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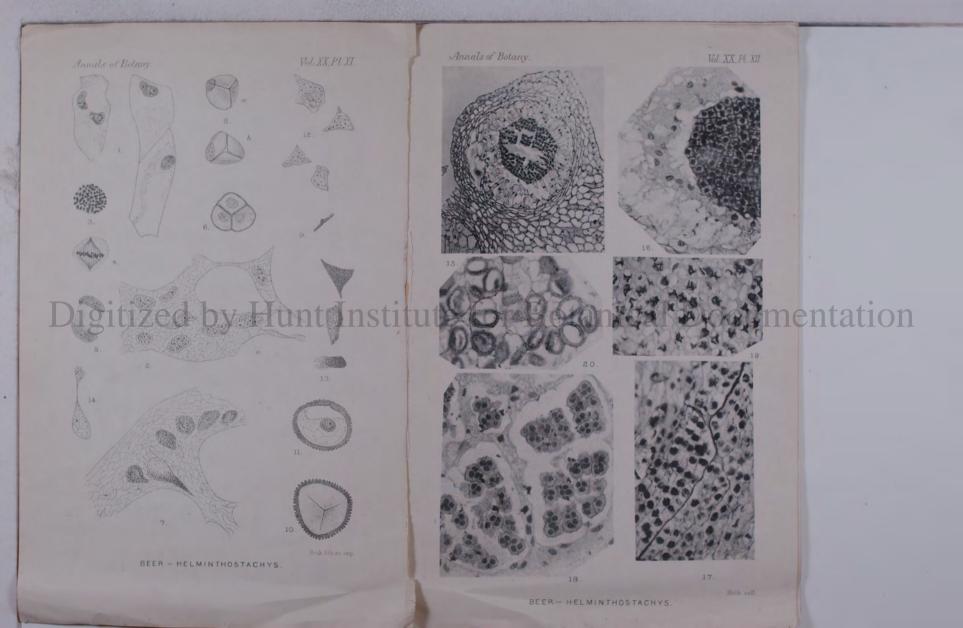
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## On the Development of the Spores of Helminthostachys zeylanica.

#### RUDOLF BEER, B.Sc.

#### With Plates XI and XII.

The writings of Goebel<sup>1</sup>, Holtzman<sup>2</sup>, Campbell<sup>3</sup>, and Bower<sup>4</sup> have made us familiar with the early history of the sporangium of the Ophioglossaceae. The actual structure of the spore, however, and the later stages of development, leading up to the establishment of that structure, have, hitherto, only been very imperfectly described for any member of this Order. Recently the publication of two papers, by Cardiff<sup>3</sup> and Stevens<sup>6</sup> respectively, have added considerably to our knowledge of certain stages, in the history of the spores of *Potrpetium*. Through the kindness of Prof. F. W. Oliver I have had the opport

imentation

Through the kindness of Prof. F. W. Oliver I have had the opportunity of examining a number of fertile spikes of *Helminthostachys zeylanica* which had been preserved in spirit.

Although many important cytological details can only be determined with certainty by the study of material which has been specially fixed for the purpose, it was thought that the following notes might not be without their interest.

The first stages in the development of the sporangium of *Helmintho-stachys zeylanica* have been followed by Bower, who found the sporangium to be derived from a single superficial cell.

The first periclinal division of the cell defines the sporogenous from the protective portions of the sporangium; 'the sporogenous mass

<sup>1</sup> Goebel, 'Belträge zur vergleichenden Entwickelungsgeschichte der Sporangien,' Bot. Zeit. 1880, Bd. xxxviii.

<sup>2</sup> Holizman, 'On the apical growth of the stem and the development of the Sporangium of Bolry-hium Virginianum,' Bot. Gazette, 1892, vol. xvii.

· Campbell, ' Mosses and Ferns,' 1895.

\* Bower, ' Studies in the Morphology of Spore-producing members,' II, 1896.

<sup>b</sup> Cardiff, 'Development of Sporangium in Bolrychium,' Bot. Gazette, May 1905, vol. xxxix.

<sup>6</sup> Stevens, 'Spore Formation in *Botrychium Virginianum*,' Annals of Botany, October 1905,

vol. xix,

[Annals of Botany, Vol. XX. No. LXXVIII. April, 1906.]

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increases rapidly in bulk, whilst the cells surrounding the sporogenous mass, to the extent of several layers, assume the character of a tapetum which gradually becomes disorganised; finally the sporogenous cells separate and the majority of them divide into tetrads; but a considerable portion of them, scattered throughout the sporogenous mass, become disorganised without undergoing division; this is similar to what has been observed in the case of *Ophioglossum*<sup>1</sup>.

My own observations begin with a stage at which the sporogenous cells have reached their full number, but in which the tapetum has not yet become disorganized (Pl. XII, Fig. 15). The tapetal layer is composed of radially elongated cells many of which have divided once or, more rarely, twice by perielinal divisions (Pl. XI, Fig. 1, and Pl. XII, Fig. 15). In *Botrychium* Cardiff observed periclinal divisions to follow one another until the tapetum became four or five cells in thickness. Stevens, however, does not confirm this statement and finds the tapetal layer of *B. virginianum* to be, as a rule, two cells in thickness, which would be more nearly in accordance with what I have seen in *Helminthostachys*.

The membranes of the tapetal cells are delicate, but clearly give the reactions of both cellulose and pectose. In this feature the tapetum of *Helminthostachys* apparently differs from that of *Botrychium*, in which Stevens found each tapetal cell to be deliminated by a plasmatic membrane mergly. Although Cardiff does not actually describe the cell-walls of the tapetum, his words certainly imply the occurrence of a cell-wall other than a plasmatic membrane.

The sporogenous cells which lie within the tapetal layer are mostly square or oblong cells which measure about  $12-18 \ \mu$  across (Figs. 16 and 17). Their walls give the characteristic pectic reactions, but neither cellulose nor callose is to be found in them. The cytoplasm of these cells is fairly dense and contains a number of plastids usually crowded with starch. In slightly older sporangia the tapetal walls begin to break down, and in these disorganizing membranes I no longer succeeded in obtaining the cellulose reactions, although pectose was still clearly demonstrable<sup>2</sup>.

The cytoplasm derived from the disorganized cells flows together (Figs. 2 and 16) and increases in amount, whilst the nuclei, which are mostly gathered together in little nests or groups at the inner or outer periphery of the tapetal layer, have become more numerous. The groups of nuclei are no doubt partly formed by the approximation of nuclei from several disorganized cells, but there can be little doubt that they also represent, to some extent, a number of daughter-nuclei which have origin-

<sup>1</sup> Spore-producing members, Part II, 1896, p. 35.

<sup>2</sup> I state this fact for what it is worth, although the failure of the cellulose reactions may be due to the increased difficulty of demonstration rather than to the real absence of this substance.

ated from the amitotic division of one or more mother-nuclei. The occurrence of constricted nuclei, such as I have represented in Fig. 2, bears out this view. Neither here, nor at any subsequent stage, have I seen tapetal nuclei dividing by mitosis.

The tapetal cytoplasm at this stage contains a quantity of starch and, in iron-haematoxylin-bismarck-brown preparations, presents a finely vacuolated appearance.

Up to the present the sporogenous cells have been united together in one coherent mass, but now they show the first signs of separating from one another (Fig. 17). The separation takes place in 'blocks' quite comparable with those described by Cardiff in *Botrychium*<sup>1</sup>. In *Helminthostachys*, as in *Botrychium*, the separation appears to take place approximately 'in the order in which the original walls were laid down in the archesporium and young sporogenous mass.' The nuclei of the sporogenous cells of *Helminthostachys* are still in the resting condition when the separation of the 'blocks' commences.

Delicate and undifferentiated as the walls of the sporogenous cells appear, they must nevertheless possess a greater complexity of structure than becomes apparent with our instruments, for during the process of the separation of the sporogenous cells from one another a middle lamella becomes mucilaginous and is finally dissolved, whilst a secondary thickening layer remains unatcred round each sporogenous cell. The entire sporogenous membrane, both before and after the mucilaginous alteration of the middle lamella, gives no other reactions than those of pectic bodies. If I understand Stevens correctly, he found the mother-cells of *Botrychium* to be demarcated from one another and from the tapetum by a delicate plasmatic membrane alone, but Cardiff's statements do not bear this out and it certainly does not apply to the mother-cells of *Helminthestachys*. The sporogenous cells now divide into the spore-tetrads.

In the first division of the spore-mother-cells the chromosomes (which presumably occur in the reduced number) are seen to be small in size and rather crowded upon the spindle (Fig. 3). I have not succeeded in accurately counting their number, but I should very roughly estimate this to be between forty and sixty. Stevens has not recorded the number of chromosomes which occur in *Botrychium*. The spindle was surprisingly well preserved in the alcohol material and could be seen to terminate in very sharp, almost acuminate apices at which no traces of centrosomes were ever observed. The spindle extends to the very periphery of the mother-cell and is free from any radiations at its poles (Fig. 4). The daughter-nuclei, resulting from the first division, have in some of my preparations a curiously flattened, almost hemispherical shape when viewed in profile<sup>2</sup> (Fig. 5). They appear, like those of *Botrychium*, to enter into a resting

<sup>1</sup> Cardiff, I. c., p. 342. <sup>2</sup> Compare this with Stevens' Plate XXIX, Fig. 28.

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stage before passing on to the second division. The spore-mother-cells do not divide simultaneously, but all stages of division can be found in one sporangium. A mother-cell with its nucleus in the prophases of the first division not uncommonly occurs side by side with one which has completed its second division and has already developed the tetrad- (specialmother-cell) walls. In *Botrychium* Cardiff found that the cells in the same 'block' are always in the same stage, but in *Helminthostachys* the separation of the mother-cells has, apparently, been more complete before they enter upon the maiotic divisions, so that they have reached greater independence from one another than is the case in *Botrychium*.

Bower found that a considerable proportion of sporogenous cells became disorganized without undergoing division. In *Botrychium* Cardiff found no such disorganization to occur, and my preparations of *Helminthostachyss* are quite in accordance with his observations and show no abortion of mother-cells. When the two divisions of the mother-cell have been completed the four cells of the tetrad are enveloped by the tetrad-wall (special-mother-cell wall) (Fig. 18).

This membrane is very delicate and in this respect forms a striking contrast to the conspicuous tetrad-walls of nearly all the other plants that I have examined (e.g. *Riccia, Anthoceros,* Lycopodiaceae, *Osmunda* &c.). It gives the pectic reactions very clearly, whilst several times I obtained a rather faint workt pink reaction with calcium chloride-iodine solution. For some unexplained reason the calcium chloride-iodine solution does not always produce the same result; the tetrad-wall in some cases colouring violet-pink, in others yellow.

While we may, therefore, conclude with certainty that this wall contains pectose, we must leave it undecided whether or not this substance is accompanied by cellulose<sup>1</sup>. The young spore-membrane colours, although rather faintly, with bismarck-brown, methylene blue, and similar dyes, and appears to be more or less cuticularized from a very early stage. It is a new formation and is not derived from the transformation of the special mother-cell wall, which can still be recognized outside the spore-membrane (Fig. 6). In the meanwhile the disorganization of the tapetal walls has become complete, whilst the tapetal cytoplasm, which now forms a sort of plasmodium around the sporogenous cells (Fig. 16), has increased considerably in amount. The numerous nuclei which it contains still tend to be aggregated in groups.

We now find the plasmodial envelope sending finger-like processes into the cavity of the sporangium between the separated sporogenous cells, These cytoplasmic processes spread further and further between the sporogenous cells, branching and anastomosing with one another and separating

<sup>3</sup> No other cellulose reaction, but only the inconstant appearance of the violet-pink colour with calcium-chloride-iodine, was given by these walls.

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the young tetrads more and more completely from one another (Fig. 18). At first these plasmodial strands consist solely of cytoplasm, unaccompanied by nuclei, which remain in the peripheral envelope. Soon, however, we can observe the nuclei dipping into the broader arms of cytoplasm and making their way into the interior of the sporangial cavity. As the round or oval nucleus passes into the strand of cytoplasm it usually alters its shape and becomes drawn-out, often into a very fine point, at its anterior extremity (Fig. 7). It usually stains more deeply at this beak-like process than it does at its blunt, posterior end. As it moves further along the strand and passes between the spore-tetrads it not infrequently assumes an elongated form, sometimes almost justifying the term vermiform, until it temporarily comes to rest at this or that spot, when it reassumes a more iso-diametric shape. The tapetal plasma, which has a finely vacuolated structure in successful preparations, contains an abundant supply of starch. All the cells composing the walls of the sporangium are rich in starch; the membranes of these cells give the pectic and cellulose reactions. The spores remain associated together in groups of four throughout the greater part of their development. They now rapidly increase in size, but their walls remain very thin and the cytoplasm they enclose is scanty. In consequence of this the spores, at this stage, are nearly all collapsed in the alcohol material (Fig. 19). The young spores are tetrahedral in shape and the edges of those surfaces which adjoin one another in the neighbouring mentation spores of a tetrad are raised up into ridges. In this way each spore develops three ridges upon its surface, and these ridges converge to a common point (Fig. 8, a).

Early in the history of the spore one can see that the sides of the tetrahedron which meet at the ridges are only very loosely joined together. The action of various reagents, and even the pressure of the microtome knife, often leaves the spore with three flaps entirely separated from one another at the ridges, as is shown in Fig. 8,  $\delta$ . The cuticularized wall of the spore now grows somewhat in thickness, and at about this time a new layer—the endospore—makes its appearance within it. This layer, which gives the reactions of a pectic body, can be traced as a continuous, although delicate, layer over the entire inner surface of the spore-wall.

In microtome sections which have passed through the apex<sup>1</sup> of the spore we can trace a little process or fold of the endospore which has pushed between the ridges of the exospore and which reaches to the outer surface (Fig. 9). In spores measuring about  $23 \mu$  across their longest diameter the exospore has become sufficiently thick to prevent the collapse of the spore in alcohol. The surface of the spore is still smooth and its protoplast poor in substance<sup>2</sup>.

<sup>1</sup> Viz., the point at which the three ridges meet. <sup>2</sup> It usually contains a very little starch, however,

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The further growth in thickness of the exospore continues to leave the fold of endospore uncovered at the apex of the spore. The smooth surface of the exospore now becomes slightly wavy in outline, and this soon leads to the formation of the reticular sculpture which is characteristic of the mature spore.

The fully grown spore measures, in section, about  $33 \mu$  by  $28 \mu$ . The exospore is thicker at the apex than over the rest of the spore, but is perforated by a cleft through which the fold of endospore reaches the exterior. This cleft is particularly well seen after treatment with a mixture of sulphuric and chromic acids or with sulphuric acid alone, but it can be quite readily distinguished in sections which have been stained with methylene blue, or bismarck-brown without the previous action of acid. Chlor.-zinc-iodine solution, to which more iodine than usual has been added, also very clearly shows the endospore—coloured brown—penetrating the excespore and reaching the exterior. The reticular sculpturing upon the surface of the exospore appears in section as a series of blunt processes with flattened summits (Figs. 10 and 11). The protoplast of the spore has now become somewhat richer in substance and usually includes starch, but not in any quantity.

Nothing in the nature of an epispore is to be found in the spores of *Helminthostathys*. The exispore and, most probably, also the endospore are in the first place deposited by the scoreory activity of the spore-protoplast. There is no possibility of the former layer being derived from the transformation of part or all of the mother-cell-wall, since this at all times very delicate wall can still be distinguished, in apparently undiminished thickness, over the surface of the young exospore (Fig. 6).

The fact that the exospore and endospore are inseparably united together at first suggests the probability of the two layers being derived from the differentiation of an originally single, homogeneous membrane. As Fitting<sup>1</sup> has already pointed out, however, the existence of a close fusion between two layers of a wall does not necessarily indicate their common origin by differentiation, and there are several well-known cases in which two lamellae are intimately bound together, but which unquestionably have been separately deposited by the cell-protoplast. In the present instance, moreover, it is difficult to understand how a cuticularized membrane can be so differentiated as to give rise to a pure pectic lamella on one of its faces. The development of the little fold of endospore at the apex of the spore is also more readily explained as a new formation than as a product of differentiation. Shortly after the exospore has been formed round the young spore and throughout the time that this layer is growing in thickness and in surface the tapetal plasmodium which envelops the spore-tetrads shows unmistakable signs of metabolic activity.

1 11. Fitting, Bot. Zeitung, Bd. Iviii, 1900, p. 126.

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It is impossible to escape the conclusion that this metabolism is concerned with the elaboration of material for the growth of the exospore. That such material must be forthcoming is of course obvious, and the two sources to which one naturally looks for this supply are the spore-protoplast on the one hand and the tapetum upon the other. In Helminthostachys (at least in my spirit material) the spore protoplast, after the first formation of the exospore, is very poor in substance, and it is very unlikely that it can furnish sufficient plastic material for the growth of the wall. When one turns to the tapetum, however, one finds here a gradual utilization of reserve-materials which can fully account for the substance which is being employed in the growth of the exospore. Moreover, if we deny that the tapetal material is being utilized for this purpose it is not easy to see what the fate of this substance may be. The growth of the wall of the sporangium is amply provided for by the reserve-materials contained in its cells, whilst the protoplasts of the spores do not increase in size or substance during this time, so that we are driven to associate the disappearance of starch and cytoplasm from the tapetum with the only other demonstrable utilization of material, viz., that which is adding to the size and thickness of the spore-coats.

It is impossible at present, however, to decide whether the material elaborated in the tapetum is directly built up in the spore-walls or whether it first passes, in a liquid form into the spore-protoplast which, mentation either with or without further elaboration, distributes it to the walls.

In view of the important part that the tapetal plasmodium plays in the growth of the spore-walls it will be necessary at this point to look more closely at its appearance and behaviour. We find that starch is abundant in the tapetal cytoplasm before the walls of the tapetum disorganize and that it continues to exist there in considerable quantities until the period during which the exospore undergoes its most active growth in thickness. During this time the tapetal plasma is either very poor in starch or, more often, this substance is quite absent from it. When the exospore has completed its growth in thickness and the spore-protoplast is about to add to its substance, starch can usually again be seen in the tapetal cytoplasm but never in quantities that can compare with those which occurred in the early stages of sporangial development.

The tapetal cytoplasm at first increases somewhat in amount (immediately after the disorganization of the tapetal cells), but during the later history of development it slowly but steadily decreases in quantity (compare Figs. 18, 19, and 20). Where it abuts upon a vacuole (such as that which encloses a spore or spore-tetrad) the cytoplast has a very clearly marked plasmatic membrane; elsewhere it is finely vacuolated in structure. It is the vacuolar cytoplasm which is gradually utilized in the metabolic processes of the plasmodium, and the plasmatic membranes,

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which limit neighbouring vacuoles in consequence approach one another more and more closely. In sporangia, shortly before their dehiscence, the vacuolar cytoplasm has almost completely disappeared, but the plasmatic membranes are still present between the spores (Fig. 20). These can then, in nearly all cases, be seen to be double in structure owing to the complete approximation of the plasmatic membrane of adjoining vacuoles<sup>1</sup>. Starch grains can not infrequently be seen flattened out between the two adpressed membranes and, at the angles formed by the meeting of several vacuoles, nuclei occur.

The nuclei show, at all stages, considerable variation in size, in form, and in character. In *Botrychium* Cardiff found that the nuclei underwent a very considerable increase in size during development; thus in young sporangia he gives their size as  $8\mu$ , whilst at a later stage he found them to measure  $15-20\mu$ . In *Helminthostachys* the general average of size of the tapetal nuclei appears to remain about the same at different periods of development.

(a) Tapetal cells still intact		=	IJXIIµ
(b) Young spores with thin, smooth exospore .		=	13.4×10µ
(c) Spores with immature sculpturing on exospore		=	12.6×9.6 µ
(d) Spores just before dehiscence of sporangium	•	=	13×11µ.

The above figures are the averages calculated from a large number of countings and they show practically no alteration in the size of the tapetal nuclei.

The form of the nuclei is very various. In the tapetal cells before disorganization and in the tapetal plasmodium before this flows between the sporogenous cells the nuclei are more or less oval in shape. The peculiar alteration in form which they often undergo as they pass within the sporangial cavity has already been described. In the later periods of development these nuclei are sometimes oval or spherical in outline; often they are irregular in shape and occasionally their form is angular (Fig. 12). They possess, in most cases, a distinct nuclear membrane and are usually rich in chromatin; several nucleoil can generally be seen in each nucleus. The chromatic granules are often closely packed (and the nucleus in consequence deep-staining) in the smaller nuclei, whilst in the larger nuclei the chromatin-grains are frequently separated more widely from one another and the nucleus contains more non-staining material.

In *Botrychium* Cardiff found that 'as the spores commence to separate in the tetrad, the tapetal cytoplasm has entirely filled the sporangium and many of the nuclei have begun to disorganize, though they seem unusually persistent and many are found after the tetrad is fully formed. Later,

<sup>1</sup> In consequence of the entire disappearance of the vacuolar cytoplasm which separated them from one another at an earlier stage.

when the spores are entirely separated and mature, the tapetum disappears.' In *Helminthostachys* the tapetum and tapetal nuclei are evidently more persistent than Cardiff found to be the case in *Botrychium*<sup>1</sup>. It has already been mentioned that the plasmatic membranes and many nuclei are still to be found between spores which are quite or nearly mature, and I have rarely found nuclei which were degenerating except in sporangia which were almost ready to dehisce. In such sporangia the majority of the nuclei stained intensely and were closely packed with chromatic granules, whilst here and there a nucleus was seen in which the nuclear membrane had disappeared and the granules were becoming dispersed (Fig. 13). Mitotic divisions of the tapetal nuclei were at no time seen.

Cardiff records frequent amitotic divisions of the tapetal nuclei of *Botrychium*, but Stevens was unable to confirm this observation.

In Helminthostachys I have found reason to believe that in the early stages of sporangial development, immediately after the tapetal membranes have disintegrated, the nuclei do multiply by direct division, but in older sporangia it is much more difficult to find any evidence of such divisions. Irregularities in outline, which sometimes lead to a more or less constricted shape, not uncommonly occur, but most of these are undoubtedly to be found in nuclei that are taking part in active metabolic processes. Occasionally these changes of form may actually lead to a division of the nucleus, but in my material this is certainly rare. An isolated instance of such a division is shown in Fig. 14. Very possibly the conditions which prevail at the time when the material is collected may exert an influence upon the tapetal nuclei and may explain the differences which occur in the accounts of Cardiff and Stevens.

The principal features, therefore, which the tapetal plasmodium presents during the period of exospore growth are (a) an almost or quite complete disappearance of starch, (b) a gradual diminution of the finely vacuolar cytoplasm, and (c) the richly chromatic nuclei which often show irregularities of outline.

These features, I think, bear out the view already expressed that the tapetal plasmodium is the centre of metabolic activities in which a substance is elaborated from the raw materials contained in the tapetum. For reasons already stated we may further conclude that this substance is, directly or indirectly, employed in the growth of the spore-wall.

<sup>1</sup> Stevens also records much greater persistence of the nuclei in the *Botrychium* examined by him.

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## EXPLANATION OF PLATES XI AND XII

Illustrating Mr. Beer's paper on the Spores of Helminthostachys.

#### PLATE XL DRAWINGS.

Fig. 1. Tapetal cells. × 660.

Fig. 2. Nest of nuclei lying at the inner periphery of the disintegrating tapetam. *a*, a constricted nucleus which is probably dividing amitotically. × 850.

Fig. 3. Chromosomes during first division of the spore-mother-cell. x 1100.

Fig. 4. Spindle and chromosomes during first division of the spore-mother-cell. x 850.

Fig. 5. Daughter-nuclei resulting from first division of the spore-mother-cell. × 1100.

Fig. 6. Tetrad showing the very young spore-wall (left white) surrounded by the specialmother-cell wall (drawn black). × 850.

Fig. 7. Tapetal plasmodium. Nuclei passing into one of the cytoplasmic strands which reach into the interior of the sporangium.  $\times$  850,

Fig. 8, a and b. Young spores. In b the wall has separated into three flaps at the ridges.  $\times$  850.

Fig. 9. Apex of young spore; showing little fold of endospore in section. x 850.

Figs. 10 and 11. Nearly mature spores. Fig. 10 looked at from above; Fig. 11 in section. Both x Sto.

Poth x 830. 1 Re- 42. Tepent nucles from a sportmeium in which sports had thickened; but still smooth titute for Botanical Documentation Propries 12. Nuclei from typetum of mature sportmeium.

Fig. 14. Nucleus from tapetal plasmodium of a sporangium in which the young spores were surrounded by a thin exospore. Amitosis  $(7) \times 850$ .

#### PLATE XII. PHOTOGRAPHS.

Fig. 15. Young sporangium; tapetal cells surrounding the spore-mother-cells. The break in the mass of mother-cells is caused in preparing the section and does not indicate a natural separation of these.

Fig. 16. Spore-mother-cells surrounded by the tapetal plasmodium. The tapetal membranes have all disintegrated.

Fig. 17. Early stage in the separation of the spore-mother-cells from one another. The first lines of the mucilaginous degeneration of the middle lamellae are shown.

Fig. 18. The young tetrads (special-mother-cells) with tapetal plasmodium gradually flowing between them.

Fig. 19. Older stage, showing distribution of tapetal plasmodium between the spore-tetrads. The young spores have all collapsed in the alcohol.

Fig. 20. Almost mature spores. The plasmatic membranes of the tapetal plasma still surround the spores. The spores to the extreme right of the figure show the little fold of endospore penetrating the eleft in the apex of the excesspore.

B6a On the development of the Spores of Helminthostachys Zealenica The writings of Goebel", Holtzman", Campbell<sup>(1)</sup> & Bower " here made as familiar with the early history of the Sporangium of the Ophiogloss aceae. The actual Structure of the spore, I the later stages of development leading up to the establishment of that Structure, here, nig been very imperfectly described up to the procent for any member of this Order. Recently the fized by Hunt Institute for Botanical Doc omenstico publication of two papers & Cardiff Stevens respectively have added considerably to our in knowledge of certain stages in the history of the spores of Botrychium but we are still for (5) Cardiff "Development of Sporangium in Botrychium" Bet. Hygarette 7905. Val 39. (6) Stevens "Spore Formation in Botrychium Vorginianum" annals of Bot. The October 1905 Vol XIX (1) Gaebel " Beiträge zur Vergleichenden Entwickelungsgeschichte der Sporangien" Bot. Zsit. 1880 - Vol 38. (2) Holtyman " on the apical growth of the stem of the develop. of the sporangeum. of Botry chium Virginianum" Bot. gazette 1892 Vol 17. 1009 (4) Bower "Studies in the morphology of spore-producing members II 1896

from having a complete account the que of this Jenus. Through the kindness of Prof. 7. W. Oliver I have had the apportanity of examining a number of fertile spikes of Helminthostachys Zealance which had been preserved in Spirit. Although many important cytological details can only be determined in they the Study of Instinal which has been specially fixed for the purposes it was thought that the following Digitizers by might Institute for Botanicat Documentation The first stages in the development of The Sporangeum of Helminthostacky's Zealancia have been followed by Bower who found the Sporangeum to be dereved from a single Superficial cell. The first periclinal division of the cell defines the sporogenous from the protective portions of the Sporangium; "the Sporogenons

" mass increases rapidly in bulk, whilst the " cells surrounding the Sporagenous mass, to the " Satent of several layers, assume the character of a tapetum which gradually becomes desorganised; finally the sporogenous cells reparate + the majority of them devide into tetrads; but a considerable portion of them, " Scattered throughout the Sporogenous mass "become desorganised without undergoing " division ; this is Semilar to what has been Digital by dunt obstitute for Banica Dogu phiglosiz My oon observations begin with a stage at which the Spor ogenous cells have reached this fall humber but in which the tapetum has not yet & become desorganised (Photo The tapetal lagor is composed of radially clongaled cells which have twided once or twee by pericanal, divisions. Each cell of the tapetum appears to be uni-nucleate (1) Spore Producing members Part II 1896 p.35

The tapetal layer is composed of radeally clorgated cells many of which have divided on ce on more rarely, twice by periclinal devisions of the Batry chim Cardiff observed periclinal divisions to follow one another until the tapetum became four or five cells in thickness. I tevens, however, does not conform this statement + fends the tapetal layer of B. Virginianum to be as a rule two cells in thickness which would be more nearly domes with ohat I have seen in Helminthostaclys. "The membranes of Iften la petal Bellaniard Delegate bation clearly give the reactions of both cellulore & pectore. In this feature the tapetain of Helminthostacky's apparently deffers from that of Botry chim in ouch Stevens found Each tapetal cell to be "deliminated by a plasmatic membrane merely although Cardiff does not actually describe the cell - walls of the tapetien than certainly his words certainly imply the occurrence of a cele-wall other than a plasmatic membrane.

45 \* includes only scanty cytoplasm but , Sine the readitions of bath pectore & cellula The sporagenous cells which his written the lapital lager are mostly Square or ablong cells which measure about 12-18 pe across their walls give the characteristic peeter reactions but neither cellulose nor cellose is to be found in them. The cyloplatim of these cells is fairly dense + contains a number of plastides Hundulligtu the Batthicastarchimentation the Slightly older Sporangia the tapetal walls begin to break down Fin these disorfanising the cellulose reactions although pectore was Still clearly demonstrable. The cytoplasm derived from the disorganised alls flows together + increases in amount which are mostly gathered obilist the muclii, hacome much have (1) I state this fact for what it is worth although the failure along of the cellulone reactions may be due to the increased difficulty of demonstration rather them to the real absence of the substance.

together in lettle nests or groups at the uner or onter perphery of the tapetal layer, have become more numerons. The groups of nuclei are no doubt partly formed by the approximation of nuclei from Several desorganized cells but I there can be little doubt that they also represent, to some extent a number of daughter-huclei which have oregenated from the ametater division of one or more mother - madei. The occurrence of Constructed muclii, Such as I have represented in tized by Hunt Institute for Botanical Dogumentation FS 2 bears out this Plant. hor at any Subsequent Stage, here I deen Capital nuclei deviding by mitosis. The lapetal cytoplasm at this stage contains a quantity of sterch + in iron - heematoxylin -- bismarck brown preparations presents a finely Vacuolated appearance Up to the present the sporogenous cells have been united together in one

\$ 7 coherent mass but now they show the first signs of separating from one another, The reparation takes place in blocks' quite comparable with those described by Cardiff in Botry chium " In Helminthostackys, as in Botry chium, the deparation appears to take place approximately " in the order " in which the original walls were laid down " in the archesporium + young doors mass" The nuclei of the sporogenous cells of DigHilminth butackystitere fstilletanicte Destingerstagen condition when the reparation of the "blocks" Commences. Delecate I undifferentiated as the wells of the Sporagemons cells appear they must nevertheles possess a greater complexity of structure than becomes apparent with our instruments for during the process of the separation of the sporagenous cells from one another a middle the Cardiff I. J. " Decelopent of Sporengum in Batrychium () Cardiff L. c. p. 342

lamella becomes mucileginous + 15 fenally devolved whilst a secondary theckening layer remains unaltered round each sporogenors cell. The entire Sporogenous membrane, both before & after to the mucelegenons alteration of the middle lamella genes no other reactions than there of pectre boties If I understand Stevens correctly he found the y sotrychim demarked from one another + from the tapetum ley a delecate plasmatic membrane alone but Cardiff's observations do not bear stuzed by Hutt Histicer Ports classication the mother - cells of Helminthostaclys. The Sporagenon's cells now devide with the Spore - tetrads. In the first division of the spore - mother cells the chromosomes ( which presumably occur in the reduced member) are seen to be small in Sizer rather Crowded upon the spindle ~ Herry mindows. In spite of repeated attempts I have not Succeeded in accurately counting their number & neither, apparently has stenand done so in the case of Batryching

I have not duceeded in a counting their number but I should troughly estimate this to lie between 40 + 60. Iterens has not recorded the number of chromosomes which occur is Botry chim . The spindle was surprisingly well preserved in the alcohol material & could be seen to lerminate in & very Sharp, almost a cuminate apices at which no traces of centrodomes were ever observed. The spindle satends to the very periphery of the mother cell + is free from any Pradiations lat 1/50 poles (Fig 4) tathe daughter mailin appear to pass through a resting stage, lake three of Boby the daughter - unclei, resulting from the first division, have in Some of any preparations a curronaly flattened, almost hemispherical Shape when viewed in profile. I they appear like those of Botry chium, to suter into a resting Stage before passing on to the second division

(1) compare this with Stevens' Plate XXIX 7; 28.

910 The spindle was voy closed, surprisingly well preserved in the elephon material + could be Seen to terminate in & very sharp, almost accumunate, apres at which he traces of centrosomes were Ever observed. The spindle extends to the very perphase of the matterefel & do free from any radiations at it Doles. The Spore - matter cells do not & devide simultaness in sportingion but all stages of division Digitizen by Hupt institute for Bosporal placumentation matter - cell with its uncleas in the prophases of the forst devision not uncommonly occurs side by side with one which has completed to decond division & Thes already developed the tebrad - ( Special - mother - cell) wells. In Botry chim, Cardiff found, that the cells in the Same "block" are always in the Same Stage but in Helminthostaclys Un reparation of the reather cells has, apparently,

been more complete before they enter upon the macotic divisions so that they have reached greater independence from one another that is the case in Botrychium. Bower found that a considerable proportion of sporogenous cells became desorganised without underforme division. In Botrychim Cardiff found no such desorganisation to occur I my preparations of Helminthosladys are quete in accordance with his observations When the two divisions of the mother - cells. here been completed the tobad four cells of the tebrad are enveloped by the tetrad well special - mother cell wall that membrane a Very difficult to demonstrate clearly as it is estimate the cate of is readily confused with the proper Itope-membrane which makes its appearance at an the sarly period. The clearest defferentiation between motion all

12 This membrane is very delecate + in this respect forms a striking contrast to hearly the conspiluous detrad - walls of nearly all the alter plants that I have Examined ( S. g. of Riccia, anthoceros, Lycopodraceae, Osmunda). It gives the pectic reactions , clearly whilet Several times I obtained a rather faint allow -fink reaction with calcium - chloride - I adme Solution " The latter reaction was homenon to constant + Digitized by Hunt betieter for Batanical Determentation For some unexplained reason A cal eum - chloridecodine Solution de not always produce the Same result, the telrad - wall in some Ceses colouring Violet-penk in alters yellow. I conclude, therefore, that whilst this wall certainly contains pectore and the is probably associated with cellulon which is more in less marked by I must remain undecided whether this is associated with cellulore or not.

Whilst we may therefore, conclude with certainty that this wall contains pectore we must leave it undecided whether or not this Substance is accompanied by cellulose". The young spore-membrane colours rather faintly, with bismarck-brown, meltylene blue, there of appears to be more or less catecularised from a vory early stage. if not for the very first. appresence. It is a new formation + is not derived from the transformation of the Special-Digitized by Hunt Institute to Batanical Istill mentation recognised outside the spore-well. (74g6) In the meanwhile the desorganesation of the lapetal walls has become complete whilst the lapetal cytoplasm, have a which now forme a sort of plasmodum around the sporogenous cells, has increased considerably in amount. The new humerous unclei which it contains still lend to be aggregated in groups. () ho other cellulare reaction, but only the inconstant appearance of the violet - pink colom with calcuim - chlowie - Ionie are seen by these walls. Delulity is supremasive broast too

Alter is some pridence to show that the are stoppes. I continue to show sudent some We now find the plasmodial envelope sending finger - like processes and the between the Deparated sporogenous cells. These cytoplasmic processes spread further to further between the young teles to the former the parety the young tetrads more + more completely from one another (cp. Hout First these Digitized platanodral stillrends Bollowisist Desalely nopio cytoplasm, unaccompanied by unclei Stand, which remain in the peripheral suvelope. Soon, however, we can observe an muchin depping into the broader arms of cytoplasm + making ther way into the interior of the Sporengial cavity. to the pass into the process strates af cytaplain the oval or round success alters to Shape & be comen drawn out

as the round or oval huchens passes into the Strandt of cytopleom it relters its Shape & becomes drawn out after into a very fine point, at its first satremity. (Fig 7) It usually Stains more deepely at the beak-like process than it does at its blant, posterior end. As it brauls further along the Strand + passes between the spore-tetrads it assumes an elongated form, Sometimes almost justifying the term Digitize en form Institute der Betarlem porarige comeson to rest at this or that Spat when it reassumes a more iso-déametrie shape. ten abundant Supply of Starch is contained. - the tapetal plasma, which has a finely vacualated Structure in Successful preparations, contains an abundant Supply of Starch. all the cells composing the walls of the Sporangum are rech in Starch, the membranes of these cells give the pectre + cellulore reactions.

The spores remain associated together in groups of four stronghout the greater part of their development. They now rapidly increase in size but the that any their then walls remain very this of the cytoplasm they Enclose is scenty. In consequence of this the Spores, show at this stage, are hearly all collapsed in the alcohol material The pores are tetrahedral in Shape & the edges of those surfaces which adjoin one Digiaizedary Huntenstitutgabon Bigtansopher Docamentation are raised up into redges. In this way Each Spore developes three ridges upon its Surface & these redges converge to a common point. (Fig 8 a) Early in the history of the spore one can see that the the pass redes of the tetrahedra which meet at the redges are set only very loosely joined together. a actually der free from one another. The action of reagents + Suen

16

the pressure of the mecrotome knife after leave the spore with three flaps, separated from one another at the ridges as is form in Fig 86. The cuticularised well of the apprendent of in the character of a somewhat of in the character of the a new leger - the interes makes its appearance within it . This layer, which gives at the reactions of a pectic body, to first deen just hereath the open Digitized by Hunt Institute and Botanical Documentation Continuous, although standy delicate, layor our the Entere uner Surface of the spore wall In microtome sections after space which here passed through the apex of the spore we Can trace a little process on fold of the endospore which the has pushed sport between the redges of the Exception & bluch reaches to the outer Surface. (Fig 9) (1) Viz the point at which the three ridges meet.

In spores measuring about 23 per across them longest diameter the exospore has become Dufficiently thick to prevent the collapse of the Spore in alcohol. The Surface of the spore is still smooth & the solution maller which to is contained in its protoplast the poor in Solice Substance ." The further growth in theckness of the exception continues to leave the projection of fold of endospore uncovered at the apese of the spore. Digitized by funt Institute for Bothical Cocumentation the exceptore non becomes slightly waay in sutline & this soon leads to the formation of the reticalar Scalplure which is characteristic of the mature spore. The fully grow spore measures, in Section, about 300 by 28 p. The easpore the is techer at the apex them our the rest of the spore but is perforated by a eleft through which the fold of endopou (1) It usually contains an little starch, which however.

Ba 19 Kotton reaches the exterior . This eleft is particularly well seen after breatment with a meature of Dulphurie & chromic acids or with Sulphuric aced alone but it can be quite readely destinguished in sections which have been stained with methylene blue or beswarck brown without , previous action of acid. Chlor- Tinc - indine Solution to which more codine then usual bas been added, also Very clearly shows the Endoapore - coloured brown -Digitized brating int the title to pore tapic reaching the exterior The retecular sculpturing upon the Durface of the exceptre appears in section as a series of blunt processes with flattened Summits. The protoplast of the spore has now become somewhat richer in Substance & usually includes starch but not in any quantity.

19 (continued) reaches the exterior . This cleft in the exospore is particularly well seen after treatment with dilute sulphuric acid but it can be quite clearly distinguished in Sections which have been Stained with pretty lene blue or besmarch brown without previously tripting them with a cit. Nothing of the nature of an epispore is to be found in the spores of Helminthostachys. We have already seen that It has already been said that both the excepter the Distized by Hunt Institute for Botanical Documentation mat probably also Bretto Ette exospore of the endospore are in the first place deposited by the Secretary no possibility of the spore - protoplast. There can derived from the transformation of part or all of the mother-cell wall since this, at all times very delicate, can still be distinguished, in apparently underunished theckness, over the surface of the young saoopore.

The fact that the exospore & endospore are probablist inseparably united together, at first suggests the probablist of the two lagers being derived from the defferentiation of an originally single homogeneous membrane. As the filting has already pointed out, however, the Existence fact of a close fusion between two layers of a wall does not becersarely endicate their common origen by differentiation & there are several & well known cases in which two lawellas are internately bound together but, unquestionably have been In the present enstance, moreover, it is defficalt to understand how a cute calarised membrane can he to differentieted as to give rise to a pure pectic lemella on one of its faces. The ongen development of the little fold of endoapore at the apea of the Spore is also more readily explained as a new formation than a product of differentiation Shortly after the easepore has been formed round the young spore & throughout the time that this lager is growing in thickness + in Surface the tapital plasmodium which envelopes the spore-tetrade shows unmistakable signs (1) Fitting. H. Bot. Zeitung Bd 58 1900 p. 126.

of metabolic activity. It is dufficient to escape the conclusion that this metabolism is concerned with the elaboration of material for the growth of the excopore. That such material must he forth coming is of course obvious + the two most natural sources to which one plooks for the Sapply are the Spore-protoplast on the one hand + the tapetum upon the other . In Helminthostachys (at least in my Spirit material) the Spore-protoplast, after the first fore day Hugt the stients pore Botanical engeupocatation Substance + et is very unlikely llat it can furnish sufficient plastic material for the growth of the wall. The next turns to the tapetune to read and a gradual etilisation of reserve - materials which fally fully accounts for the substance which is being employed in the growth of the exception moreover, if we deny that the tapetal material is being utilised for this manner A at least is any pleaked at pile and dentifient but thing is the spit the stand of the diministry of the always scante explosion there is the loss of the stand of the diministry of the always scante explosion there is the time of the sport route such a match append to find of the append for the protoperst were the time of the stand of the growth.

of this Substance it is dofficilt to see what the fate may be The growth of the wall of the sporangum is amply proveded for by the reserve - materials contained in its ells of the protoplasts of the Spores do not increase in anount de size or substance during this time so that we are dreven to addoceate the disappearance of Starch & cytoplasm from the tapetum inth the only a demonstrable utilisation of matinal Viz that which is the utilisation to the substance Digiogracity for the size of this part of the substance of adding to the size of this part of the substance It is impossible at present, however, to decide whether the material elaborated in ten tapetum is directly built up in the spore- wells or whether it first passes, into the sporeprotoplast which, setter with or without further elaboration, distributes it to the walls. In view of the emportant part that the taketal plasmodrum plays in the growth of

the spore - walls it will be necessary to look more closely at its appearance + behaviour. We find that Starch is abundant in the tapetal cytoplasm before the wells of the tapetum desorganise + chat it continues to & exist there in considerable quantities until the period during which the exospore undergoes its most active growth in thickness. During this time the lapetal plasma is rether very poor in starch or, more after, this Substance is quite absent Digpized by Hunt Worken textor Bestonpore D has monipleter 15 growth in thekness + the Spore protoplast is about to add to its Dubstance Starch can usually again he seen in the tapetal cytoplatur but never in quantities that can compare with those that occurred in the early Stages of Sporangial development

The cytoplasm at first increases asmealut in amount ( unmedially after the deaorgenisation of the tapetal cells) but during the and later history of development (component story of the point). Steadily decreases in quantity A Where it abuts upon a Vacade ( such as the while ancloses a spore or spore-tetrad) that has a very clearly marked plasmat-membrant; alsowhere I is finely Vacuated a Structure. It is the Vacuolar cytoplasm which is gradually until tili and for Pta anicetaller inprotection of the plasmodum & while the plasmatic membranes, in consequence which timit neighbouring Vacuoles, approach one another more & more closely. In Sporangia, Shortly before their debescence, the Vacaolar cytoplasm has almost completely disappeared but the plasmatic-but the plasmatic-between the spores have can in nearly all. cases, he seen to be double in Structure twing

24 to the complete approximation of the plaonatic membranes of adjoining Vacuales". Flattened in hetween these nombrand' Starch grains Can not infrequently be seen flattened out between the two ad pressed membranes & at the angles formed by the meeting of Deverel Vacuoles nuclei occur. The nuclei show, at all stages, considerable variation in Dize, in form + in character. In Bobrychium Cardiff found that the Dignizediby Hundrinstitlite foraBoringican Dedeublentationa in size during development; thus in yoing sporangia he gives their size as 8 ge whilst at a later Stage her found them to measure 15-20pc In Helminthostadys the general average of size of the tapetal unclei may perhaps, increase Very elight bolinen the carly & middle slages of development but the mercan does not approach the which Cardiff recorded (1) Le consequence of the entire disappearance of the vacualar cyloplasme which separated them from me another at an varlier Stage.

26 the have to great significance. The following figures give the accage dimensions at different stages. appears to remain about the Same at the different periods of development. (a) Tapetal cells still a intact = 13 × 11 pc (b) young spores wilt then smooth mospore = 13.4 ×10 (c) Spores with commettere Sculpturing on mospor= 12.6 × 9.6 p (d) Spores just before debescence of sporengin = 13×11 pc The above figures are the averages calendated from a large number of countings & they Show practically no alterations in the size of the tapeter under. It has already The form of the nuclei is very various. Le the tapetal cells before disorganisation & in the lapital plasmo from before this flows between the Sporagenous cells the nuclei are more or less over in shake. The peculiar alteration in form which they

27 often undergo as they pass writin the Sporangiak centy has abready been described. In the later periods of development these nuclei are cometines oual or Spherical in tuttine; and after they are irregular + almost amorbid in Shape + frequently they are their form is plankty angular. (Fig 12). They posses, in most cases, a distinct nuclear - membrane & hang a grander Structure. In the Smaller uncling the gran chrometic are usually rich in Chromating; everal nucleoli can generally he seen in Each uncleus. The chromatic granules are then andrile, closely packed for the nucleus in consequence deep - Staining) in the Smaller unclei, whilst in the larger nuclei the generally generally more widely from one another + the uncleus contain more non-staining material. them rarely found suclim which were

29 degenerating Sacept in Sporangia which were about to deline In Botrychium Cardiff found that " as the Spores commence to separate in the tebrad, the tapital cytoplasm has entirely filled the "Sporangium + many of the nuclei have begun to desorganize, though they seen unichally " persestent & nenny are found after the tetrad is fully formed . Later, when the Spores are entirely reparated + mature, the git la petien Hickes appetar for Botanical Ich Helminterstrug the tapetum & tapetal nuclie are evidently more persistent than Cardiff found in Boby chuin the plasmatic membranes & many muchi are Still to be found between the Spores which are quite or nearly mature & I have rarely found nuclei which were degenerating Except almost ready to dehisce were autore. In Such Sporengia the megority of the unclei (1) Stevens also records much greater persistence of the muching the Botrychium Examined by him.

29 stained intensely & were and closely packed with chromatic granules whilst here & there a nucleus was seen in which the nuclear membran had desappeared & the granules were beening dispersed (7ig 13) Milotre divisions of the tapetal nuclei were there seen . Cardiff records prequent amitotic divisions of the lapetal nuclei of Botry chium but Stevens was unable to confirm this observation In Helminthostadys & I have found reason Digits deliver Hunteter tute the Botanical Dostantition Sporangial development, at the two when the taptal membranes are disintegratedy, the nuclei do multiply by direct division but I have never been able to satisfy my self that such divisions take place in older sporangia. Racing chan dregularities in outline which may sometimes lead to a more or less constructed shape occur but I do not find that there are result in the actual. We apply with to inply that can differ observation of amiting in the actual in the division of the nucleus : they is apply with to inply that can differ observation of amiting in the actual is interested to inply that can differ observation of amiting in the and in the apply that his interest in a protocol in the fortune of the first the standed manual the mission the interest made and the first the

In Helminthostachys I here found reason to believe that in the early stages of Sporangial development, immediatly after the taketal membranes have desintegrated, the nuclei do multiply by derect diversion but in older sporangea it is much more deflecalt to fend any evedence of Such devisions, Harrow Dregularities in outline, which sometimes lead to a more or less constructed shape, accor not uncommonly occur but most of these are undoubtedly to be Diginizferred Hoint care itu patring ot changes of uponintati which are so frequently to be found in muchei that are taking part in active metabolic processes. Occasionally these changes of form may actually lead to a division of the unders but in my material this is certainly rare. an isolated instance of such a division is shown in Fig 14 . Very possibly the conditions which prevail at the time when the material is collected may have an influence

upon the tapetal nuclei & may account for the different results which Cordeff & the defferences which occur in the accounts of Cardiff & Stenans. The principal features, therefore, which the tapetal runding plasmadeum presents during the period of exospore growth are (1) an almost or quite complete desaper desappearance of starch, (2) a gradual deministeon of the finely Vacaslar cytoplasm, + (3) the reckly chromatec nuclei which after show Digitize Subsities int Astitute for Botanical Documentation These features, I think, bear out the view already already expressed that the tapetal plasmodium is the centre of metabolic activities in which a Substance is eleborated from the raw meteriels contained in the lapetim. For reasons already slaled we may further conclude that this substance is, directly or indirectly, employed in the growth of the spore - wall

Rudolf Beer Bickley

32 Explanation of Plates A. Frawings A. Frawings Tapetal cells. y many × 660 Fig 1 7.52 hest of nuclei lying at the unner periphery of the disintegrating tapetum (a) a construction nucleus which is probably dividing amitate cally. Chromosomes during first division of × 850 Fig 3 the spore-mother cell. × 1100 Fig 4 Spendle & chromosomes during forst division of Spore - mother cell × 850 Fig 5 Daughter - nuclei resulting from first Digitized bailtant Institute sport otanital Decurrent and Fig. 6. Tetrad showing the very young spore-wall (left white) surrounded by the special moltur cell well ( drawn black). X 850 Figy Tapetal plasmodium. Nuclei passing into along one of the cytoplasmic strands which reach into the interior of the Sporangium Fig 8 a + b. Young spores. In (b) the × 850 wall have has deparated into three flaps at the ridges. X 850

7:59 apex of young spore; showing little fold of endospore in section × 850 Figo 10 + 11. Rearly mature spores. Fig 10 looked at from above; Fig 11 in dection. Both × 850 7.8 12 Tapetal nuclei from a sporangin in which spores had theckened, but Still Smooth Exceptes × 850 75 13 & Nuclei from mature lapitum of mature sporengin . One epitim Type lastitute for Establica plasmosentation Dightzed Sporangium in which the going spores were surrounded by a this savepore. amitosis (?). X 850 B. Photographs Photo Fig 15. Young sporangum; tapetal cells Surrounding the Spore-mother cells . The break in the mass of mother cells is caused indicate natural separation of these.

34 7.8 16. Spore-mother- cells surrounded by the tapetal plasmodium. Tapetal membranes have all desintegrated . 71817 Early Stage in the Reparation of the Spore-mother cells from one another. The first lines of the mucillaginous degeneration of the meddle lamellae are shown. The young tetrads ( special-matter cells) with tapetal plasmodium flowing unbetween Botanical Documentation 7ig 18 7ig 19 Older stage showing distribution of lapetal plasmodrum. between the sporetetrado. The young spores have all collapsed in the alcohol . 7:5 20 almost mature spores. The plasmatic - membrenes which are of the lapetal plasma still surround (tu spores. Spores to the satrene left of the figure shows the eleft into 2000 little fold of endoepore penetreling the cleft in the apex of the earspore.