

METHODS OF INFILTRATING AND STAINING IN TOTO THE HEADS OF VERNONIA.

By E. H. HEACOCK.

In beginning a study of the development of the embryo sac of *Vernonia*, two difficulties at once present themselves. The first is to properly stain the head in toto, and the second to infiltrate with paraffine so as to be able to section properly.

The form is an ordinary composite, having twenty or more flowers in each head. The ovary is surrounded by a thick, solid integument several layers of cells deep, and the difficulty lies in penetrating this coat.

Before staining or infiltrating, all parts of the head above the achenes were cut off, thus securing a smaller body and a more ready penetration. Heads dehydrated by 96 per cent. alcohol were washed out in distilled water, the water being frequently changed, until they sank to the bottom of the vial. They were then stained for seventy-two hours in alum cochineal (Czoker's Formulae). When sectioned they showed but a faint trace of stain. Heads dehydrated by absolute alcohol and stained for seventy-two hours in borax carmine, Kleinberg's and Delafield's hamatoxylin gave no better results.

Heads which had been dehydrated by absolute alcohol, washed out in distilled water and stained for seven days in alum cochineal (Czoker's Formulae) gave good results. The general tone of the stain is dim, but under high power the differentiation is very fine. Heads dehydrated by absolute alcohol and stained for seven days in borax carmine gave very fair results. These results point to the fact that with long time treatment the heads may be successfully stained.

The second difficulty: infiltrating with paraffine. The first medium used was turpentine. Heads dehydrated by 96 per cent. alcohol were placed in turpentine for twenty-four hours, then into a mixture of one-half turpentine and one-half paraffine for twenty-four hours, thence into pure paraffine (48° C.) for twenty-four to forty-eight hours. On sectioning the sections were found to tear out in the center, thus proving that paraffine had not reached the center of the head. Heads dehydrated by absolute alcohol were given the same treatment. In this series the small heads showed an improvement, but still the normal sized heads were not properly infiltrated.

The next medium used was a mixture consisting of one-half cedar oil and one-half xylol. Heads dehydrated by absolute alcohol were placed in this medium for twenty-four hours, then into a mixture of one-half the medium and one-half

paraffine for twenty-four hours, then into pure paraffine (48° C.) for twenty-four hours. On being sectioned this material showed but little, if any, improvement over the material treated with turpentine.

The next medium used was xylol. Heads dehydrated by absolute alcohol were placed in xylol for twenty-four hours, then into a mixture of one-half xylol and one-half paraffine for twenty-four hours, then into pure paraffine (48° C.) for twenty-four hours. When sectioned on a Heidelberg microtome the heads were found to be well infiltrated and made fine ribbons. A series of experiments was then begun on heads dehydrated by absolute alcohol, giving them shorter time periods. It was found that heads treated with xylol for three and one-half hours, then to a mixture of one-half xylol and one-half paraffine for two hours, then to pure paraffine (48° C.) for two and one-half hours, were infiltrated, and sectioned just as well as heads which had had the extended time treatment.

The conclusions to be drawn seem to be, first, that large heads of composites may be stained successfully in toto, but to insure success a long time is necessary. Incidentally it may be said that, so far as tests made have gone, alum cochineal gives decidedly the best differential stain.

Second, that successful infiltration can be made in a time as short as eight hours by the use of xylol, a longer treatment being unnecessary. That a treatment with turpentine and a mixture of cedar oil and xylol, as far as *Vernonia* is concerned, gives unsuccessful results with the normal sized heads. Cedar oil alone was not tried, nor are the experiments as to methods yet completed. They are given, however, in the hope that suggestions may be made that will extend their scope and lead to more definite conclusions.

EMBRYOLOGY OF THE RANUNCULACEAE. BY D. W. MOTTIER.

CERTAIN CHEMICAL FEATURES IN THE SEEDS OF *PLANTAGO VIRGINICA* AND *P. PATAGONICA*. BY ALIDA M. CUNNINGHAM.

In the study of the genus *Plantago*, to ascertain the value of seed characters in determining specific rank, the peculiarities hereinafter described were noticed as among the results of some of the experiments. These results, in themselves, are perhaps of little or no value in determining the question under investigation, yet, they are so closely connected with the experiments, and altogether so peculiar as to warrant a somewhat extended research.