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A CONTRIBUTION TO THE MORPHOLOGY OF *AGATHIS OVATA* (MOORE) WARB.

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Introduction.

The material, on the investigation of which this paper is based, was collected by Professor R. H. Compton in New Caledonia in 1914, and kindly placed by him at the disposal of Professor Seward of Cambridge University, who gave me the privilege of examining it.

My attention was primarily directed to the reproductive structures rather than to the vegetative ones. The latter were studied as a rule only in connection with certain points which have been brought up from time to time as having some phylogenetic importance.

Anatomy of the Vegetative Structures.

1. THE STEM.

The pith is generally large, and in young twigs consists of three kinds of cells, (*a*) ordinary thin-walled parenchyma cells, (*b*) secretory cells. (*c*) sclerotic cells (fig. 1). In older twigs the sclerotic cells practically replace all the other kinds of cells.

The medullary-rays are long and narrow and as a rule uniseriate. They are generally three cells deep. Near the primary xylem the cells have no constant shape and they may be elongated in the vertical direction. Thomson (9) has described the change of shape, which the medullary-ray cells undergo in passing out from the pith. He says that in doing so the vertically long cells develop a pair of opposite horizontal processes, which elongate at the expense of the vertical arms and in this way form the horizontally elongated ray-cells. Such 'irregularly elongated or four-armed cells' were very commonly

seen in young twigs, roots, and peduncles. That these vertically elongated cells belong to the medullary-ray system and are not tracheids is shown by the fact that they often possess a nucleus.

In the wood the transitional area between the primary and the secondary xylem is very broad, as one type of element gives place to the other very gradually. In the secondary wood the pitting near the primary xylem is not quite definite in arrangement. The bordered pits may sometimes be opposite, but more often they are alternate. In older wood the pitting is never of the opposite type, and is often confined to the ends of the tracheids. The torus was always found to be absent.

The annual rings are well marked; even small twigs show these quite distinctly. The autumn tracheids especially those at the periphery of the ring, often show tangential pits. The leaf-trace is persistent and double, with secondary wood separating the two parts. The arrangement of pits in it, though generally alternate, is occasionally opposite.

2. The Root.

The sclerotic cells of the cortex are aggregated together in a layer just under the periderm. The pericycle is broad and has thickened elements and sclerotic canals. The primary wood is diarch. There are distinct annual rings, but they are not so clearly marked as in the stem. Occasionally the tangential walls of the autumn-wood are pitted. In the metaxylem the bordered pits are slightly smaller than those of the stem. The pores are generally oblong and oblique. The vertically elongated cells with nuclei, which as mentioned above, belong to the medullary-ray system, are often seen in sections of the wood.

3. The Leaf.

The leaf has a thick cuticle and its epidermis is made up of quadrangular cells with deeply-coloured contents. The cells of the spongy parenchyma are a little elongated laterally; with them are a few sclerotic cells. There are resin-canals in the palisade and spongy tissues alternating with the veins.

There is very little transfusion tissue in the basal and the middle portion of the leaf, but towards the tip it is well-marked (Fig. 2). Seward and Ford (8) have recorded 'centripetal xylem above the protoxylem' in some cases of *Agathis*. In the ordinary leaves of *A. ovata* this is not so clearly seen, but in leaves situated on the peduncle, about a centimeter below the female cone, these centripetal xylem elements are fairly clearly seen (Fig. 3). Thomson (9) in common with Worsdell (10) and Bernard (1), thinks that the transfusion cells above and opposite the protoxylem represent the centripetal

wood. He has shown that in *Agathis* the transfusion cells generally take the place of the centripetal xylem as we go from the base towards the apex.

Reproductive Structures.

1. Female Flowers.

The youngest flower in the collection was only 2·5 c.m. long and 1 c.m. broad. It was clearly differentiated and could easily be distinguished from a vegetative bud. This cone was collected in October and showed the earliest beginnings of ovule-formation on the scales. There was no sign of pollination having taken place Other cones collected at the same time showed two different sizes and stages of development. Some were 2·25 c.m. in diameter and showed early stages of the development of the gametophyte. In these pollination had taken place and pollen-tubes had penetrated into the nucellus and branched there. The megaspore-wall was seen to be uniformly thick all round. The oldest cones collected at the same time were 3·5 c.m. in diameter, and showed fertilisation and beginnings of embryo-formation. These observations fully confirm Eames's (4) conclusions drawn from his observations on *A. australis*, that the female cones appear about October, take a year to form the female gametophyte, and another year elapses before fertilisation. Cones collected in February showed well-advanced embryos. They were about 4 c.m. in diameter. No older cones were available.

(*a*) The Axis of the Female Cone.

In the axis there is a relatively larger pith and the sclerotic cells are fewer than in the stem. Here also the wood may have nucleated and vertically elongated cells, which belong to the medullary-ray system.

The tracheids of the secondary wood sometimes show opposite arrangement of pits, but the 'rims of Sanio' do not seem to be present, at least not in their typical form. Resin-canals, such as have been mentioned by Jeffrey (5) in *A. Bidwilli*, were never seen. Radial rays are more marked than in the stem.

(*b*) Vascular Anatomy of the Female Cone-scale.

In a fertile scale three kinds of arrangements have been observed :—

1. A single trace enters the base of the scale. It divides into 6–10 bundles; the two central strands give off a branch each and this becomes inverted. One of the central stands may again give off another inverted bundle, so that three inverted bundles are produced, all of which enter the base of the ovule.

2. The single trace which enters the scale divides into 4 or 5. The central bundle gives off two inverted strands, one after the other.

These move apart and face respectively the two bundles on the sides of the central one. Higher up two more inverted strands are produced by horizontal splitting of the two bundles which are separated by one or two strands from the central bundle. The four inverted strands thus produced enter the base of the ovule.

3. The single trace on entering the scale splits by a horizontal plane into two bundles with their xylem portions facing each other. The lower strand (with normal orientation) branches into many, three of which cut off bundles with opposed orientation. Here again four inverted strands are produced but by a different process. All these four bundles pass into the base of the ovule.

(c) The Ovule.

The ovule first appears as a protuberance at the base of the cone-scale. It is interesting to note that at this stage there is only one series of bundles in the scale. No inverted bundles were found in any of the sections.

The next available stage in the development of the ovule showed the integument and the nucellus. The middle cells of the nucellus had died off, thus producing a cavity. In the next stages the free nuclei which later form the female gametophyte were found. These nuclei tend to arrange themselves in a parietal layer parallel to the wall of the cavity. Even at this early stage the pollen-tubes are seen to have penetrated the nucellar tissue. In later stages a slight protuberance of the nucellus through the broad micropyle is clearly seen. In one series of sections a kind of 'pavement' or feeding tissue was seen just below the developing female gametophyte (Fig. 4). This has not been mentioned before in any Araucarians. Coulter and Chamberlain (3) mention and figure such a tissue in the ovules of *Gnetum Gnemon*. It consists of closely packed and fairly large parenchymatous cells with prominent nuclei and dense contents. This tissue does not seem to exist very long in the ovule, as it was found in only one ovule.

In the next stage young archegonia had been developed and the megaspore wall, which is thicker at the top, had been burst by the branching pollen-tubes. This bursting was seen to have occurred even though the archegonia were quite young, thus showing that the thickening of the wall at the apex was not produced, as Eames (4) thinks, for the special function of protecting the young archegonium from the depredations of the pollen-tubes. It was also found that the archegonia in a gametophyte developed practically simultaneously. I could not, for instance, discover a very young archegonium in a gametophyte in which others showed a later stage.

The mature female gametophyte is an elongated structure, narrow at the base and somewhat expanded at the top. The archegonia are confined approximately to the upper third part of the prothallus. In many cases a regular zone of flattened cells was observed, which divided the fertile from the sterile portion (Fig. 5.) In no case was any archegonium found below this. This layer seems to suggest a meristematic zone.

(*d*) The Archegonium.

The archegonia occur in groups. Sometimes two archegonia are separated only by a membrane. In development they do not seem to follow acropetal succession as all the archegonia in a gametophyte show practically the same stage of development. The megaspore wall is ruptured by the pollen-tubes very early. A ventral-canal nucleus is cut off (Fig. 6). The egg-nucleus is very large, about 110μ in diameter.

2. Male Flowers.

The microstrobili were all collected in October, hence they were rather young and showed practically the same stage of development. They were about 3.25 c.m. long and about 1.25 c.m. broad.

The wall of the microsporangium is many-layered. The outermost layer consists of broad cells with dark-coloured contents. The tissue between this layer and the tapetum is made up of thin-walled parenchymatous cells, some of which are secretory. Well-defined resin-canals were often seen in this tissue. This fact does not seem to have been recorded before. Seward and Ford (8) have mentioned the secretory cells in this tissue, but they do not mention any resin-canals in the microsporangial walls. The pollen grains found inside the sporangia all show the uninucleate stage.

(*a*) The Male Gametophyte.

Since the available microsporangia showed only the uninucleate stage of the pollen-grains, earlier stages of the development of the male gametophyte could not be studied. An examination, however, of some pollen-tubes taken from the tips of the ovules revealed some very interesting facts. Fig. 14 shows a pollen-tube containing a large cell with dense protoplasm. The cytoplasm of this cell is partially enclosed by a thin membrane which has been ruptured. Several small nuclei are seen close to the ruptured membrane. Evidently the large cell is the body-cell which has produced many nuclei, two of which have become larger to form the male nuclei, while most of the rest have passed out of the cell on account of the rupture of the wall. In order to obtain further evidence on the point sections were cut of the pollen-tubes which were teased out of the

ovules. It was found that beside the two male nuclei there were some other much smaller nuclei also in the body-cell. Another interesting fact in this connection is that some of the nuclei found in the pollen-tubes, which have hitherto been regarded as vegetative nuclei, are of considerable size, not infrequently reaching a diameter of 25μ. This fact supports the view that they are degenerate male nuclei. The two normal male nuclei are equal in size and are spherical or sometimes a little elongated. Fig 7 shows two such male nuclei embedded in dense protoplasm. Their separation and the development of walls round them were not observed. Eames (4), however, records the formation of walls round them. It is significant that he was not able to see the division of the male nucleus into two. This appears to be due to the fact that the body-cell nucleus divides many times, which results in the formation of the two male nuclei and the many smaller nuclei, as mentioned above. The presence of many spermatogenic nuclei beside the two male ones is very interesting. It shows the primitive character of the male gametophyte, as the cell-group produced by the body-cell is easily seen to be antheridial in nature. Lorpriore (7), who has recorded many spermatogenic nuclei in the pollen-tube of *Araucaria Bidwilli*, has already pointed out that this character reminds one of the cell-groups found in the pollen of the Cordaitales, which are also most probably antheridial in nature. Eames's objection (4) as to the primitiveness of the Araucarineae on the ground that many cells of the Cordaitalean male gametophyte might have been spermatogenic, falls to the ground in the light of the discovery of many spermatogenic nuclei in *Agathis ovata*. My observations, however, do not agree with those of Lorpriore as he found that all the spermatogenic nuclei were of equal size. He compared the nuclei of *Ar. Bidwilli* with the large number of male nuclei in *Cupressus Gowenia* (6).

3. Pollination and Fertilisation.

Eames (4) has described the branched and haustorial nature of the pollen-tubes in *A. australis*. In *A. ovata* pollen-tubes were seen to penetrate the nucellus even before the embryo-sac was differentiated. Some tubes were seen to have penetrated the scale. The body-cell remains in the extra-nucellar part of the tube for quite a long time, as has also been observed by Burlingame in *Araucaria* (2).

Figures 8–10 show fertilisation. In Fig. 8 the pollen-tube is seen to penetrate the archegonium round the neck-cells. The cavity of the archegonium is seen to be full of dense male protoplasm. Figure 9 shows the same archegonium in another section, where the fused nuclear mass is clearly seen at the bottom. Figure 10 taken from a different archegonium, shows the two nuclei clearly lying

near each other. The position of the fused nucleus is interesting in view of the fact that in *A. australis*, according to Eames (4), the fusion takes place "near the centre of the archegonium". The protoplasm is seen to be full of food-grains.

Figure 11 shows "pseudo-fertilisation", as described by Eames (4). Here a small nucleus is clearly seen to unite with the egg-cell, the chromatic material having become confluent.

4. The Embryo.

Only three stages of the young embryo were available. In the earliest stage a large number of cells was seen to have been produced, but the walls had not yet been formed. The upper cells were seen to elongate upwards and thus form a sort of cap like that mentioned by Eames (4). Figure 12 shows a later stage. The upper cells have elongated much more. They show anchorage to the top of the archegonium in some sections of the series. The walls have been formed round the proto plasts. The wall surrounding the whole embryo is also quite clearly seen. The next stage is represented in Fig. 13. Here the lower cap-cells have been differentiated and have elongated much. It is interesting to see in this figure that just round the top of the cap-cells the tissue has become corroded, suggesting their penetrating function, which has also been pointed out by Burlingame (2).

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Explanation of Figures.

Fig. 1.—Transverse section through a young branch. × 30.

Fig. 2.—Transverse section through a leaf near the tip. × 100.

Fig. 3.—Transverse section through a leaf from the peduncle. px = protoxylem. × 90.

Fig. 4.—Longitudinal section through a young ovule. × 30.

Fig. 5.—Longitudinal section through a ripe female gametophyte. × 40.

Fig. 6.—Longitudinal section of an archegonium. m = meristematic zone. × 220.

Fig. 7.—Section of a pollen-tube, showing the two male nuclei lying embedded in thick protoplasm. × 220.

Figs. 8-10.—Process of fertilisation. × 220.

Fig. 11.—'Pseudo-fertilisation'. × 175.

Figs. 12-13.—Stages in the development of the embryo. × 65.

Fig. 14.—A body-cell with two large nuclei and other smaller nuclei, as seen inside a pollen-tube. × 100.

Plate I.

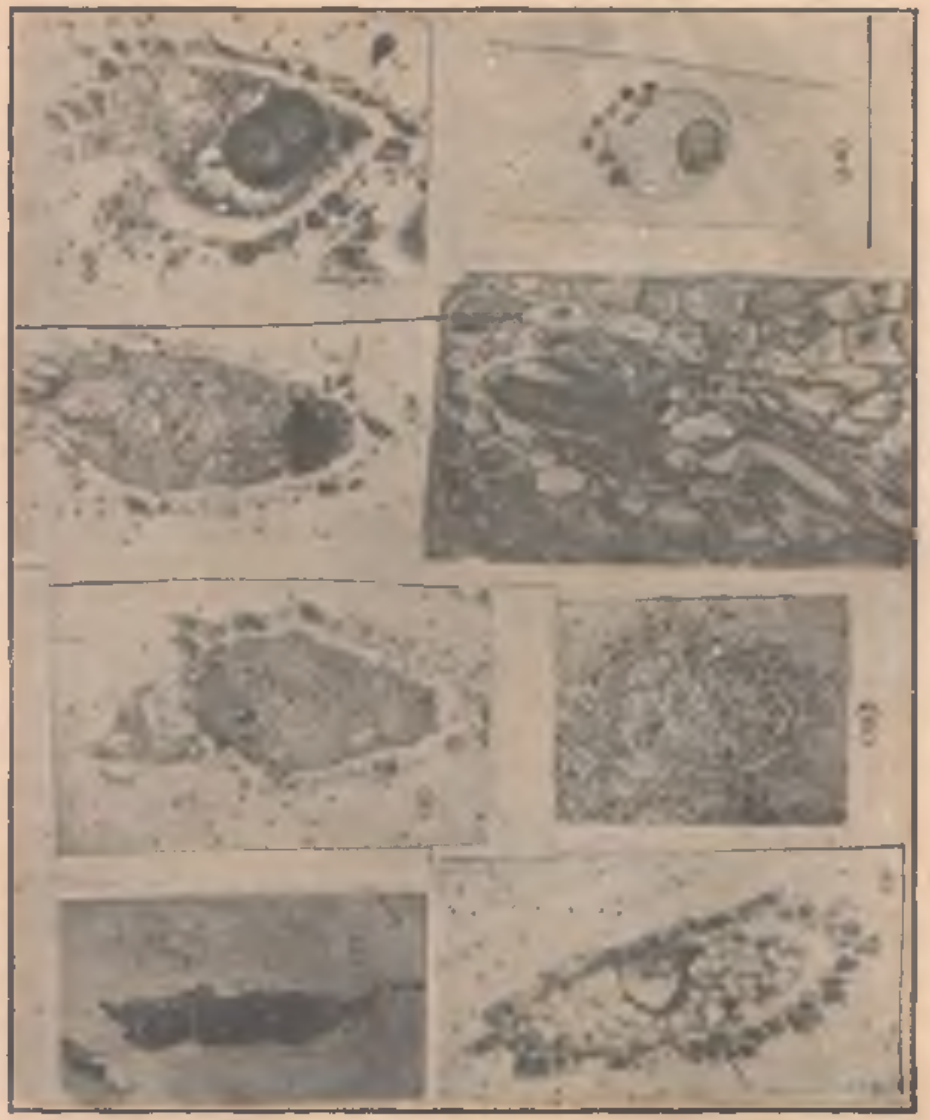

Plate II.