

difficult. For this reason, the two means of the single experiments have been taken together and two new means calculated and their difference tested for significance both by the 't' and the 'Z' tests. For variance due to treatment 'Z' = 1.426 (theoretical 'Z' for  $n_1 = 1$  and  $n_2 = 11$  is = 1.133,  $P = 0.01$ ); 'Z' for variance due to different days or occasion = 0.89641 (theoretical 'Z' for  $n_1 = 11$  and  $n_2 = 11$ , is = 0.7785,  $P = 0.01$ ). 't' = 2.2342 (theoretical 't' = 2.07,  $P = 0.05$ ).

The differences due to treatment as well as for occasion of experiment are thus both real and significant.

We may therefore, conclude from these experiments, based on 195 coleoptiles exposed and 390 test plants, that the first negative phototropic curvature is correlated with differential auxin distribution of the order of 42 per cent. on the dark side and 58 per cent. on the illuminated side.

In regard to the question as to whether exposure to light influences the total auxin-production we find that although the mean totals for controls (viz.  $6.11 + 6.14 = 12.25$ ) and for negative curvature experiments (viz.  $7.19 + 5.14 = 12.33$ ) agree very well, it is not safe to draw any conclusion since we have established from the analysis of variance that variance due to occasion is significant; the excellent agreement may, therefore, be quite fortuitous. Only experiments carried out on the same day can decide this point. Data from two such experiments, viz.

<i>Total Auxin Content</i>		
Date.	Control.	Negative curvature
18/10/35	16.36	13.30 (not significant)
20/10/35	9.74	9.57 " "

seem to suggest that there is no such effect of light.

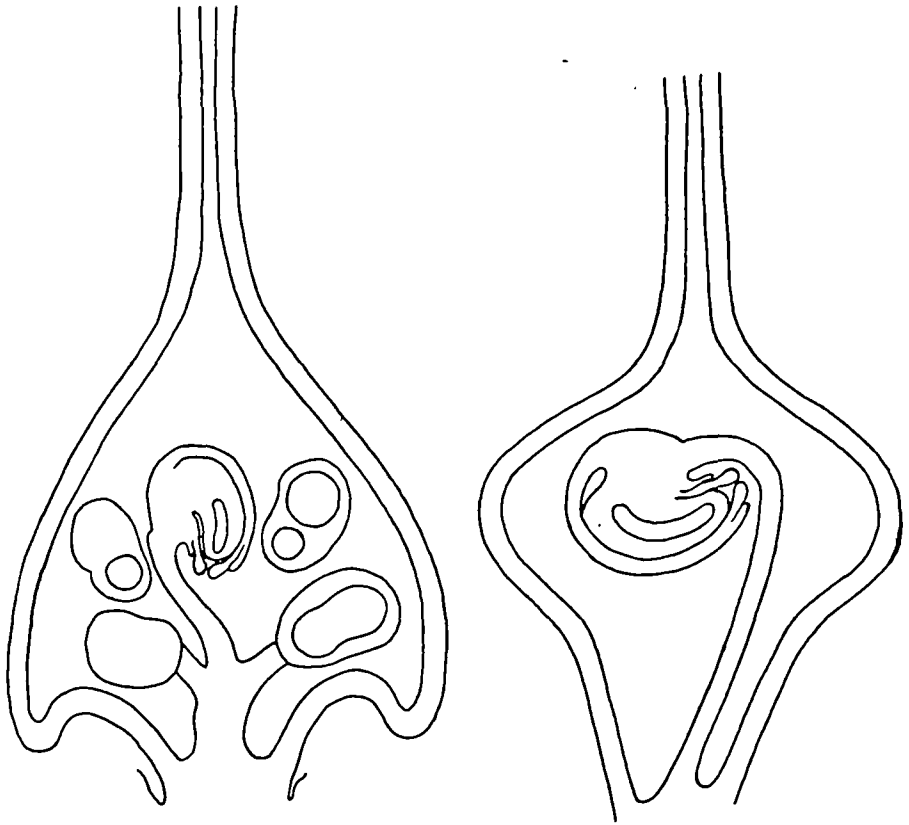
In conclusion the writer wishes to express his thanks to Prof. V. J. Koningsberger, Director of the Botanisch Laboratorium, Utrecht, for generously placing all the excellent facilities of the laboratory at his disposal and for much kindness shown during his stay at Utrecht; and to Dr. F. W. Went for suggesting this problem and for helpful discussion and criticism. The writer's best thanks are also due to other members of the Botanical Staff for unflinching courtesy and much practical guidance.

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**THE NATURE OF THE OVULAR STALK IN POLYGONACEAE AND SOME RELATED FAMILIES.**—In the Polygonaceae the ovule in most genera is nearly sessile, but in the large-flowered forms like *Brunnichia* and *Coccoloba* it possesses a well-developed stalk. This stalk has been generally called a funiculus as in other angiosperms. R. A. Laubengayer, ('Studies in the Anatomy and Morphology of the Polygonaceous flower', Amer. Journ. Bot., xxiv. 329-43, 1937), however, from his recent studies on the anatomy and morphology of the Polygonaceous flower, comes to a different conclusion. He believes that the single-ovuled gynaecium of this family has been derived by reduction from a many-ovuled condition with free central placentation, and the stalk of the ovule represents a reduced free central placenta; because its vascular supply is comparatively large and the vascular elements are so arranged as to suggest not a single morphological trace, but a product of fusion of the ventral traces of the gynaecium and the ovular traces.

It would have been easy to verify Laubengayer's view as to the exact nature of

the ovular stalk if there were forms in the Polygonaceae with many-ovuled gynaecia. It would then be possible to determine whether the ovules in such types were sessile or possessed a true funiculus. Unfortunately such forms are not to be found. The only method, therefore, of testing Laubengayer's views is to compare the structure



FIGS. 1 and 2. Longitudinal sections of ovaries showing the form and arrangement of the ovules. Fig. 1. *Celosia argentea*. Fig. 2. *Pupalia lappacea*.

of the Polygonaceous ovule with that of related families. Amarantaceae and Chenopodiaceae are two such families. Of these, the former is useful for the present purpose, because it includes some genera with many-ovuled gynaecia, while in the great majority the gynaecium, as in the Polygonaceae, is only one-ovuled. Figs. 1 and 2 illustrate the structure and arrangement of the ovules in this family. *Celosia argentea* Linn. (Fig. 1) is representative of forms with many ovules in the ovary borne on a short free central placenta. *Pupalia lappacea* Juss. (Fig. 2) is representative of uni-ovulate forms. A comparison of the two shows that there is no difference in the structure of the ovule. The stalk of the ovule in the many-ovuled forms is as well developed as in the one-ovuled types. It is therefore of the same nature in the two cases. It is a true funiculus and not a reduced free central placenta except perhaps at the basal extremity.

The study of Amarantaceae thus does not support Laubengayer's conclusion. It appears that in the Polygonaceae also the stalk of the ovule in forms like *Brunnichia* is, for the greater part of its length, a true funiculus. At the most only the basal end which is generally found fused with the ovary wall can be regarded as a vestige of the free central placenta. The unusual vascular supply of the funiculus is to be explained by the fact that the ovule in the Polygonaceae terminates the floral axis. Accordingly, the ventral traces of the carpels necessarily pass into its stalk, just as in the family Leguminosae many axial bundles pass into the solitary carpel.

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### AN IMPREGNATION METHOD FOR STAINING STARCH GRAINS.

—Structures in the cell which will not take up stains readily can sometimes be coloured by precipitation of metallic salts. Such impregnation methods are in fairly common use in preparations of animal tissues, but are less often resorted to in botanical microtechnique. A method involving the precipitation of ferric sulphide has been described ('A Plugging Substance in the Vessels of Hops', *Ann. Bot.*, xlii, no. clxviii, pp. 1027 and 1028, Oct. 1928). Similarly, silver iodide can be used for starch grains, rendering them 'brass' yellow (silver nitrate added to tincture of iodine produces a precipitate which as seen under the microscope is of the same colour), or any darker shade desired.

The procedure for this process is as follows: (1) The material should be fixed in acetic alcohol so that the leucoplasts stain well with eosin. (2) A solution of iodine in potassium iodide is left upon the section for about 20 seconds. (3) The section is washed with water, treated with silver nitrate for 20 seconds, and again washed. The process (2 and 3) is repeated. (If desired, the starch grains can be rendered darker by repetition, but in this case their centres are generally of a lighter shade than the rest of the grain. After fifteen repetitions they go nearly black. (4) It is then better to dip the slide into acid hyposulphite of soda for a few seconds only, in order to remove strings of small crystals which otherwise spoil the appearance of the preparation. (If left in this solution for longer, the yellow colour gradually disappears, beginning apparently at the surface and spreading to the centre of the grains.) (5) Haematoxylin is a satisfactory stain for the cell walls and 2 per cent. eosin should be used for ten minutes for the leucoplasts. (The nuclei of the cells also take up the eosin.)

Although leucoplasts are sometimes difficult to find in fixed material, this process is particularly useful for drawing attention to them, because while they are not affected by the deposition of silver iodide their red colour is in good contrast with the yellow starch grains. The method brings out the starch grain laminations, giving to them the appearance of rough 'turning' marks (e.g. in *Phajus grandiflora*).

No success was achieved with the use of other metals which form iodides. Treatment with potassium iodide, in which there is no dissolved iodine, followed by silver nitrate produces no result, while the use of tincture of iodine with this silver salt does give the characteristic yellow colour to the grains. This yellow colour is not