Botany. — "*The development of the ovule, embryo-sac and egg in Podostemaceae*". By Prof. F. A. F. C. WENT.

During my voyage to the West-Indies I had an opportunity of visiting in Surinam some of the rapids where *Podostemaceae* grow, namely the Armina falls of the Marowyne river. There I collected material of these remarkable plants, and at a later date I received an abundant supply obtained by the various expeditions, which of late years have investigated the interior of the colony. This material, preserved in alcohol, has suggested to me an investigation of the above order. I hope soon to publish the results *in extenso*, but wish in this place to deal briefly with one point, namely the development of the ovule, the embryo-sac and the egg.

As was mentioned above, the material was fixed in alcohol, but the fixation nevertheless proved to be good enough to allow of many cytological details being made out with a sufficient degree of certainty in stained preparations. In this preliminary communication I do not propose to discuss the method of treatment of the preparations, but merely record, that Messrs. A. H. BLAAUW and J. KUYPER have assisted me. A complete developmental series could only be obtained in the case of a few species, namely of *Oenone Imthurni Goebel* and *Mourera fluviatilis Aubl.* Of eight other species only a few stages of the development were examined, and of *Tristicha hypnoides Spr.* I only had the ripe seeds.

It soon became evident that the whole development of the ovule in this order departs very widely from the ordinary type of *Angiosperms*, but that within the limits of the order there is an extraordinary degree of uniformity, so that the differences between the species, which have been investigated, are so slight, that they may be passed over in silence in this preliminary notice. The description which follows, therefore applies to all the species.

The ovules are anatropous; in the youngest stage examined, the curvature had already taken place. In this stage the nucellus was still alone present and consisted of a central row of four cells surrounded by a single layer of peripheral cells. Of the central row the uppermost cell, which is therefore still surrounded by a cap of epidermal cells, becomes the spore mother-cell. Accordingly this cell is not only soon distinguished from all the other cells of the nucellus by its size, but also by its dense protoplasmic contents and by its large nucleus. The subsequent behaviour of this spore mother-cell will be further discussed below.

We may now consider how the integuments are formed. The

outer one arises *first* and here we find the first deviation e normal course of development in Angiosperms. This integument simply arises as an annular fold on the nucellus, with which it remains connected by the chalaza, while for the rest it grows round pretty loosely. Finally there remains at the point, where its borders meet, a very narrow micropyle, which can only be seen properly in truly medial sections.

After the outer integument has already surrounded half the ovule, the inner one begins to develop. Cell divisions are seen to occur in a few epidermal cells of the nucellus, immediately above the point of attachment of the outer integument. These divisions take place in such a manner, that a wall arises in one of the basal cells of each longitudinal row of the epidermis; this wall forms an angle of 45° with thè longitudinal direction of the ovule, so that each of the cells is divided into two. The upper half remains an epidermal cell of the nucellus, while the lower half develops to form the inner integument. In a transverse section the number of epidermal cells, counted on the periphery, is seen to be 5, occasionally 6 or 7. At first the inner integument will therefore show in transverse section an equal number of cells. Dividing walls soon arise, however, which make this inner integument two cells thick. More than two layers do not develop, as no further tangential walls are formed, but other walls, both radial and transverse to the long axis of the ovule are developed. Especially the number of radial walls is very different in the two cell layers; it is large in the outer layer, but on the other hand small in the inner layer. As a result, the number of cells of the inner layer of the inner integument is generally little more than five, when counted in transverse section. When afterwards the cells of the inner integument increase in size and often acquire dimensions, which make them very noticeable, it is the inner cells which are especially large. This growth is often accompanied by strong thickening of the walls.

The transverse walls, which arise in the cells of the inner integument, enable the latter to grow longitudinally. In this process the top of the nucellus remains free however, and is only surrounded by the outer integument, so that it lies in the endostomium; the strong longitudinal growth of the inner integument is chiefly directed downwards. At its base, near the chalaza, it of course remains connected with the nucellar tissue.

Now it is very remarkable, that the nucellar tissue does not participate by cell division in this strong longitudinal growth of the ovule. The portion of the nucellus, which projects beyond the inner integument, remains unaltered, except for certain changes, which

the spore mother-cell undergoes, and which will be discussed below. We may however at once point out, that in the formation of embryo-sac and egg-cell, the whole apparatus remains in the same place, and is therefore never surrounded by the inner integument.

The portion of the nucellus lying below this, is now elongated by the extreme stretching of a single cell (or in some cases perhaps two cells) in the central and in each of the 5, 6 or 7 peripheral rows of cells, of which it consists. The nuclei often also assume an extended shape, so that one gets the impression that a passive stretching has taken place. At the same time a digestion of the longitudinal walls occurs, and finally the protoplasts also coalesce more or less. In this way a great cavity arises, containing protoplasm, often in a peripheral layer and with 6, 7 or 8 nuclei, perhaps sometimes even more in consequence of nuclear fragmentation, which seems to occur.

If an ovule is examined in this stage, without the history of its development having been traced, this cavity is inevitably regarded as the embryo-sac, and the real embryo-sac, which lies above it, is then taken for the egg-apparatus. It is in this way that WARMING, who, for want of the necessary material could only trace part of the development of the ovule, has regarded things.[1]) This pseudo-embryo-sac remains in existence during the further development of the ovule to the seed, and is only compressed more or less in some cases by the large increase in size of the cells of the inner integument, which has already been dealt with above. When the embryo begins to develop it grows out into this pseudo embryo-sac, in the same way as would happen with a true embryo-sac.

We may now pass on to consider the fate of the spore mother-cell. At a certain period its nucleus shows a clear synapsis stage. In the division, which follows this, the reduction of the number of chromosomes therefore probably takes place. The fixation was not sufficient to allow one to conclude with certainty that a hetero-typic division of the nucleus occurs (the nuclei are moreover extremely minute); such observations as were made, leave very little doubt, however, when considered in connection with the preceding synapsis, that the haploid generation begins here. This nuclear division is followed by a cell division and the formation of a dividing wall. The upper of the two cells, which are thus formed, gradually degenerates and becomes more and more flattened by compression; remnants of it

[1]) EUG. WARMING Familien Podostemaceae. II. Afhandling. Kgl. Danske Videnskab. Selsk. Skr. 6te Raekke, naturv. og math. Afd. 2det Bd. III. Kjöbenhavn, 1882. Compare e.g. p. 65 (107).

may nevertheless still be observed for a long time. In some cases the nucleus of this cell divides once more, in a plane perpendicular to that of the previous division, so that the equatorial plane of the second division is in the longitudinal direction, with respect to the ovule. Perhaps this division also takes place in other cases, in which the two nuclei cannot be seen on account of the unfavourable direction of the section and in consequence of the rapid degeneration of the cell. Only in a single instance I have thought that I observed a cell division following the division of the nucleus in the upper cell.

The lower of the above-mentioned two cells is the embryo-sac. Having regard to the size of the pseudo-embryo-sac, it is remarkable, that the real embryo-sac increases but little in size, and always remains situated in that upper part of the nucellus, which projects beyond the inner integument; it remains of course surrounded by the layer of epidermal cells, which later are only compressed and flattened more and more, so that they become scarcely visible.

The nucleus of the embryo-sac soon divides again. Only a single division was observed, and then the fixation did not allow many details to be made out; it can hardly be doubted, however, that this must be a homoiotypic division of the nucleus. The axis of this spindle is longitudinal with respect to the ovule and therefore also with respect to the embryo-sac. The lower of the two nuclei, which are formed, is seen to degenerate in the anaphases of the division, by a strong clumping of the chromatin masses, so that the latter come to lie at the base of the embryo-sac as a structureless chromatin-like clump, which stains deeply. This is evidently all, that can here be seen of the antipodal apparatus and of the lower polar nucleus. I shall call this nucleus the antipodal nucleus of the embryo-sac.

In contra-distinction to the last-named, the other nucleus assumes a normal shape and is prominent on account of its size. Soon afterwards there follows another division, of which I have been able to see the various stages. The axis of the spindle is this time also longitudinal to the embryo-sac and ovule. This division is not at first followed by a cell division, but afterwards each of the two daughter nuclei divides again. The actual process of division I have not observed, but have only found four nuclei; the second division evidently takes place very rapidly, for I have looked through hundreds of preparations of about this age, without getting the actual stage of division. This second division takes place in such a manner, that the axes of division are perpendicular to each other; for the upper

pair of nuclei the axis is at right angles to the length of the embryo-sac, and for the lower pair it is parallel to it.

Before this last division has taken place, the embryo-sac is still seen to be a single cell, as was already stated above; after this division four cells, each with its nucleus, may be observed. It is of course possible, since I have not seen the actual nuclear division, that the latter is preceded by a cell-division, in such a way, that each cell contains a nucleus, and that afterwards each of these two cells divides again, after its nucleus has divided. However this may be, there are finally four cells, which, it should further be noticed, are not separated by cell-walls — four naked protoplasts therefore Of these four two, the synergids, lie at the top, next to each other; then follow the other two, one under the other, the upper one of the pair being the egg and the lower one all that remains of the embryo-sac with the upper polar nucleus.

Considering this lower cell first, we observe, that it remains small and that pretty soon its nucleus clumps to a little ball of chromatin, in which structure can no longer be discerned; often the antipodal nucleus may be seen at the same time. In other cases no remnants of it can be observed; I imagine that in such cases it has so far degenerated, that it can no longer be rendered visible. Yet another hypothesis might be suggested, namely, that these nuclei fuse like two polar nuclei. I regard this, however, as extremely improbable, for the very reason that the two nuclei are so clearly in a state of degeneration. Indeed, all the rest of the embryo-sac does not come to much; endosperm is not formed; the cell is still seen for some time, until it disappears with the developing embryo.

For some time the egg and the synergids undergo no further changes, and are ready for fertilisation. This process I have only been able to follow accurately in *Mourera fluviatilis Aubl.*; in a few other cases I found a young embryo, or sometimes pollen-grains, which had germinated on the stigma and had developed pollen-tubes. In a new species of *Apinagia*, still to be described, there occur, in addition to the normal hermaphrodite flowers, others, which have abortive stamens, and which remain inside the closed spathella, at least as far as I have been able to observe in the material at my disposal. Whether the latter flowers can also furnish ripe seeds, without fertilisation, I cannot say, as they had not developed beyond the stage, here described. In the numerous preparations of various *Podostemaceae* which I have examined, I found moreover many ovules, which were degenerating at the above-mentioned stage, evidently because no fertilisation had taken place. It seems to me, that the chance of

regular pollination among these plants is probably not so very large, and that in consequence of this so many ovules ultimately abort.

I now pass on to describe what I have seen of the fertilisation itself, and must remark, that I have but rarely observed anything of the penetration of the pollen-tubes; to some extent this is probably a result of the process of fixation, during which such tender, thin structures readily shrivel up; at the same time the staining does not succeed well. In any case I can however state, that the pollen-tube penetrates through the micropyle, and then reaches the egg-apparatus by passing between two epidermal cells of the nucellus. In one case I observed two nuclei in the top of the pollen-tube, one of which appeared to be a generative and the other a tube-nucleus. In another case I saw a nucleus, which had a much elongated appearance, and was constricted in the middle, so that there might have equally well been two generative nuclei. Taking all the cases, which I have seen, into account, I am led to the view, that the conditions in the top of the pollen-tube are normal, so that there are two generative nuclei and one tube-nucleus. In the actual process of fertilisation, the top of the pollen-tube unites with one of the synergids; the synergid and especially also the egg undergo at the same time peculiar changes in shape, somewhat resembling amoeboid movements. What further happens in the synergid cannot readily be made out, because its contents stain very strongly and become highly refractive. I nevertheless also succeeded in this case in observing the main features of the process. At least one nucleus of the pollen-tube penetrates into the synergid and assumes, in so doing, a more or less vermiform shape. Thereupon a fusion of the synergid with the egg takes place, so that the protoplasts communicate with each other at least at one spot. This communication does not last long, but during it one of the generative nuclei evidently penetrates into the egg-cell; anyhow stages are found later, in which two nuclei lie close to each other in the egg. Still a little later these are found in contact, and afterwards they are found fused in such a manner, that the origin from two nuclei can still be seen.

The fertilized ovum now rapidly enlarges, while all other cells in its neighbourhood are crowded out. As the epidermal cells of the nucellus have generally aborted, this large cell lies more or less by itself in the endostomium, almost filling it up. By the first division wall there is formed a bladder-like basal cell, which remains in the cavity, and a smaller one, which is gradually pushed forward into the pseudo-embryosac. This cell now undergoes some divisions, in which the walls are formed perpendicular to the long axis of the

young seed. When a row of four cells has thus arisen, the three which are turned towards the micropyle become a suspensor, while the fourth divides by a wall at right angles to the previous ones and becomes the embryo proper.

I have not traced the further development of the embryo, partly for want of sufficient material, but especially because WARMING has already furnished an excellent treatise dealing with this subject, and illustrated with figures. Considering the many new facts, which WILLIS has discovered about the germination of the *Podostemaceae* of Ceylon, an investigation of the American forms in this direction would certainly repay, since through Goebel we have only learned in detail of a single case. For this an investigation on the spot is necessary, and as will appear from the full paper, I have not been able to find much that is new in this direction.

What was hitherto known about the ovules of *Podostemaceae* we owe almost exclusively to WARMING. As was said above, this author described in detail the first development of the ovules of *Mniopsis Weddelliana Tul.*, and it was only owing to the want of the exact stages, that the meaning of certain organs did not become clear to him. The development proper of the embryo-sac was completely left out of account, but the development of the embryo of this plant, beginning with the two-celled stage, was treated very thoroughly. It is quite clear from his letter-press and from his figures, that the whole development takes place in the same way as in the species examined by myself. The same can be said of the other cases, in which he has stated or figured something regarding the ovules of *Podostemaceae* namely *Castelnavia princeps* TUL et WEDD.[1]) *Hydrobryum olivaceum* GARDN.[2]) and *Tristicha hypnoides* SPRENG[3]). On the last named CARIO[4]) had already made observations which seemed to indicate an agreement with the other *Podostemaceae* as regards the development of the ovule. This is of some little importance, because this plant deviates in the structure of its flowers from the majority of the species of the order. If the development of the ovule here corresponds to what I found in the species examined by me this agreement constitutes an additional reason for supposing, that the order is extremely uniform in its embryogeny, in which it differs so widely from the other Angiosperms. I have already

1) WARMING, l.c. Plate XIV. Fig. 9—21.

2) WARMING, Ibid. 6 Raekke, Nat. og math. Afd. VII. 4. 1891, p. 37, fig. 34.

3) WARMING, Ibid. 6 Raekke, Nat. og math. Afd. IX. 2. 1899. p. 113, fig. 6.

4) R. CARIO. Anatomische Untersuchung von *Tristicha hypnoides* Spreng. Botan Zeitung. 1881 S. 73, Taf. I. Fig, 20—24.

remarked, that much to my regret, I have only ripe seeds of *Tristicha,* but no younger stages. In the 78th Versammlung Deutscher Naturforscher und Aerzte in 1906 at Stuttgart R. von Wettstein made a communication : "Ueber Entwickelung der Samenanlagen und Befruchtung der Podostemonaceen". So far he has not published anything about this, however. I have indeed found an abstract of the communication in "Naturwissenschaftliche Rundschau" of 1906, Bd. XXI, p. 615, and in it several statements occur which agree completely with what I have observed, but in other respects there are such differences, that I must assume, that the reporter did not completely understand the meaning of the reader of the paper ; I dare not therefore rely on this abstract.

The *Podostemaceae* differ on the following points from the ordinary arrangement in *Angiosperms,* as regards the development of the ovule : 1. The inner integument begins to develop after the outer ; this is perhaps connected with the fact, that the top of the nucellus remains free in the endostomium, a phenomenon, which has been observed in other plants. 2. The peculiar development of a pseudo-embryosac by the stretching and dissolution of the cell-walls of a layer of the nucellus. I am not acquainted with anything in the vegetable kingdom corresponding to this. One could only point out, in explanation, that in many cases the developing embryo-sac exercises a solvent action on the surrounding tissue of the nucellus, and that in the present case a similar action is exerted on those cells of the nucellus which are turned towards the chalaza ; these cells only disappear completely, when the embryo proceeds to develop there.

The phenomenon also suggests, that, to a certain extent, it is comparable to that of nucellar embryos. By this I mean, that these nucellar embryos prove the existence of causes, acting in the embryo-sac, which determine a developing cell to become an embryo. What these causes are, we do not know, but it is by no means inconceivable, that some day we may know them completely and even be able to imitate them, so that we may be able to produce an embryo at will. Similarly this phenomenon in *Podostemaceae* seems to me to prove, that there are causes acting in the ovule, which favour the development of such a large cavity as the embryo-sac, so that in those cases, in which the embryo-sac itself does not develop greatly, because it is enclosed and separated off in the upper part of the ovule, the cavity is formed by other cells, lying underneath the embryo-sac.

3. The development of the embryo-sac departs widely from the normal, in that no antipodal cells and no antipodal polar nucleus

are formed, on account of the early degeneration of the nucleus, which, by its divisions should have given rise to these nuclei. Further more, after the egg-apparatus has been formed, the remaining portion of the embryo-sac is only very slightly developed, so that there is no question of the formation of endosperm (what happens to the second generative nucleus, if indeed present, I have not been able to make out). It is much clearer here than in most cases, that this portion of the embryo-sac and the egg-cell are sister-cells. This agrees with the view of PORSCH [1]), according to whom the egg-apparatus of the higher plants is a reduced archegonium, the synergids being the neck canal-cells and the upper part of the embryo-sac with the upper polar nucleus being the ventral canal-cell. The latter hypothesis is however specially difficult in this case, for here the positions of egg-cell and of ventral canal-cell would be exactly reversed. A reduction in the antipodal apparatus, similar to that which occurs here, is found in *Helosis guyanensis*, according to the investigations of CHODAT and BERNARD [2]), and a still further reduction exists in *Cypripedium*, where, according to the researches of Miss PACE [3]), the lower portion of the embryo-sac has not even been laid down at all. It need scarcely be argued, that we are here concerned with a progressive differentiation, and not with the recurrence of ancestral peculiarities. Perhaps it may not be amiss to point out, in conclusion that we cannot here fall back for "explanation" on a parasitic or saprophytic mode of life of *Podostemaceae*.

Mathematics. — "*On twisted curves of genus two*". By Prof. J. DE VRIES.

1. A curve of genus *two* bears one and only one involution of pairs of points I^2. On the nodal biquadratic plane curve it is determined by a pencil of rays, having the node as vertex; its coincidences are then the points of contact of the six rays touching the curve. If we could arrange the points of the curve in a second I^2 then this I^2 would be projected out of the node by a system of rays with correspondence [2], and the above six tangents would furnish six rays of ramification whilst a [2] can have four only.

[1]) O. PORSCH. Versuch einer phylogenetischen Erklärung des Embryosackes und der doppelten Befruchtung der Angiospermen. Jena 1907.

[2]) R. CHODAT et C. BERNARD. Sur le sac embryonnaire de l'Helosis guyanensis. Journal de Botanique T. XIV. 1900. p, 72.

[3]) LULA PACE. Fertilization in Cypripedium. Botanical Gazette. XLIV. 1907. p. 353.