 3). A number of yellow and thickened leaf-tips were ground in a mortar and a very small portion of the so obtained porridge, mixed with some diluted glycerin, injected in several places in leaf-tips. 4). The same operation as in 3 was applied after the porridge had first been heated to 100° C.

The result was exactly the same in *all* cases.

After some ten days small, brown specks were visible in the wounded places, which afterwards could still increase somewhat in size. A month after the wounding the brown specks had become surrounded by a very thin, more or less transparent, yellow margin. The brown specks were formed by the cells which had died in consequence of the wounding and the walls of which had turned brown. In the yellow margin a complex was found of relatively small cells, leaving no intercellular cavities. These cells had thick walls and their protoplast still contained remnants of the chlorophyl grains. The complex was formed by hyperplasia of the whole mesophyl. On the border between this complex and the normal tissue some cells of the spongy parenchyma had become greatly enlarged, their chloroplasts having become disorganised. After another month it was noticed that the leaf-tips in the neighbourhood of the wounded spots assumed a somewhat yellow colour, which gradually became more and more distinct. Microscopically it could be stated that where externally this yellow discoloration was vesible, the tissue round the wounded spots had undergone precisely the same changes as take place round the yellow visicles, caused by the scale-insects, namely a general hypertrophy of the cells of the spongy parenchyma, while here and there even a partition wall had already been formed in the enlarged cells.

As was stated above, this result was obtained in all cases, also in those in which small wounds had been made without anything else. From which we may conclude that the leaf of *Gnetum Gnemon* may be stimulated to the formation of intumescences and hence of adventitious buds by wounding, provided this is very light and that consequently the process must be regarded as a reaction on a *wound stimulus.*

In a disease of carnations which also consists in the formation of a sort of intumescences [1]) and for which it has been shown by Woods [2]) that it is caused by the sting of aphides, among others, Woods thinks the growth of the yellow, thickened spots must be ascribed to the diffusion of an irritant, injected by the insect [3]).

That a similar hypothesis is superfluous for the „stigmonose" of *Gnetum Gnemon*, appears clearly enough from

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1) H. v. Schrenk. l. c. pag. 39.

2) A. F. Woods, Stigmonose: a disease of carnations and other pinks. (Bull. no. 19. U. S. Dept. Agr. Div. Veg. Phys. and Path. 1900).

3) l. c. pag. 24.

our experiments. The specific point about the wound, made by the suction organ of the scale-insect is only that it is so trifling. Only a few cells, namely those which are distinguished in the yellow vesicles by brown walls, have undergone the direct consequences of it, while the whole subsequent formation of the intumescences takes place as a reaction on the stimulating action, proceeding from these few wounded cells.

Küster [1]) calls all cataplasms after vulneration, as far as they have a parenchymatical character, callus. According to this terminology also the tissue of which the thickened leaf-tips of *Gnetum Gnemon* consist, is a „callus" and the buds, formed in them, are callus-buds [2]).

Why the two plants from the cooler hothouse did not form callus or buds on their leaves, is now also clear. The Aspidiotus, playing such an important part in this formation of callus, is also found in the cooler hothouse; but for callus-formation the chief condition is humidity. This condition was only to some extent fulfilled by the cooler, but completely by the hotter house, while also the higher temperature in this latter could not but favour the formation of callus with these tropical plants.

Why only the apical part of a leaf is capable of forming callus and buds, may be explained in the following manner. The small wound causes an afflux of nutrient matter in an apical direction. If now an accumulation of this matter, which is necessary for the hyperplastic formation of callus, shall be possible, the afflux must not be able to pass by, i.e. it must be stopped apically of the wound.

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1) E. Küster, Pathologische Pflanzenanatomie. Jena. 1903 pag. 154.

2) See also: E. Küster, Histologische und experimentelle Untersuchungen über Intumescenzen. (Flora oder allgem. bot. Zeitg. 96 Bd. 1906, pag. 527—537).

And this condition is normally only fulfilled in the tip of the leaf, in another part of the blade only when the organic relation with the tip has been disturbed.

Botanical laboratory at Utrecht.

EXPLANATION OF PLATES II, III AND IV,

Illustrating J. van Beusekom's paper: „On the influence of wound stimuli on the formation of adventitious buds in the leaves of Gnetum Gnemon L."

Pl. II, fig. 12 has been drawn under an Abbe's camera lucida, using Leitz' Obj. 6, Oc. III. In the same manner camera drawings were made for the figs. 8, 9, 10, 11 of Pl. II and 13, 14, 15, 16*a* of Pl. III; these drawings were photographied half size, copied from the glass plate on drawing paper with the aid of a retouching apparatus and afterwards accomplished. Figs. 16, 17 (Pl. III) and 18 (Pl. IV) were drawn with Leitz' Oc. I and Obj. 6 after the front-lens of the latter had been unscrewed, the other figs. which are not natural-sized with the hand-magnifier.

PLATE II.

Fig. 1 (magnified 4 ×). Knob with entirely suberized surface, that has opened lately; at the apex of the adventitious sprout the first pair of leaflets is seen developing.

Fig. 2 (natural size). Adventitious sprout with 1 pair of scale-shaped and 3 pairs of ordinary leaflets.

Fig. 3 (natural size). Ramified adventitious sprout.

Fig. 4 (natural size). Apical half of a leaf, the yellow and thickened tip of which is showing numerous small special thickenings and one big knob.

Fig. 5 (magnified 4 ×). Leaf-tip with some ten knobs in different stages of development; from *a* and *a*[1] a young sprout has come to light.

Fig. 6 (natural size). Photograph of the apical half of a leaf, on whose tip the biggest sprout, I have observed, can be seen. (The leaflets on the first node have been dropped).

Fig. 7 (natural size). Young specimen of a sucker. Great number of such suckers appeared in spring on the curved upper branches and the stem of the two plants from the cooler hothouse. These suckers drew the attention, because the leaflets on their first node remain scale-shaped just as they do with the adventitious sprouts.

Fig. 8 (magnified 200 ×). Section of a normal leaf-tip; *ep.* epiderm; *st.* stoma; *p.* palissade parenchyma; *subp.* subpalissade cell; *skl.* sclerenchyma fibres; *sch.* spongy parenchyma; *b.b.* vascular bundle.

Fig. 9 (magnified 200 ×). Section of a small yellow vesicle; in the centre of the drawing hypertrophical spongy parenchyma cells; surrounding the latter brown-walled cells (*x*); under the biggest hypertrophical cells some compressed cells that have died.

Fig. 10 (magnified 200 ×). Section of a big yellow vesicle; numerous cells of the spongy parenchyma and subpalissade cells are hypertrophical; to the right cells with partition walls; in the middle of the upper part of the section also enlarged palissade cells. The darker drawn walls in the spongy parenchyma are suberized.

Fig. 11 (magnified 200 ×). Section of a yellow leaf-tip that was not yet perceptibly thickened. Hypertrophy of the spongy parenchyma and of the subpalissade cells.

Fig. 12 (magnified 400 ×). Drawing of a hand-cut preparation of a yellow, thickened leaf-tip; destruction of the chloroplasts; the destruction has gone furthest in the cells of the spongy parenchyma, least far in the palissade cells.

PLATE III.

Fig. 13. (magnified 200 ×). Section of a yellow, greatly thickened leaf-tip; the intercellular cavities of the spongy parenchyma have disappeared almost entirely; the subpalissade cells have stretched themselves very much in a direction perpendicular to the surface of the leaf and are partitioned by 1 or 3 cross-walls. The palissade cells have but slightly enlarged.

Fig. 14 (magnified 200 ×). Part of a section of a knob, containing a primordium of a meristem (*m.a.*); the primordium is surrounded by compressed cells (*pr.c.*).

Fig. 15 (magnified 200 ×). Part of a knob, showing a young meristem (*m.*); above the meristem can be seen something of tracheid-formation; in the sections, preceding the one drawn in this figure, tracheids find themselves also at *.

Fig. 16 (magnified 45 ×). Two meristems (*m. 1.* and *m. 2.*) within one knob; *b.b.* vascular bundle; ep^1 dead portion of the epiderm; *k* suberized cells.

Fig. 16^a (magnified 200 ×). High power drawing of the part of the same section which in the foregoing figure is surrounded by a spotted line; *ep.* unchanged epiderm; ep^1. epidermal cells that have died; *k* suberized cells; *s* slit-shaped space brought about by cells, forming the transition between the meristem and the unchanged tissue of the knob, being dissolved.

Fig. 17 (magnified 45 ×). Left half of a section through a knob, containing two meristems; a complete procambial connection (*proc.*) has been established between the meristem (*m*), the upper side of which is lying free within a slit-shaped space (*s*) and the vascular bundle (*b.b*) of the mother-leaf.

Fig. 19 (magnified 12 ×). Section of a very big knob containing 2 adventitious buds (m_1 and m_2) that have developed allready considerably. In the axil of one of the two primary leaflets of the bud m_1 an axillary bud (*ax*) is visible.

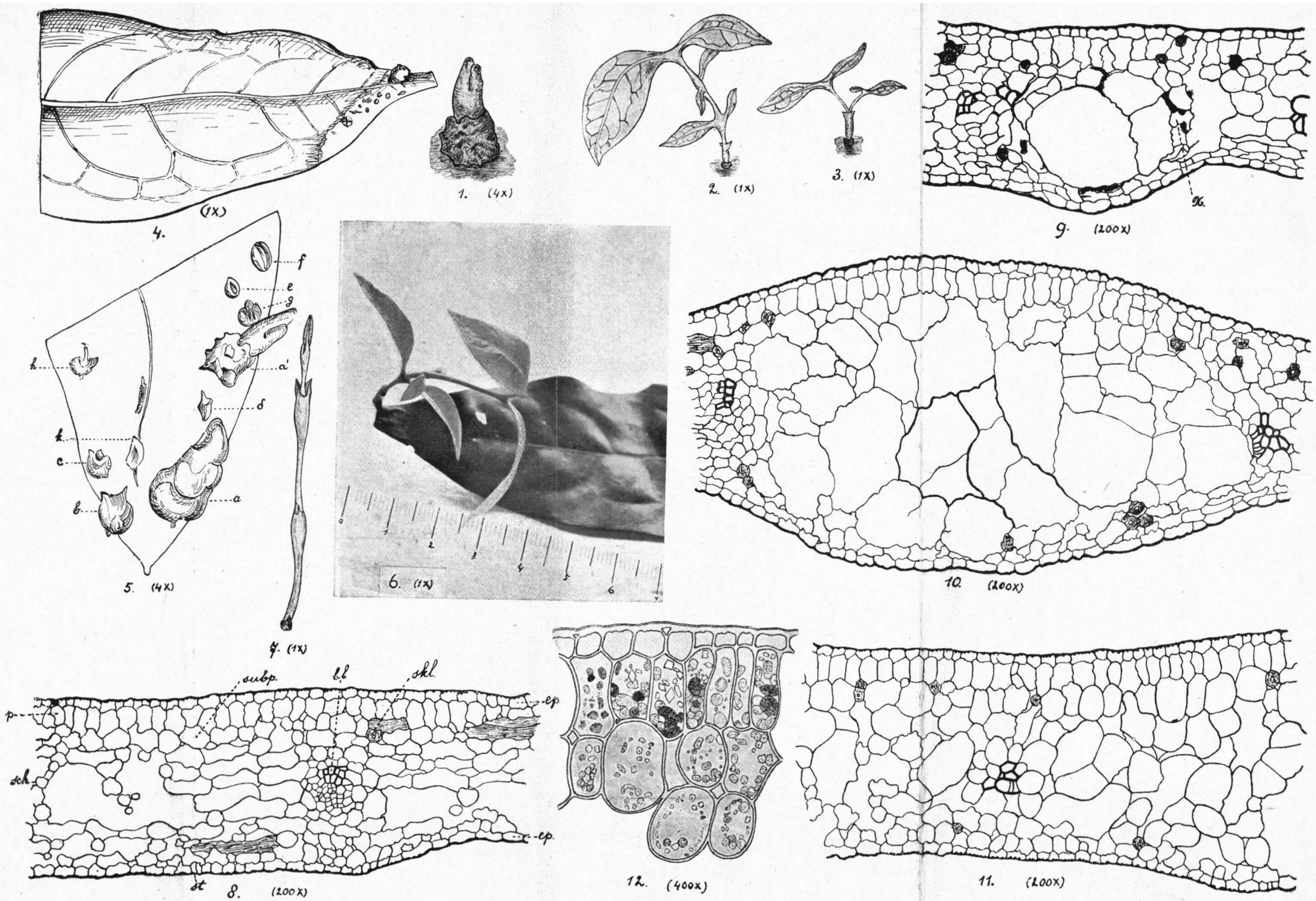
Fig. 20 (magnified 12 ×). Transverse section through a knob and the sprout, that has arisen lately from it. Within the knobs tissue between the vascular bundles of the leaf (*b.b.*) and the bundles of the sprout (*g*) some small groups of tracheids are present, but there is nothing that can be compared with the complete procambial connection of fig. 17; *ax.* axillary bud.

PLATE IV.

Fig. 18 (magnified 45 ×). Low power view of a preparation showing a bud *m* within a slit-shaped space (*s*); *pr.c.* rows of compressed cells; *b.b.* vascular bundle.

Fig. 21 (magnified 12 ×). Transverse section through the apex of the sprout that has been figured in fig. 6; *gl.* glandular tissue on the swelling (*p*) at the base of the petiole; *v.* vegetative cone; *ax.* axillary bud; *g.* vascular bundles.

Fig. 22 (magnified 12 ×). Diagrammatic drawing of the vascular connection between an adventitious sprout and its mother-leaf, reconstructed from a series of ± 130 sections through the base of the sprout figured in fig. 6. The vascular bundles α, α^1, β and β^1 (β and β^1 lying out of the plane of drawing) unite with the group of vessels and tracheids γ, which are connected with the vascular system of the mother-leaf.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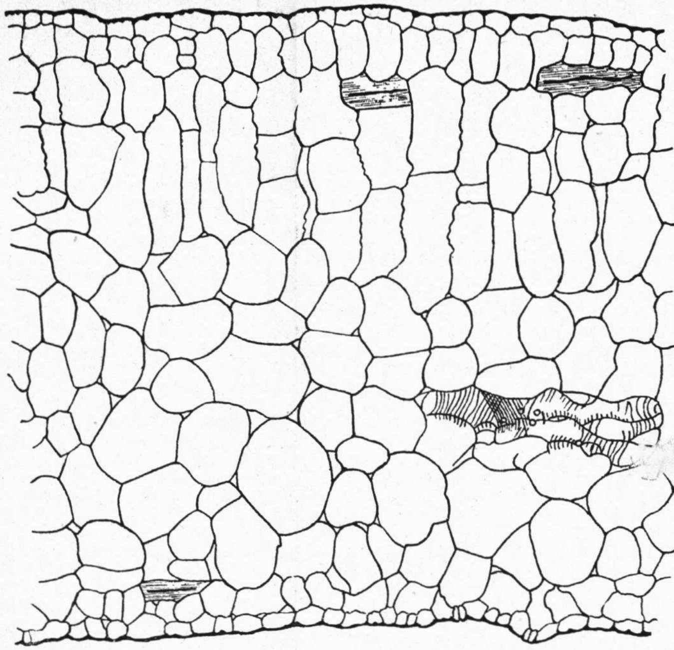

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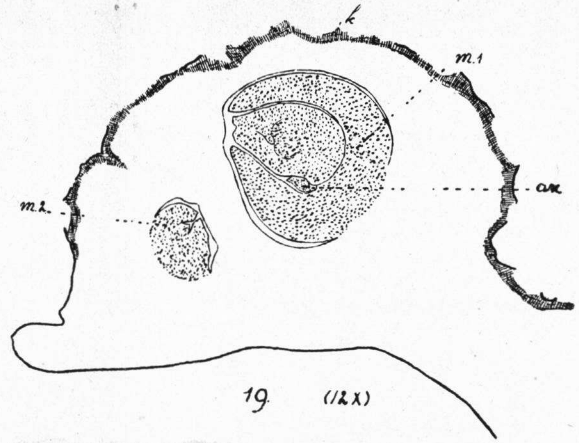

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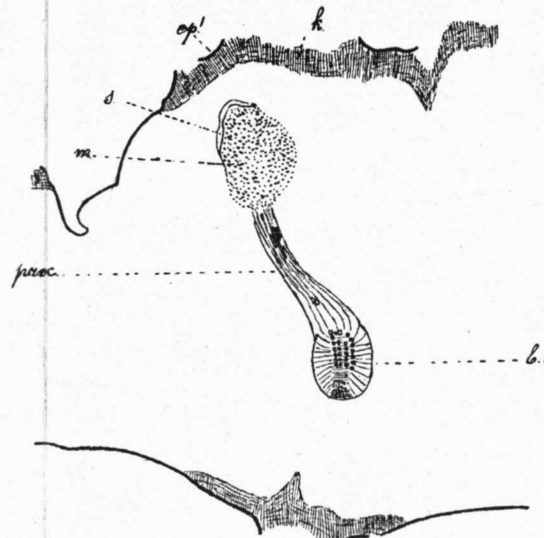

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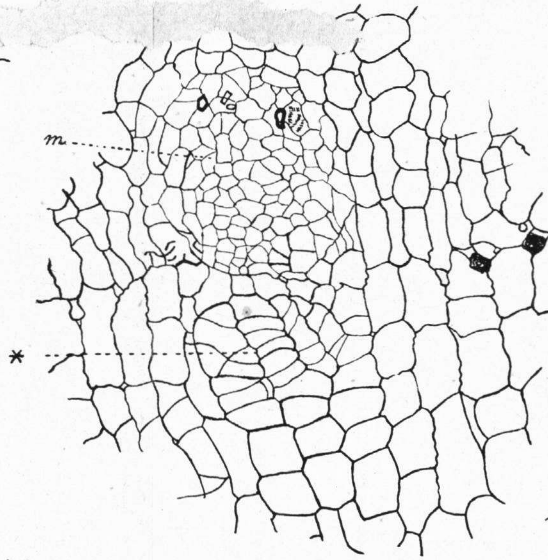

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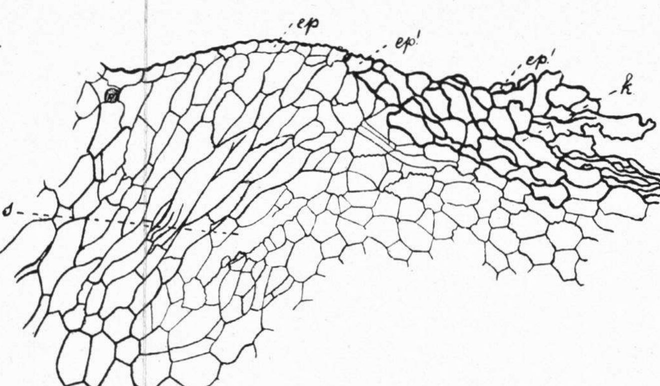

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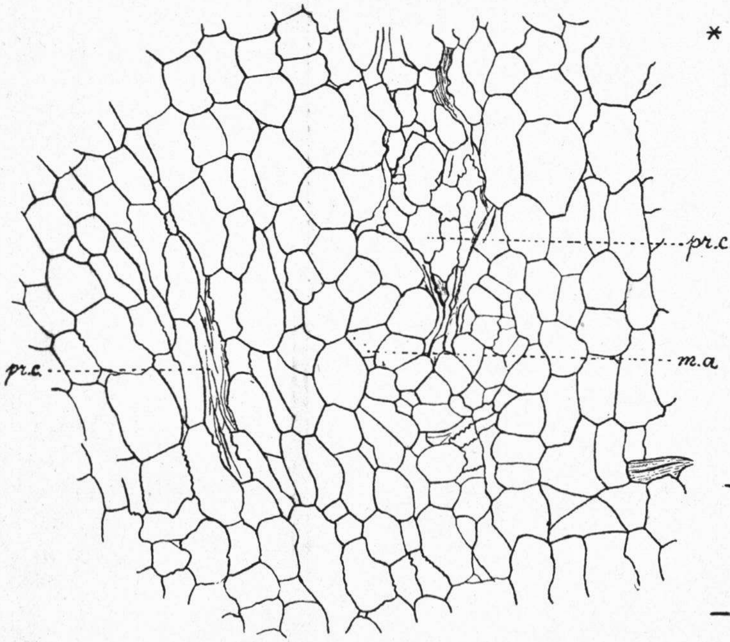

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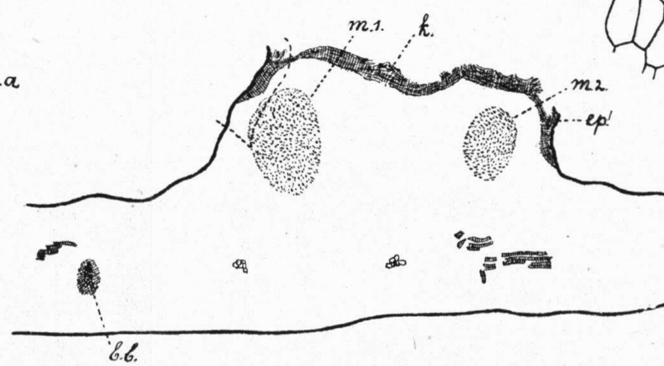

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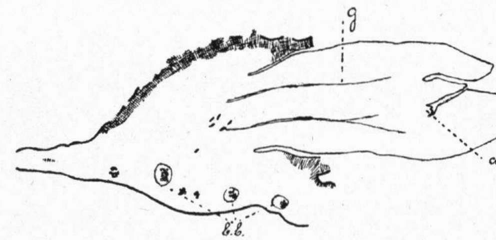

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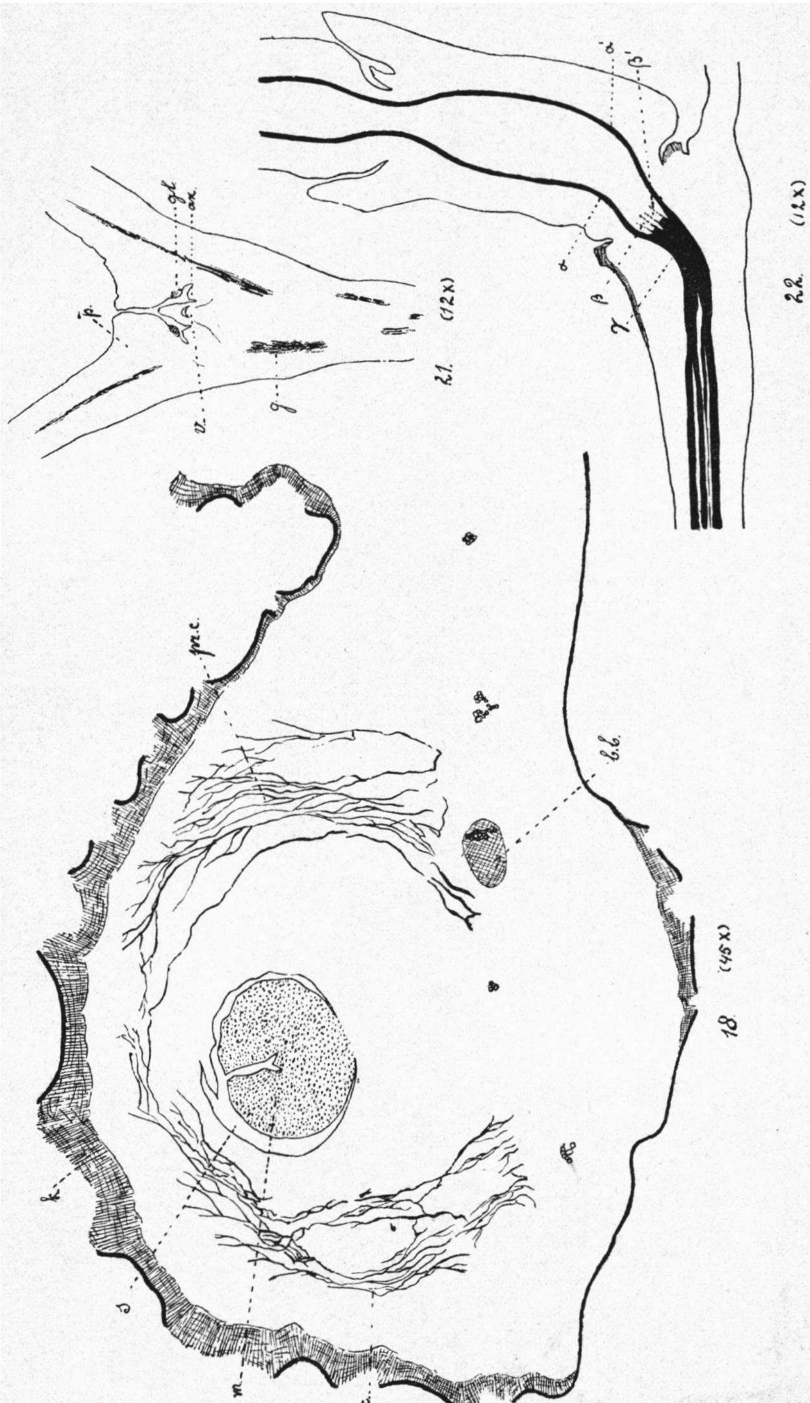
21. (12 X)
22. (12 X)
18. (45 X)
pr.c.
b.b.
k
m