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## CONTRIBUTION TO THE LIFE HISTORY OF *ASIMINA TRILOBA*.\*

WILLIAM B. HERMS.

The material for this study was collected in the outskirts of Columbus, Ohio, along the banks of the old canal, where the papaw grows quite abundantly. The young buds were killed in a one per cent. solution of chromo-acetic acid, within ten to twenty minutes after collecting them. Before killing, the sepals and petals were removed to secure better penetration.

The first collection was made Sept. 30, 1905, and continued weekly until the middle of December, when collections were made every two weeks until the middle of February, at which time weekly collections were again resumed. During the early part of June collections were made twice per week. The entire ovulary was imbedded in paraffin and serial sections cut ten microns thick with a rotary microtome. Several staining methods were employed, of which the double stain anilin-safranin followed by gentian violet was the best for the early work on the megaspores and microspores, while Delafield's Haematoxylin gave the best results for the later stages, e. g., development of the embryo sac and late microspores.

The work was carried on in the Botanical Laboratory of the Ohio State University under the direction of Professor J. H. Schaffner to whom the writer wishes to express his thanks for advice and helpful criticism freely given.

**OVULES AND MEGASPORES.** The first sections cut of Sept. 30, 1905, (Fig. 1), showed an undifferentiated condition of the ovules. No cell could be distinguished that might eventually give rise to the archesporial cell. This undifferentiated condition is retained throughout the winter as is evident from Fig. 2 (Jan. 6). The first sections showing the archesporial cell, which is

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\* Contributions from the Botanical Laboratory of the Ohio State University, XXXII.

hypodermal, were of April 14 (Fig. 3). No integuments have as yet appeared. From this time on development and differentiation is rapid. Fig. 4 (April 21) shows the archesporial cell divided into parietal cell and megasporocyte. The parietal cell has divided into two by a vertical wall, and the incipient integuments are now visible as may be seen in Fig. 5. This figure also shows a further division of the parietal layer. By April 28 (Fig. 6) the megasporocyte has divided into four degaspores which are arranged in a row, the three upper of which at once begin dissolving. An extensive parietal tissue is formed by this time (Fig. 7). In Figs. 7 and 8 the outer megaspore is divided by a vertical wall, while in Fig. 6, the division is horizontal. This latter condition was the more commonly observed. The arrangement of the megaspores in a more or less perfect tetrad indicates a rather primitive position of the plant under consideration. The same condition was noted by Surface<sup>1</sup> for *Sanguinaria canadensis* and by Shreve<sup>2</sup> for *Sarracenia purpurea*.

**EMBRYO SAC.** The functional megaspore divides about April 28, forming the two-celled embryo sac (Fig. 9). The non-functional megaspores are gradually dissolving. This date also shows some ovules having embryo sacs with conjugating polar nuclei, the three antipodals, synergids and egg (Fig. 10). The synergids are arranged in such a manner that together with the egg they form a sort of tripod (See Figs. 10, 12 and 13). The embryo sac elongates very greatly during the next week or two (Fig. 11) and the antipodals come to lie close together in the base of the sac and are still plainly visible in sections of May 19 (Fig. 15), though beginning to degenerate. This evanescent condition of the antipodals is very different from what would be expected in *Ranunculaceae*.

The polar nuclei occupy a characteristic position but remain side by side for an unusually long time, apparently about three weeks, before conjugating (Figs. 10 and 11). The various structures of the embryo sac are all clearly differentiated by staining, so that interpretation is not difficult.

Together with the long period during which the polar nuclei remain in contact without fusion, should be noted the equally long time that the oospore remains undivided after the beginning of the endosperm formation. May 19 (Fig. 16) shows the oospore still undivided, but there are already formed a dozen or more endosperm cells.

**ENDOSPERM AND EMBRYO.** In sections of May 19 (Fig. 12) the first endosperm wall was observed. This wall is transverse and divides the sac into two equal parts. The endosperm nuclei

1. Surface, Frank M., '05.—Contribution to the Life History of *Sanguinaria canadensis*. *Ohio Nat.* 6 : 1, 1905.

2. Shreve, Forrest, '06.—The Development and Anatomy of *Sarracenia purpurea*. *Bot. Gaz.* 42 : 107, 1906.

now divide rapidly as indicated by Figs. 13 and 16, forming a linear series of endosperm of about a dozen cells, with transverse walls, when a vertical wall appears in the base of the sac (Fig. 16). The endosperm grows rapidly until there is formed a narrow strip throughout the length of the seed, the upper end of which is shown in Fig. 17. The order of the endosperm division was not determined but the indications are that the divisions are not basipetal, as Strasburger<sup>3</sup> found for *Ceratophyllum*, though the first division of the sac into two halves is similar. Figs. 12, 13 and 16 show what has taken place. Apparently the endosperm divides into two cells, each of which divides again and so on until perhaps a dozen cells have been formed in a linear series when vertical division takes place as already noted.

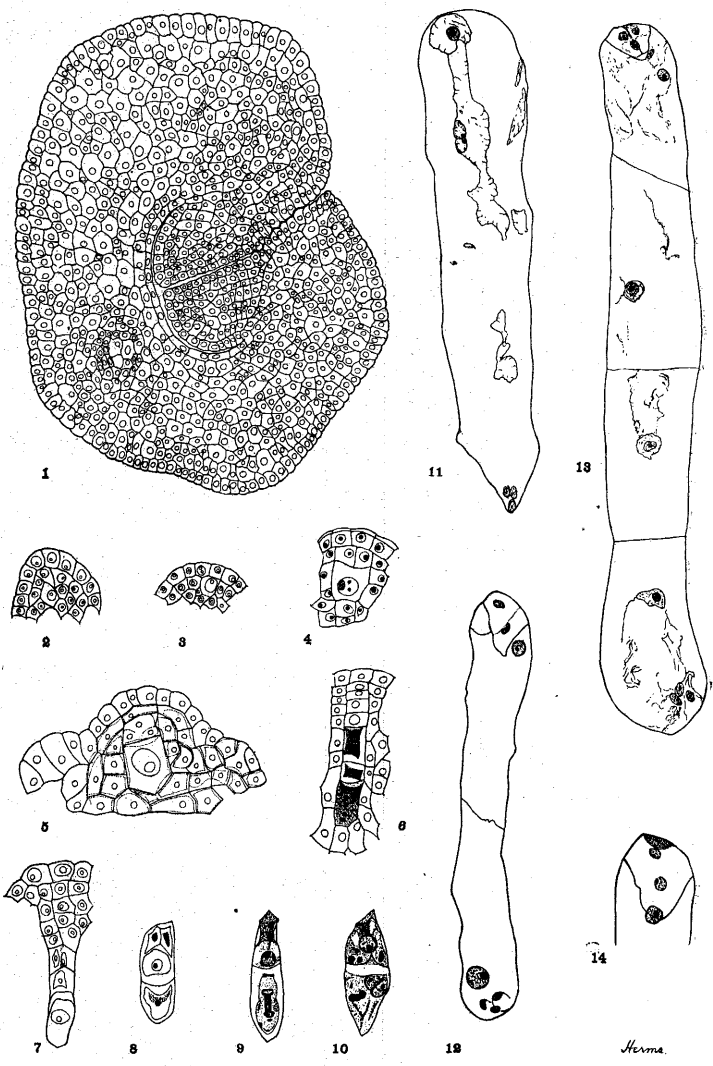
The germination of the oospore is very much delayed, as has already been pointed out. The first wall is transverse and the subsequent divisions quite irregular. The embryo in the mature seed is very minute and imperfectly developed, the greater part of the seed being occupied by a peculiar wrinkled tissue of the wall of the ovule. Fig. 17 (June 10) shows the upper end of the endosperm column with the minute embryo and remnants of the pollen tube.

**MICROSPORANGIUM AND MICROSPORE.** The first sections made (Sept. 30) showed the differentiation of the microsporophylls each with four microsporangia. Fig. 18 shows one of the microsporangia in which may be seen a number of microsporocytes in cross section, of which two show a somewhat greater development and more prominent nuclei. This condition is not altered throughout the winter as is shown by Fig. 19 of Jan. 6. Fig. 20, of March 10, shows the growth of the microspores at that date, being the earliest to show what is really taking place. It can be seen at once that some of the sporogenous tissue is breaking down and that only a few microsporocytes (usually two in cross section) are building up and growing at the expense of the surrounding cells. Fig. 21 (April 14) is interesting since here may be seen the differentiation of a bridge of tissue between the microsporocytes. The nuclei of these cells have divided as well as those of the surrounding tapetal cells. By April 21 (Fig. 22) the spore tetrads are formed and the sterile tissue in the sporangium is dissolving rapidly. By April 28 (Fig. 23) the sterile tissue and tapetum has completely dissolved. May 5 (Fig. 24) the pollen grains are ready to be shed, the generative and tube nuclei being formed.

3. Strasburger, Eduard, '02.—Ein Beitrag zur Kenntniss von *Ceratophyllum submersum* und phylogenetische Erörterungen. *Jahrb. f. wiss. Botanik.* 37: 477-526. Pls. 9-11. 1902.

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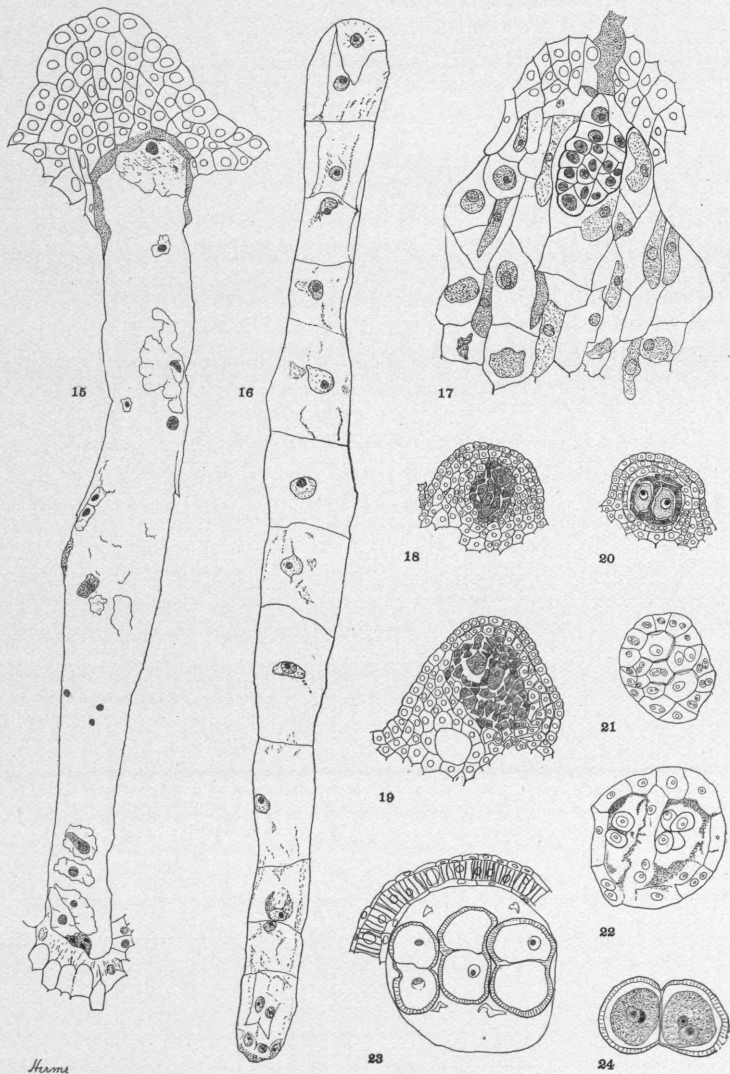
Plate XV.



HERMS on "Life History of *Asimina triloba*."

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Plate XVI.



Herms

HERMS on "Life History of *Asimina triloba*."

## SUMMARY.

1. The archesporial cell of *Asimina triloba* remains undifferentiated during the winter, no differentiation being apparent until about April 14.
2. Tetrad megaspores are of rather frequent occurrence.
3. The parietal layer of the ovule develops greatly.
4. The great elongation of the embryo sac is striking.
5. The length of time that the polar nuclei remain in contact is quite unusual (three weeks and over).
6. The evanescent condition of the antipodals is rather unexpected in this form.
7. The oospore remains undivided relatively long (between three and four weeks).
8. Endosperm forms in a peculiar manner. The first wall is transverse and divides the sac into two equal parts. The formation of a linear series of endosperm now follows, continuing until about a dozen cells are formed when a vertical division begins at the base of the sac.
9. The embryo is minute and imperfectly developed, even in the seed.
10. Comparatively few large microsporocytes are formed.
11. There is a peculiar development of sterile tapetum-like tissue in the microsporocytes.
12. The pollen grains are ready to be shed by May 5.
13. The study shows that *Asimina triloba* differs in its development from the Ranunculaceae and Papaveraceae and,
14. Resembles the Ceratophyllaceae more or less closely in its development.

## DESCRIPTION OF PLATES.

All drawings were made with the use of the camera lucida at table distance. A Bausch and Lomb microscope was used with the combination lenses indicated with each description.

FIG. 1.—Sept 30, 1905. Cross section of ovulary showing undifferentiated ovules. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 2.—Jan. 6, 1906. Section of ovule showing undifferentiated condition still present at this date. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 3.—April 14, 1906. Tip of ovule showing the differentiated archesporial cell. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 4.—April 21, 1906. Shows archesporial cell divided into parietal cell and megasporocyte. The parietal cell is divided into two by a vertical wall. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 5.—April 21, 1906. Tip of ovule showing incipient integuments and further division of parietal layer. B. & L. 1 in.— $\frac{1}{2}$  in. oil immersion.

FIG. 6.—April 28, 1906. Shows four megaspores arranged in a row; the three upper ones are dissolving. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 7.—April 28, 1906. Shows extensive parietal tissue and four megasporocytes, the two outer divided by a vertical wall. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 8.—April 28, 1906. Same as Fig. 7.—Four megaspores, the two outer ones divided by a vertical wall. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 9.—April 28, 1906. Two celled embryo sac with three megaspores above. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 10.—April 28, 1906. Shows conjugating polar nuclei, three antipodals and egg apparatus. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 11.—May 19, 1906. Mature embryo sac showing conjugating polar nuclei, the egg and antipodals. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 12.—May 19, 1906. Embryo sac with two endosperm cells, separated by a wall, also the vanishing antipodals, oospore and synergids. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 13.—May 19, 1906. Embryo sac showing three endosperm walls, three vanishing antipodals and complete egg apparatus. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 14.—May 19, 1906. Showing oospore with one endosperm nucleus and remnants of pollen tube. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 15.—May 26, 1906. Embryo sac without definite endosperm walls and with dissolving antipodals. (The delicate walls have probably been destroyed by the technique.) B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 16.—May 19, 1906. Embryo sac showing thirteen endosperm cells, the lowest one divided by a vertical wall. The egg is still in the oocelled stage and the antipodals are still distinct. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 17.—June 10, 1906. Showing young embryo surrounded by endosperm tissue. The remnants of the pollen tube are visible. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 18.—September 30, 1905. Section of sporophyll showing microsporocytes, of which two are somewhat larger than the others. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 19.—Jan. 6, 1906. Same as Fig. 18, except that there is a very slight enlargement of the two microsporocytes. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 20.—March 10, 1906. Showing two microsporocytes developed and others undeveloped. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 21.—April 14, 1906. Microsporocytes with a bridge of sterile tissue between them. Note the dividing nuclei of the sterile tissue, also of the tapetal layer. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 22.—April 21, 1906. Spore tetrads. On the one side only two are visible. The sterile tissue surrounding the tetrads is dissolving. B. & L. 1 in.— $\frac{1}{8}$  in.

FIG. 23.—April 28, 1906. Spore tetrad. The sterile tissue and tapetal layer have completely dissolved. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.

FIG. 24.—May 5, 1906. Pollen grains with generative and tube nuclei. B. & L.  $\frac{1}{2}$  in.— $\frac{1}{8}$  in.