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## CONTRIBUTION TO THE LIFE HISTORY OF SANGUINARIA CANADENSIS.*

FRANK M. SURFACE.

Sanguinaria is a monotypic genus of the Eastern United States belonging to the family Papaveraceae. Apparently no very great amount of morphological work has been done on the members of this family. Shaw (3) in a recent paper gives some observations on certain Papaveraceae among which is Sanguinaria. Some of the conclusions in the following paper do not agree with his. This may possibly be due to a difference in climatic conditions between the two stations where material was collected, although Shaw does not state definitely, the locality from which his material came.

The following study of the development of Sanguinaria canadensis L. was begun in April, 1904. Material was collected at intervals of about one week until the middle of June when the seeds were mature and had fallen to the ground. In the fall material was collected in October, November and December, while in the Spring of 1905 young capsules were taken at intervals of about two weeks from March 1st to the middle of April.

The material was killed and fixed in chromo-acetic acid, passed through the alcohols and imbedded in paraffin. Sections were cut on a rotary microtome 10–12 microns thick. In the younger stages the entire ovulary was cut, either transversely or longitudinally but in the older stages the individual ovules were sectioned. The orientation of the ovule was not difficult owing

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to the distinct crest developed along one side. In the oldest stages it was found necessary to remove the hard covering of the seed before cutting. Several stains were employed among which were anilin-safranin followed by genetian violet, Delafield's haematoxylin and Haidenhein's iron-alum-haematoxylin. The latter well known stain proved to be the most successful in the stages of the development of the megaspores and embryo-sac, while Delafield's stain gave the best results with the microspores.

The flower of Sanguinaria begins its development early in the summer and the ovules and stamens are considerably advanced by the end of September. Material taken on Oct. 6th showed the single, hypodermal, archesporial cell distinctly differentiated (Fig. 2). The ovules at this time were just beginning to curve but no traces of the integuments were discernable (Fig.1). At this time the microsporocytes were just separating (Fig. 19) and a few of the tapetal cells already had two nuclei. There are three intermediate layers between the epidermis and tapetum (Fig. 19). Material taken on Nov. 9th showed but little change in the development of the ovules, except that they had elongated and the curving was much more marked. The integuments were just beginning to make their appearance but the archesporial cell was still undivided. At this time the microsporocytes had undergone some division and some tetrads were observed. The tapetal cells contained two nuclei (Fig. 20).

By Dec. 9th the archesporial cell had divided twice giving rise to the megasporocyte with two parietal cells above (Fig. 3). The curving of the ovule was much more marked than in the previous stage and the beginnings of both integuments could be readily seen in central sections. The division of the microsporocytes must have proceeded rather rapidly for at this time the microspores were present with large resting nuclei and the tapetum was dissolving (Fig. 21). In this stage the microspores pass the winter.

No material was taken from December until the first of March when it seemed that the weather conditions were favorable for the continued growth of the plant. Sections of material fixed March 1st showed that there had been but little activity during the winter months. One of the parietal cells had again divided so as to form a row of three cells above the large megasporocyte (Fig. 4). In some instances transverse division had taken place forming a considerable tissue (Fig. 5). The integuments had increased quite a little in size and now reached more than half way around the developing ovule. The curving of the ovule was complete at this time. The microspores were in practically the same stage in which they were found in December. The nucleus was undivided but the vacuole in the center

of the microspore had enlarged so that the nucleus was forced to one side of the cell. Material taken on March 13th showed but little change from that taken on the previous date. The megasporocyte had enlarged quite a little and seemed to be preparing to divide (Fig. 4), and a few spindles were observed (Fig. 6). The microspores showed no change.

Material fixed on March 22nd showed that the megasporocyte had divided into four megaspores the lowest being the large functional one (Fig. 7). The arrangement of the megaspores varied considerably. In some cases the transverse walls were nearly parallel forming a row of cells but frequently they were very irregular (Figs. 7, 8). These divisions are interesting in that they show a great similarity to the divisions of the microsporocyte and often result in a nearly typical tetrad (Fig. 8). Above the megaspores the rows of parietal cells could be distinctly seen (Fig. 7). These varied in number but it is evident that considerable division has occurred in the three original parietal cells. Vesque (1) states that Papaver orientale has no parietal cells. The nuclei of the microspores were divided at this time (Fig. 21). Pollen grains taken at later dates did not seem to show any further division. It is probable that the generative cell does not divide until after the tube has been formed. Strasburger (2) however, reports that in Papaver the generative nucleus divides in the pollen grain.

From this time the development of the functional megaspore is rather rapid. Material taken on March 28th showed the complete eight celled embryo-sac and often the two polar nuclei had already fused to form the definitive nucleus (Fig. 9). The antipotals are rather large at this stage and each has a single nucleus. Material taken on April 10th showed the oosphere and definitive nucleus still undivided but the antipodals were very large and each contained two nuclei (Fig. 10). This stage corresponded with material taken the year before at about the same date. The egg then seems to be fertilized about the first week in April. Many of the sections show remains of the pollen tube which is very prominent (Figs. 10, 11). The synergids seem to disappear early. Remains of one or more of them could usually be seen at this stage, lying to one side of the oospore and staining very dark (Figs. 10, 11). The early divisions of the embryo seem to occur very slowly for it was not until May 12th that the first division was observed (Fig. 13). This makes a remarkably long period of rest for the fertilized egg. In material killed May 16th the three celled embryo was found (Fig. 14). The divisions of the definitive nucleus had begun in early April and by the latter part of that month the endosperm had 20 to 30 nuclei.

The development of the embryo from this stage on was not traced very closely but the divisions must occur rather rapidly for on May 23rd the embryo had reached about the 12-celled stage (Fig. 15). On May 29th a large spherical embryo was present (Fig. 16). The suspensor had lengthened considerably and sections showed a double row of cells (Fig. 16). At this time the antipodals were degenerating (Fig. 17). They lie in a broad depression at the base of the sac which is greatly enlarged and filled with endosperm. The behavior of the antipodals recalls the condition of these cells in certain Ranunculaceae. On June 6th the ovules had matured and were falling to the ground. The integument is very hard and it was found necessary to remove it before imbedding in paraffin. At this time the embryo was much larger and the two cotelydons were well developed (Fig. 18). The suspensor was still present but showed signs of degeneration.

SUMMARY.

The flowers of Sanguinaria begin to develop very early in the summer previous to the year in which they blossom.

The development of the microsporocytes and microspores is much more rapid than that of the megaspores for the microspores are formed before the winter season begins.

The ovule passes the winter in the megasporocyte stage and during March its development is very rapid while the microspore does not renew activity until the last of March and early April.

Three parietal cells are formed and later these divide forming a parietal tissue of considerab'e size.

The division of the megasporocyte frequently results in rather typical tetrads.

The generative nucleus does not appear to divide in the pollen grain.

The long resting period of the oosphere is especially interesting.

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## DESCRIPTIONS OF PLATES.

All drawings were outlined under a camera lucida with the following optical combinations:

Bausch and Lomb—1 oc., $\frac{2}{3}$ obj.—Fig. 1.
Bausch and Lomb—1 oc., $\frac{1}{6}$ obj.—Figs. 20, 21.
Bausch and Lomb— $\frac{3}{4}$ oc., $\frac{1}{6}$ obj.—Figs. 2-8, 13, 15-17, 22, 23.
Bausch and Lomb—2 oc., $\frac{1}{6}$ obj.—Figs. 18, 19.
Leitz—4 oc., 7 obj.—Fig. 10.
Leitz—6 oc., 7 obj.—Fig. 14.
Zeiss—18 oc., Leitz 7 obj.—Figs. 9, 12.
Zeiss—18 oc., Leitz 3 obj.—Fig. 11.

Fig. 1. Section of young ovulary, showing incipient ovules. Killed 10–6–'04.
Fig. 2. Archesporial cell. Killed 10–6–'04.
Fig. 3 Megasporocyte with two parietal cells. Killed 12–9–'04.
Fig. 4. Megasporocyte with three parietal cells. Killed 3–13–'05.
Fig. 5. Megasporocyte with parietals divided. Killed 3–1–'05.
Fig. 6. First division of megasporocyte. Killed 3–13–'05.
Fig. 7. Four megaspores and parietal cells. Killed 3–22–'05.
Fig. 8. Four megaspores showing tetrad arrangement. Killed 3–22–'05.
Fig. 9. Eight celled embryo-sac. Killed 3–28–'05.
Fig. 10. Embryo-sac with oospore. Antipodals with two nuclei. Killed 4–16–'04.
Fig. 11. Outline of ovule with oospore. Killed 4–10–'04.
Fig. 12. Oospore with remains of synergids. Killed 4–16–'04.
Fig. 13. Two-celled embryo. Killed 5–12–'04.
Fig. 14. Three-celled embryo. Killed 5–16–'04.
Fig. 15. Young embryo showing eight cells in section. Killed 5–23–'04.
Fig. 16. Spherical embryo with long suspensor. Killed 5–29–'04.
Fig. 17. Degenerating antipodals. Killed 5–29–'04.
Fig. 18. Embryo showing large suspensor and cotyledons at the time the seed falls. Killed 6–6–'04.
Fig. 19. Section of stamen with microsporocytes and tapetal cells. Killed 10–6–'05.
Fig. 20. Section of microsporangium showing microsporocytes just before formation of tetrads. Tapetal cells with two nuclei. Killed 11–9–'05
Fig. 21. Microspores with remains of tapetum. Killed 12–9–'05.
Fig. 22. Single microspore. Killed 12–9–'05.
Fig. 23. Pollen grain. Killed 3–22–'05.

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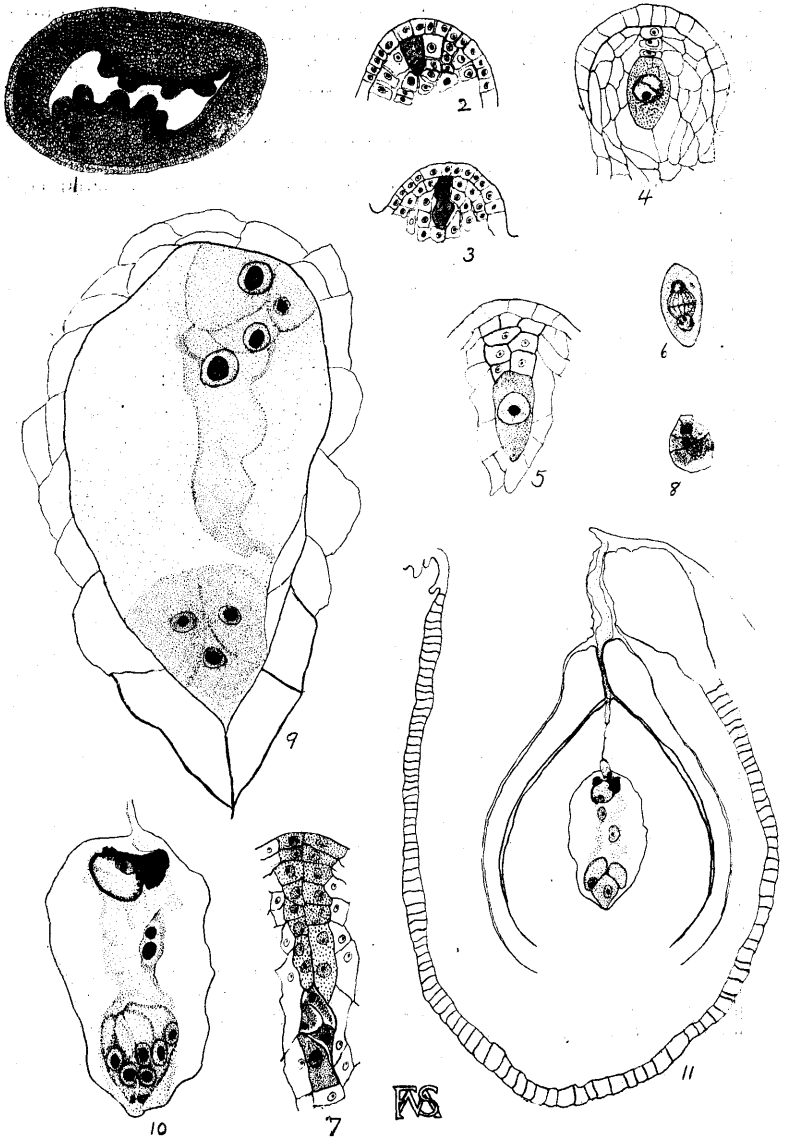
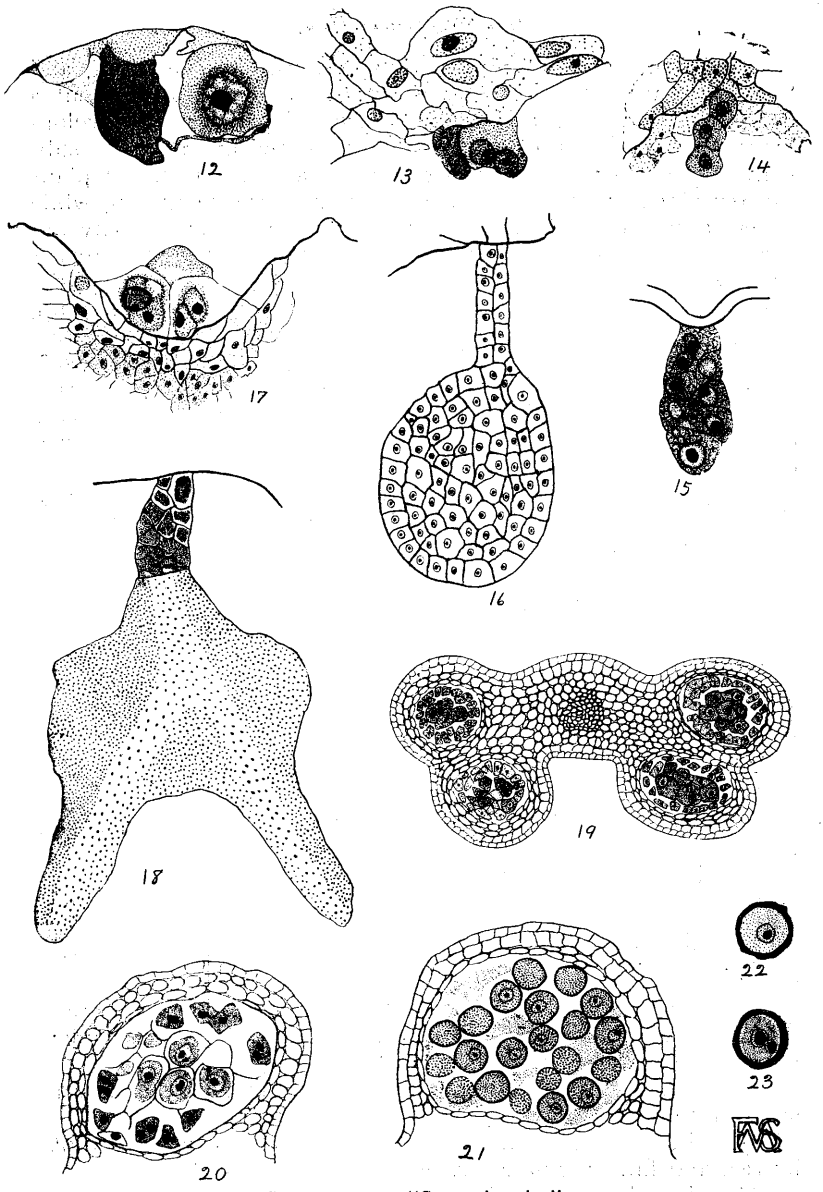



SURFACE on "Sanguinaria."




SURFACE on "Sanguinaria"