Proceedings of the Iowa Academy of Science

Volume 1 | Part 4, 1893; (1887) - Article 33

1893

The Paraffine Method Applied to the Study of the Embryology of the Flowering Plants

H. W. Norris

PODASPHÆRA OXYACANTHÆ (D C), Duby on *Prunus Cerasus.*
MICROSPHÆRA RUSSELII, Clinton on *Oxalis corniculata* var. *stricta.*
*M. GROSSULARIÆ (Wallr.) Lev. on *Sambucus Canadensis.* New.
M. EUPHORBIÆ (Peck), B. & C. on *Euphorbia corollata.* New.
M. ALNI (D C), Winter on *Viburnum lentago, Syringia vulgaris.*
M. QUERCINA, (Schw.) Burrill on *Quercus rubra.*

THE PARAFFINE METHOD APPLIED TO THE STUDY OF THE EMBRYOLOGY OF THE FLOWERING PLANTS.

BY H. W. NORRIS.

These few notes are given, not that they contain much if anything new, but simply as the record of a year's experimenting. The difficulties connected with the use of paraffine in the sectioning of plant tissue are well known to all students in botanical microscopy. The cutin, cork, etc. of the cell wall resist penetration. The heat necessary to melt paraffine often renders the tissue too hard and brittle for successful manipulation. Free-hand sectioning is often the only available method. Frequently this is all sufficient. Celloidin (or collodion) is available for imbedding young and soft tissues, requires no heat and its general cleanliness and easy manipulation recommends its use whenever possible. But many plant tissues are of too firm and resisting a structure to render the use of celloidin even possible. Seeds in their mature condition, will not permit the use of celloidin, and seem to almost defy the penetration of paraffine.

In attempting to study the development of ovule in the Compositæ, I was led to find some way of obtaining perfect series of sections through the flower. The forms studied were *Grindelia squarrosa, Helianthus annuus,* and a cultivated species of *Ageratum.* In most of the Compositæ the tissues of the flower become very resistant to the section knife, even at an early period. The testa of the seed is not easily penetrated by reagents. The peculiar structure of the ovule found in many Compositæ, called *endodermis* by Hegelmaier, becomes very hard and brittle on application of heat.

Rowlee[1] obtained good sections of ripe seeds by the paraffine method, after first soaking them in water twenty-four hours before dehydration. Having seen his sections I determined to try some modification of his method. As I did not study the mature condition of the ovule, I did not soak any of the material in water.

The tissue was hardened first in 25% and then 50% alcohol, and preserved in the latter. Then as material was needed it was dehydrated in a Schultze's dehydrating apparatus into 95% alcohol, then placed in the following substances successively, one to several days each: 95% alcohol and

[1]Imbedding and Sectioning Mature Seeds, Proceedings American Society Microscopists, 1890.

chloroform equal parts, pure chloroform, chloroform with a small per cent of paraffine dissolved, increasing the percentage of paraffine from time to time, using just heat enough to to keep the solution a liquid, "soft" melted parafine, finally "hard" melted paraffine. The time required for the process was sometimes two to three weeks, but with the younger tissue, much less. As will be seen, I followed the ordinary method, but used more time. I am satisfied that many of the so-called insuperable difficulties connected with paraffine infiltration can be overcome by patience and time-serving.

Turpentine, I did not find as satisfactory a reagent as chloroform, probably because the latter will penetrate even if dehydration is not complete. I find alcohol a stisfactory hardening reagent. McClatchie recommends the use of chromic acid in hardening plant tissue. I failed to see its superiority over alcohol.

The staining was done mostly on the slide. Most of the ordinary nuclear stains worked well. The most satisfactory stains all arouud were Czokor's Alum Cochineal for the nucleus, and an alcoholic solution of bismarck brown for the cell wall. When managed properly saffranin gave most beautiful results. Alum-cochineal, borax-carmine, saffranin, haematoxylin, fuchsin, and picro-carmine utterly failed to penetrate the specimens in mass. Orth's lithium-picro-carmine was the only stain that penetrated in mass enough to differentiate the structure of the embryo-sac.

THE DEVELOPMENT OF THE AUDITORY VESICLE IN NECTURUS.

BY H. W. NORRIS.

Owing to the lack of a complete series of embryos, I have been unable to trace the earlier stages of the development of the ear. In all the Amphibia, so far as studied, unless we except the species of Axolotl figured by Houssay, and he was doubtless in error, the ear arises as a differentiation of the inner of the two layers into which the ectoderm is early divided. This inner sensory layer thickens on each side of the head so as to form a small sensory tract, the *anlage* of the ear, closely analogous, if not homologous, in formation to the lateral line sense organs. An ingrowth or inpushing of the thickened ectoderm results in the formation of a pit. The outer layer of indifferent ectoderm takes no share in the formation of the auditory vesicle, but it is slightly involuted into the opening of the pit. The pit deepens, its edges approach each other until the pit becomes a closed vesicle. This description applies to development of the ear of the frog as studied by Villy[1] and of the salamander, Amblystoma, as studied by myself[2].

[1] Development of the Ear and Accessory Organs of the Frog, Quart. Jour. Mic. Sci., No. CXX., 1890.

[2] Development of the Ear of Amblystoma. Jour. Morph., Vol. VII, No. 1, 1892.