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A study of multiple embryo development in the netleaf hackberry (celtis reticulata)

Gary Porter Lawrence
Brigham Young University - Provo

A STUDY OF MULTIPLE EMBRYO DEVELOPMENT IN THE NETLEAF HACKBERRY (CELTIS RETICULATA)

A Thesis

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INTRODUCTION

Statement of the Problem

For many years plants have been known to exhibit evidence of multiple embryoed seeds. (An explanation and definition of multiple embryos will be discussed later in this work.) For some time multiple embryos were considered to be rare and very unusual, but in 1901 Ernst compiled much of the information dealing with multiple embryos. He found that the occurrence of multiple embryos was not rare, and that multiple embryos were important in breeding and perpetuating new forms of plant life. No longer was the study of multiple embryos considered an interesting but unfruitful study of plant monstrosities, but rather it was, and is, an important part of biology and agriculture (Webber, 1940).

Multiple embryos in Celtis reticulata Torr. were observed by Dr. Earl M. Christensen, Professor of Botany, Brigham Young University. While Dr. Christensen was conducting seed germination experiments, he found that C. reticulata had rather frequent occurrences of twinning, and one seed was observed that produced three seedlings. He found that numerous seeds contained multiple embryos. He also observed double epicotyls growing from single hypocotyls in several seedlings and occurrences of four cotyledons on a single seedling (personal communication). This study was undertaken to determine the methods by which these multiple embryos are formed.

Classification of the Plant

The following synonomy is adapted from Little, 1953:

Celtis reticulata Torr. Netleaf hackberry

C. douglasii Planch., C. laevigata S. Wats., C. occidentalis L. var. reticulata (Torr.) Sarg., C. mississippiensis var. reticulata (Torr.) Sarg., C. rugulosa Rydb., C. laevigata var. brevipes (S. Wats.) Sarg., C. reticulata var. vestita Sarg., C. villosula Rydb., C. laevigata Willd. var. reticulata (Torr.) L. Benson.

In Utah, C. reticulata occurs on rocky foothills or canyon slopes ranging in elevation from 2700 to 5900 feet. It is a shrub or small tree, and grows in scattered patches (see Figures 1-3). The twigs are slender, reddish-brown, with light-colored lenticels. The very young twigs are somewhat pubescent. Clusters of small branches called witches-broom often occur on the branches.

Figure 1

Figure 2

Figure 3

The seeds are borne in drupes approximately 5 mm in diameter. The drupes turn reddish-brown and become very sweet upon ripening, as do the fruits of some other species of Celtis. The sugarberry (Celtis laevigata Willd.) is well known for its edible qualities.*

C. reticulata is of little economic importance, and except for its occasional use as a fence post, it is of value only to birds who use the drupes as a source of food (Erdman, 1961).

Literature Review

Celtis reticulata

The literature concerning C. reticulata is very limited, and there are no published records of polyembryony in C. reticulata.

The occurrence of polyembryony is not uncommon in the Ulmaceae. In Ulmus americana embryos have been formed from both antipodals and synergids. Polyembryony has also been known to occur in other species of Ulmus. The entrance of more than one pollen tube into the ovule has also been noted to occur in Ulmus (Johansen, 1950; Maheshrvari, 1950).

The flowers in C. reticulata appear soon after the unfolding of the leaves. The flowers are apetalous and monoecious, but often the flowers of both sexes contain rudimentary organs of the other sex; also some dioecious flowers can be found.

The fruit is an ovoid or globose drupe, covered with a thin pulpy skin. Within the pulpy covering is a thick, bony or glassy-walled nutlet. The seed is single-celled, with one ovule. The embryo is curved or folded in little or no endosperm. The fruit ripens in early fall, but

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*In some ancient cave deposits at Choukoutien in North China, some specimens of the endocarps of the Asiatic hackberry, C. barbouri, have been found, indicating that Peking Man may have used these seeds as food (Chaney, 1955).

often persists on the tree until midwinter (Martin, 1946 and 1961; Browne, 1846; Peattie, 1966).

Martin has diagramed the embryo of C. reticulata, and a drawing of the seed and embryo of C. occidentalis appears in the Woody-Plant Seed Manual.

Fossilized endocarps (fruits) of C. reticulata have been found in sediments of the Pliocene Period in Texas. Twelve of the endocarps were examined for embryos, and all were found to contain an embryo. Fossilized endocarps were compared to aged, present-day endocarps, and the two were found to look identical (Segal, 1966). A drawing of the endocarp is presented by Segal.

Polyembryony

The literature dealing with multiple embryos or polyembryony is very abundant. For the most part, the continuing literature review will be a discussion and explanation of the different types and occurrences of polyembryony.

Polyembryony is described by Johansen (1950) as being of three types: true polyembryony, false polyembryony, and "so-called 'true polyembryony'." Johansen states that true polyembryony occurs when (a) the egg gives rise to a multiple cellular body and in turn this multiple cellular body gives rise to several embryos; (b) the terminal cell of the proembryo splits longitudinally, thus forming two embryo initials; and (c) the zygote may divide longitudinally, forming two separate embryos.

False polyembryony, according to Johansen, occurs in the following ways: (a) two or more ovules are developed, each having only one embryo; (b) nucellar embryos are developed during the ontogeny of the ovule; (c) the production of two or more megagametophytes may arise

from a multicellular archesporium (i.e. different megaspore mother cells), or from the same megaspore mother cell; and (d) when an apomictic embryo develops within the same ovule as a normal embryo. Johansen does not specify as to how the apomictic embryo may develop.

Johansen signifies that what he terms "so-called 'true polyembryony'" occurs in three ways: (a) when there is no differentiation between the egg and the two synergids; (b) when two or more potential eggs develop within the single megagametophyte; and (c) when embryos are formed from nuclei in the megagametophyte other than the egg. These other nuclei are the antipodals, endosperm, and synergids.

Webber (1940) states that true polyembryony occurs when two or more embryos occur within the same megagametophyte, and that false polyembryony is when a single embryo arises from the megagametophyte but there are two or more megagametophytes within a single ovule. He goes on to explain the ways in which true polyembryony may occur. True polyembryony, according to Webber, occurs in four ways: (a) Simple polyembryony--the fertilization of the endosperm, synergids or antipodals by extra generative nuclei. This is very similar to the frequent occurrence of plural eggs and sperms by the Gymnosperms. (b) Sporophytic polyembryony--embryos formed by budding of either the nucellus or integument tissue. (c) Euploid polyembryony--embryos are formed having the following sets of chromosomes: haploid-haploid, haploid-diploid, haploid-triploid, diploid-triploid, diploid-tetraploid, triploid-triploid. (d) Cleavage polyembryony--when the zygote or embryo splits or divides to form two or more embryos.

Webber and Johansen seem to be in agreement as to how the various pluralities of embryos may occur; but, they do not agree upon the

terminolgy given to each of the various ways. Also, Webber is the only one to list budding of the integument as one of the ways. It should be noted that the synergid may give rise to the second embryo by apogamy or by being fertilized (Lebeque, 1952).

Schurhoff (1928) gives an explanation as to why the synergid often becomes egg-like. He states:

> The synergid and the egg cell are sister cells and therefore, this synergid is to be interpreted as a ventral canal cell. It is concluded that the second synergid is the sister cell of the upper polar nucleus. These two cells represent a second archegonium. This interpretation satisfactorily explains the persistance of the two equal male nuclei in Angiosperms, whereas in Gymnosperms the second male nucleus tends to be dwarfed except in cases where the two sperms may fertilize two neighboring archegonia, as in Sequoia, Juniperus and Gnetum.

In addition to the above mentioned ways for polyembryony to occur, Crete (1938) has also stated that polyembryony occurs when the suspensor cells give rise to a second embryo.

METHODS AND PROCEDURES

Collection of Material

Material was collected during 1965, 1966, and 1967. The 1965 collection began February 24 and ended August 12. Material was collected approximately once a week during this period. In 1966 a single collection was made during the time of flowering. This was on April 27. Due to the knowledge gained from the previous years' collections, the 1967 collection period began later, April 5, and ended earler, July 10. However, collections during this time were made at 3 to 6 day intervals. Appendix I contains a detailed report on dates of collection.

During the fall of 1965 and the fall of 1966, mature seeds were collected. Some of these seeds were used in germination studies and dissections were made to determine the number of multiple embryos in mature seeds.

Preservation of Material

Upon collecting the plant specimens, they were placed in a solution of 70 per cent formalin-aceto-alcohol (FAA) (Johansen, 1940; Appendix II). The material was aspirated for approximately 5 to 15 minutes, and was then stored in the solution of FAA.

The dehydrating and embedding process used was the tertiary butyl alcohol method (Johansen, 1940; Appendix III).

The material was cut on a hand-operated rotary microtome at thicknesses varying between 7 and 20 microns. The author found that most of the material could be cut at a thickness of 7 microns, but a thickness

of 10 microns proved to be the most satisfactory thickness. Some of the more mature seeds, however, were too hard to cut at thicknesses below 20 microns. The material was then affixed to the microscope slide with Haupt's adhesive.

Staining Procedure

The slides were stained in lots of 30, using metal racks. Metal and polyethylene food containers were used as staining vessels. The staining procedure used was Conant's Quadruple. Approximately 1500 slides were prepared.

Seed Germination Studies

Seed germination studies were conducted in the following manner. At first, 450 seeds were placed in hot tap water and were allowed to soak for 3 days. These seeds were those collected in the late fall of 1965. They were then planted in flats of 150 seeds per flat. The seeds were allowed to germinate in the greenhouse. They were kept moist. The germination period required 3 to 4 months time. The next planting was of 300 seeds in flats of 100 seeds per flat. These seeds were those collected during the late fall of 1966. These seeds were first scarified and they soaked for 24 hours in 500 ppm of Giberellic acid.

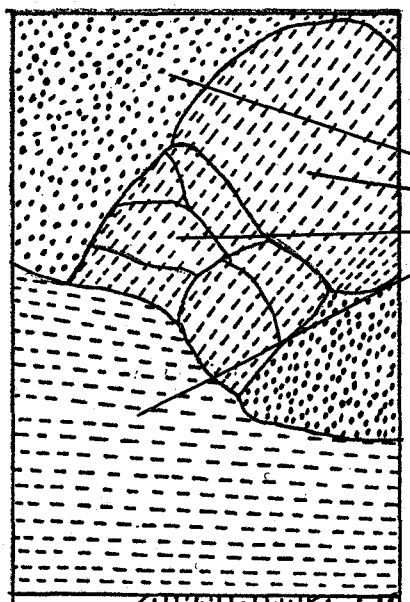


Figure 4b

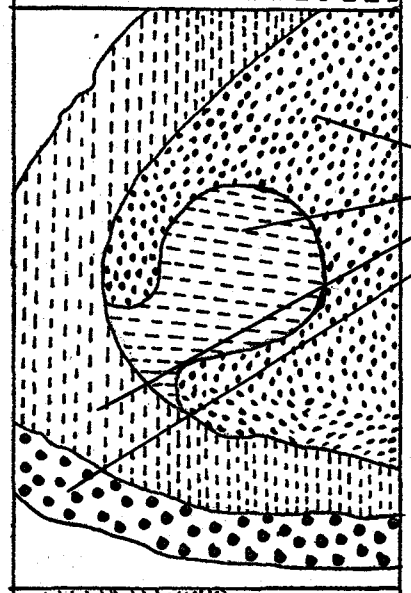


Figure 5b

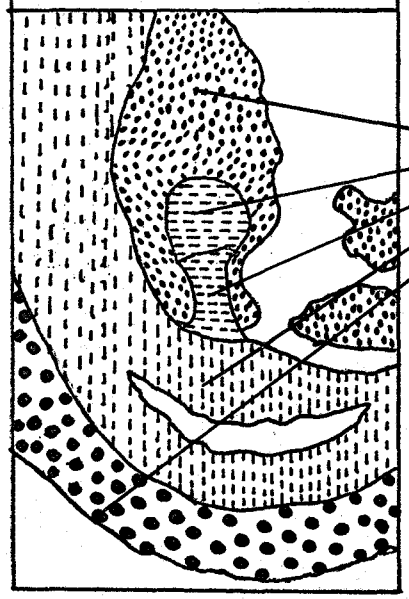


Figure 6b

RESULTS AND DISCUSSION

Single Embryos

Figures 4-6 show single embryo development. As can be seen in the figures, the suspensor cells of C. reticulata are two cells thick. As is shown in Figure 6, C. reticulata has cellular endosperm in the embryo sac.

Figure 4a

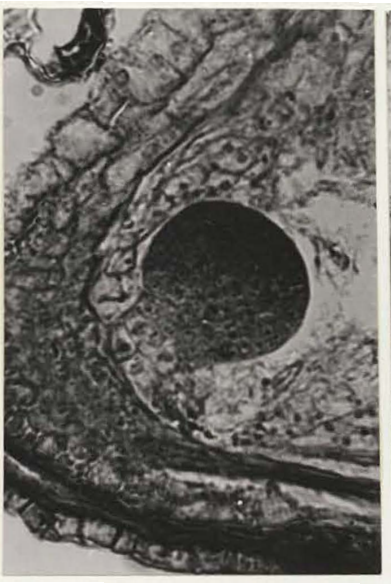


Figure 5a

Figure 6a

Figure 7 is a single embryo which is quite well developed. Why it is not connected to the wall of the embryo sac could be due to two reasons: (1) the embryo was torn away during sectioning, or (2) this is not a median section and possibly the median section would have been connected. (The other sections of this embryo were lost during sectioning.)

Multiple Embryos

The results indicate four probable processes by which the multiple

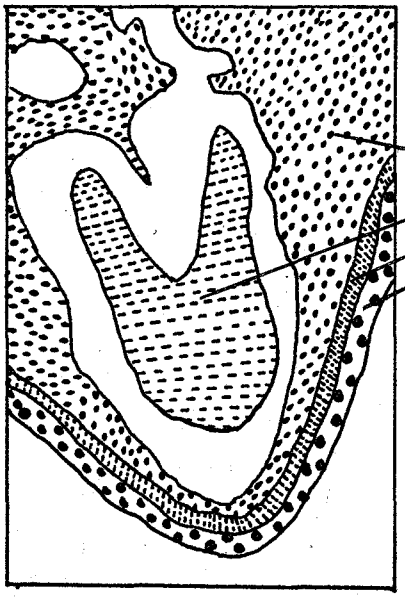


Figure 7b

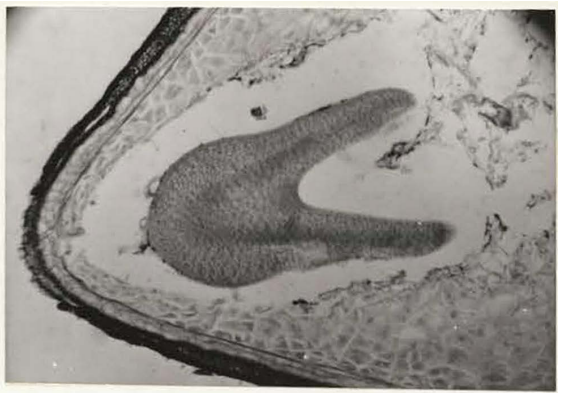


Figure 7a

embryos are formed. These are: (1) development of an extra egg in the embryo sac--probably a synergid; (2) splitting of the embryo; (3) budding of the suspensor cells; and (4) budding of the nucellar tissue.

Formation of Extra Egg-like Cell

The development of an extra egg-like cell in the ovule appears to be quite a frequent occurrence and is probably the method by which most of the extra embryos are formed. From the position of the egg-like cell next to the embryo and the two embryos developing close to each other, it is presumed by the author that the egg-like cell is a synergid. It is also presumed that this extra "egg" becomes fertilized to develop the extra embryo; however, the author has no proof that the extra "egg" becomes fertilized. This method of development can be explained by Schurhoff's discussion of a synergid's being a sister cell to the egg, and is in agreement with Johansen's discussion of "so-called 'true polyembryony'" where embryos are formed from other nuclei in the megagametophyte other than the egg, these other nuclei being antipodals, endosperm, and synergids. It is also in agreement with Webber's discussion of true polyembryony which he calls simple polyembryony or the fertilization of the endosperm, synergids, or antipodals by extra generative nuclei.

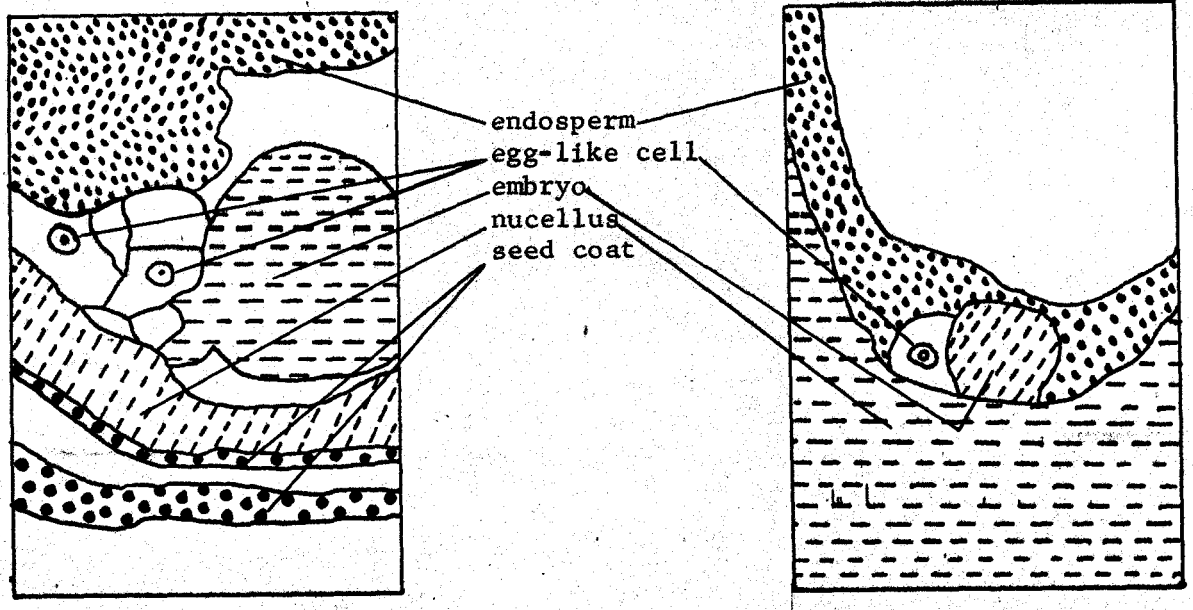


Figure 8b

Figure 9b

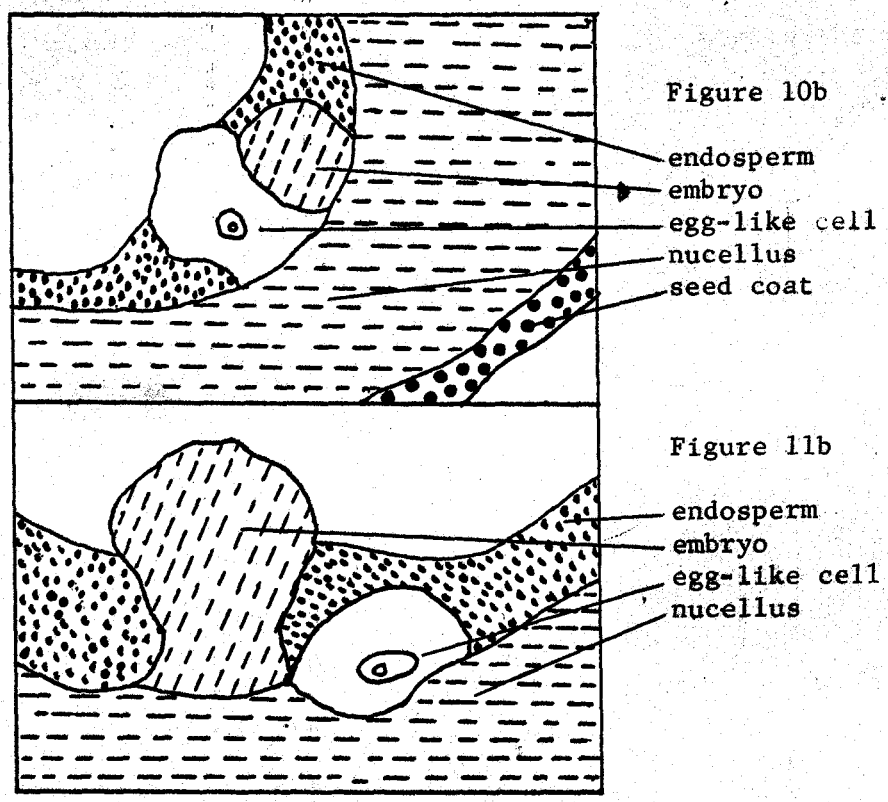


Figure 10b

Figure 11b

Figure 8 is a single embryo with at least two large egg-like cells beside it. Figures 9-11 are small embryos, each with a large egg-like cell beside the embryo In Figure 10, the "egg" cell is larger than the single embryo.

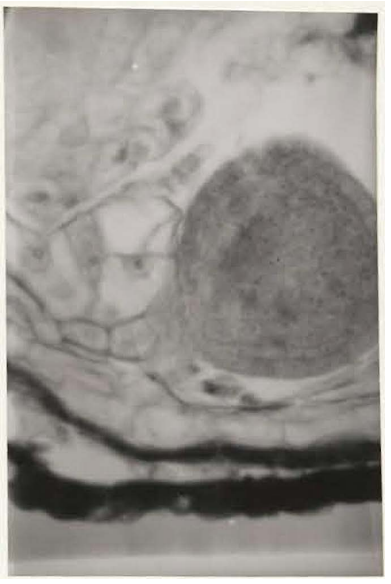


Figure 8a

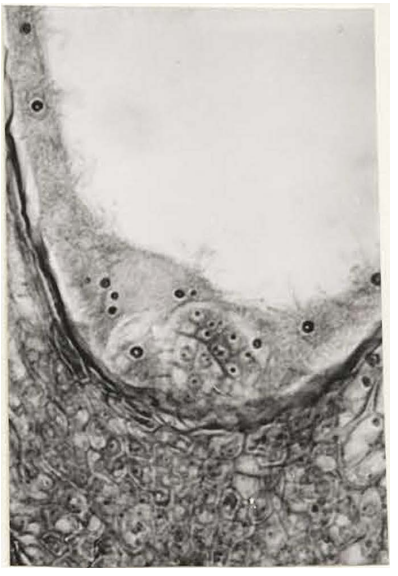


Figure 9a

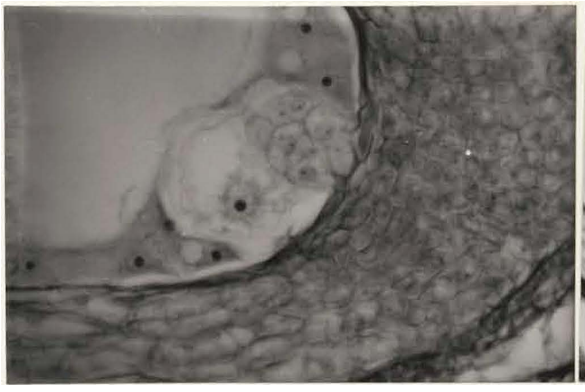


Figure 10a

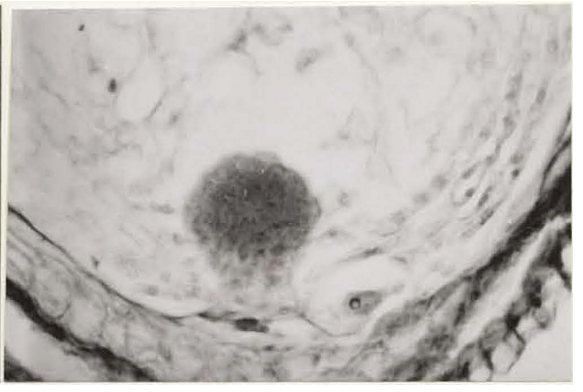


Figure 11a

Figures 12-14 are serial sections showing three successive sections through an embryo and an egg-like cell. In Figure 12, the embryo can be seen with only an outline of the large cell. Figure 13 has the embryo as well as the large cell. Figure 14 shows the embryo and also the large

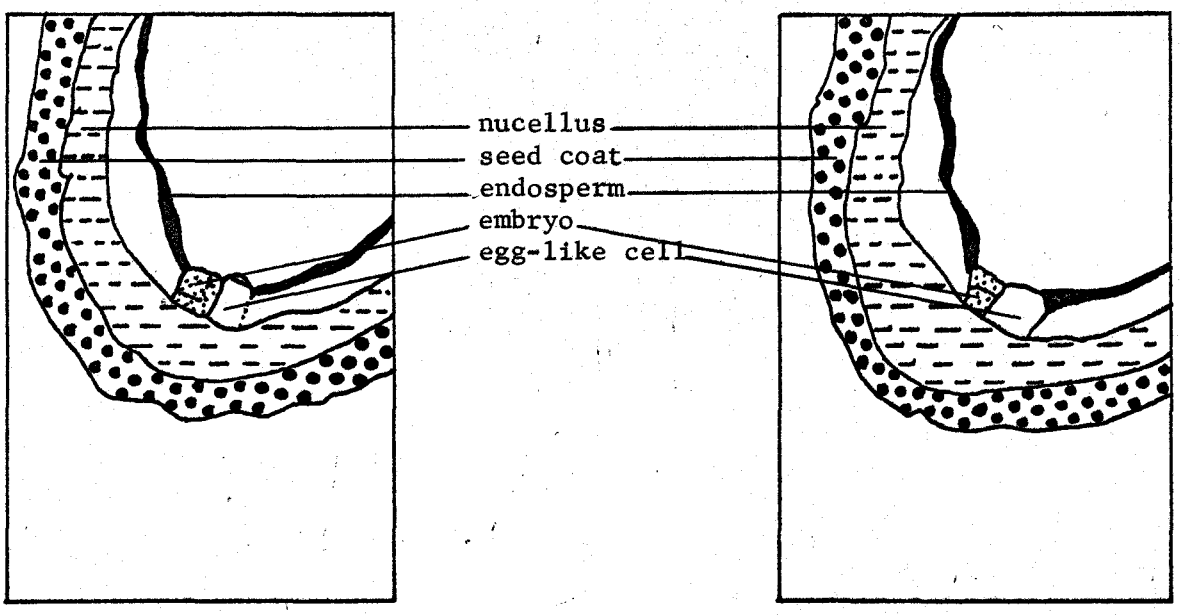
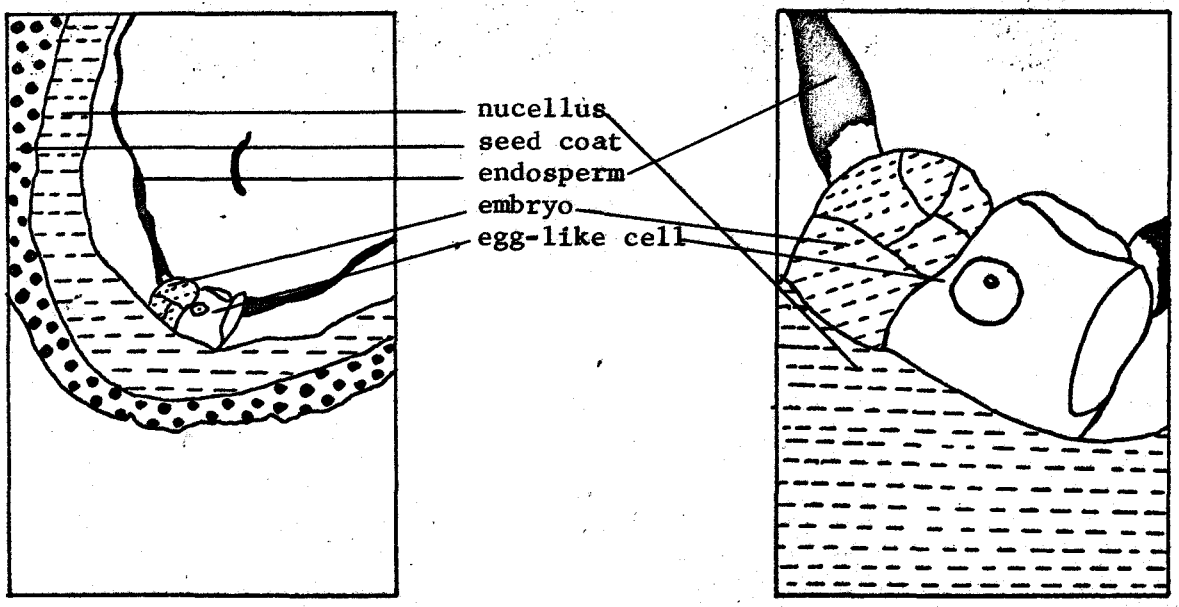


Figure 12b

Figure 13b

Figure 14b

Figure 15b



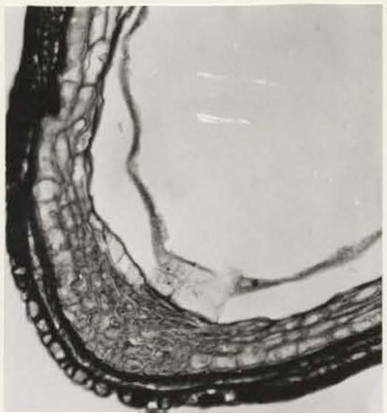


Figure 12a Figure 13a Figure 14a

nucleus within the egg-like cell.

Figure 15 is an enlargement of Figure 14.

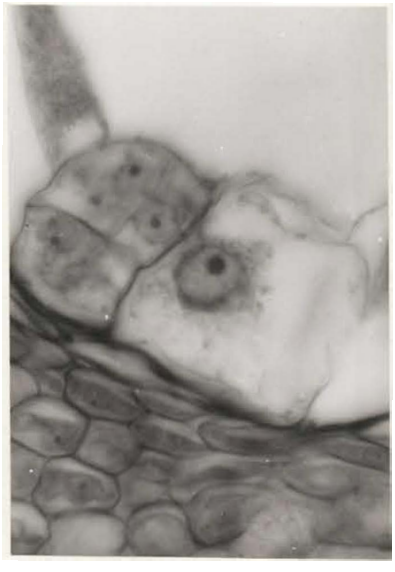


Figure 15a

Figure 16 has two large egg-like cells, one of which appears to be trinucleate; this could be one of the "giant" cells discussed by Wells (1920) (see Appendix IV).

Figure 17 shows two embryos growing beside each other. These

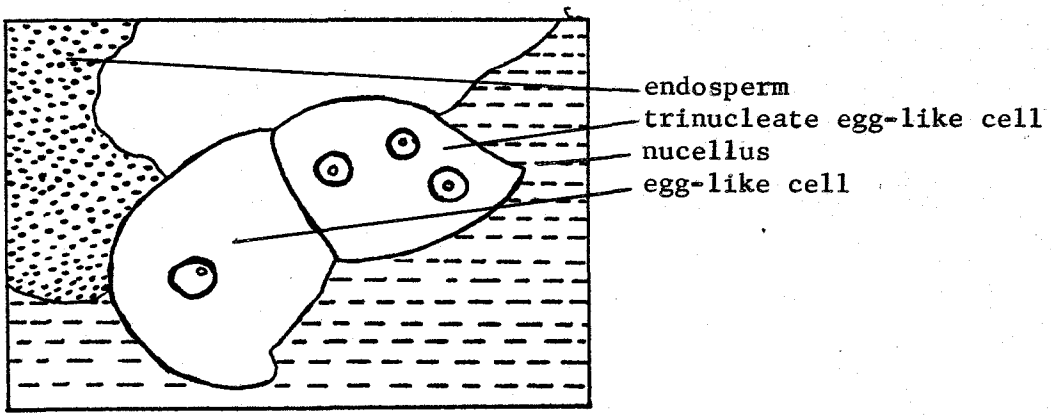


Figure 16b

Figure 17b

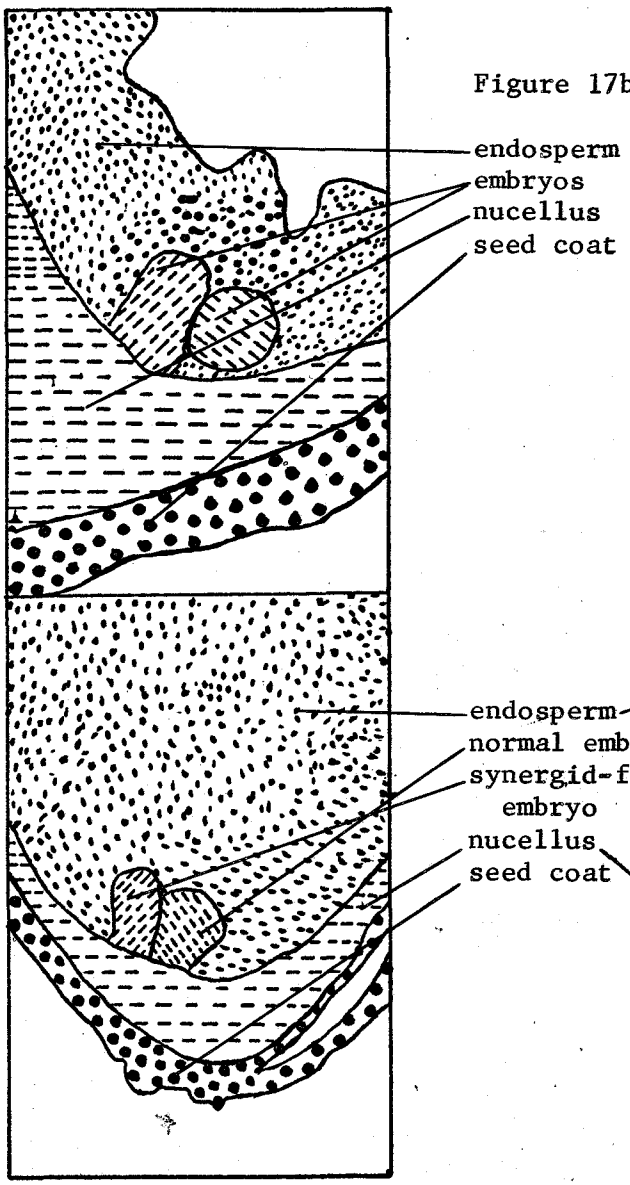


Figure 18b

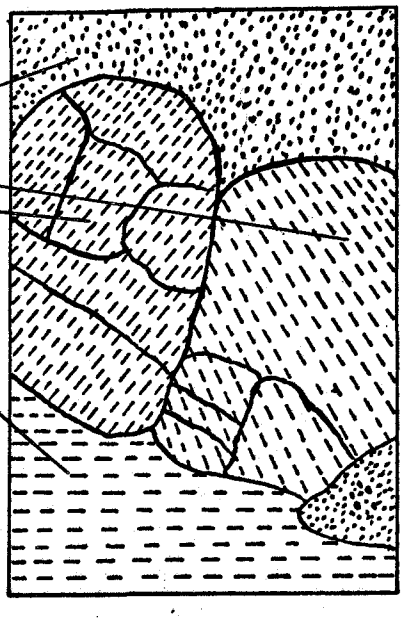


Figure 19b

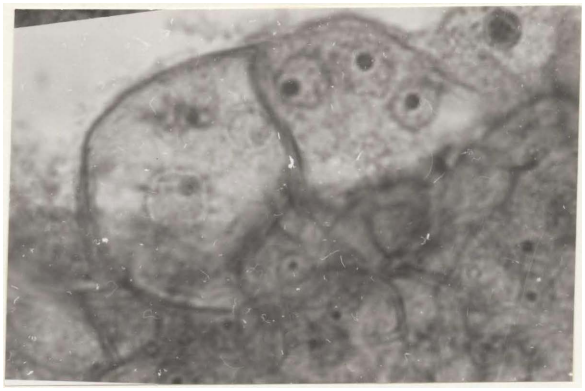
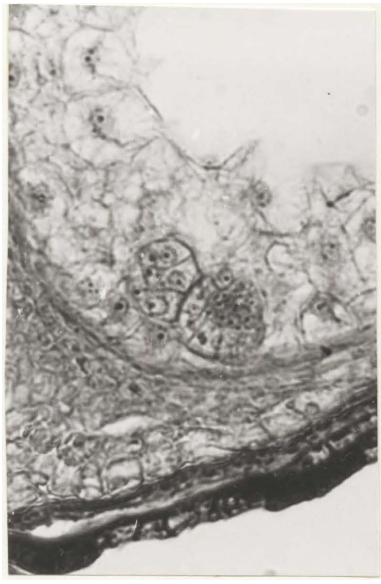
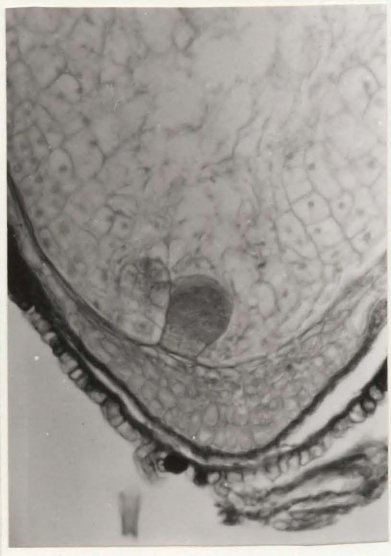


Figure 16a

embryos do not appear to be well developed; their irregular appearance could be due to the way in which this section was cut.





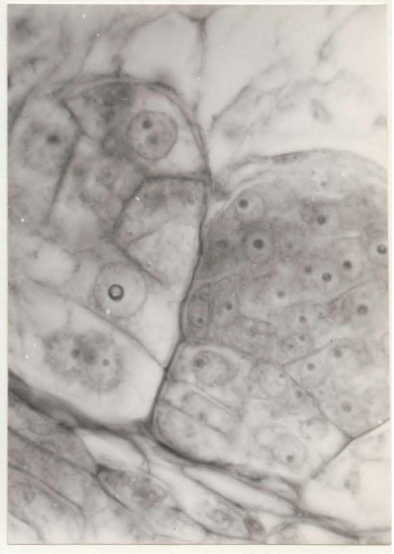


Figure 17a Figure 18a Figure 19a

Figure 18 shows two small but nicely developed embryos. Because the lower cells of the embryo on the right are in two rows, it is the author's belief that this embryo is the one formed from the egg, while the embryo on the left was formed from the synergid. Figure 19 is an enlargement of Figure 18.

Figures 20-23 show three sets of two embryos at various stages of development. In Figure 20, both embryos appear to be nearly identical,

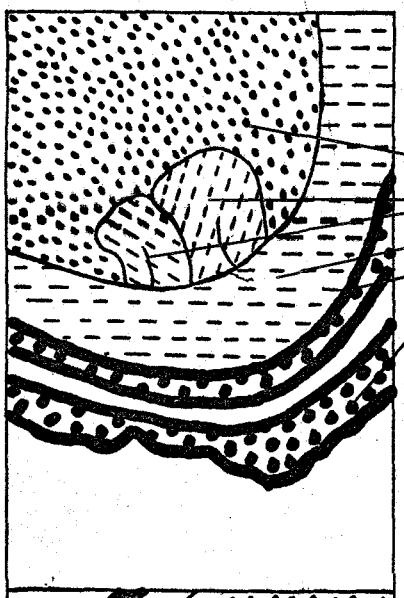


Figure 20b

endosperm
embryos
nucellus
seed coat

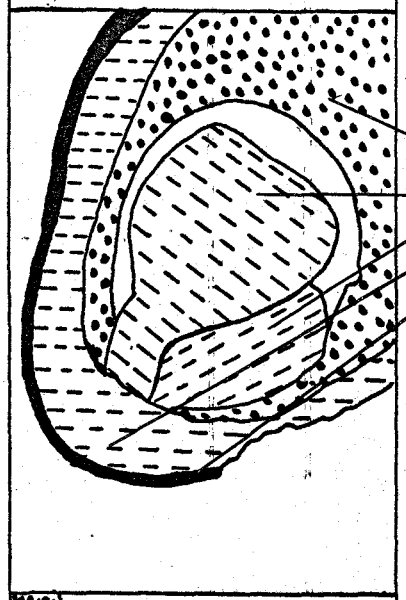


Figure 21b

endosperm
embryos
nucellus
seed coat

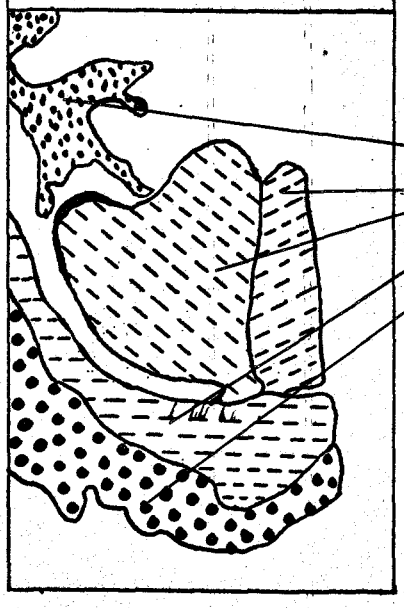


Figure 22b

endosperm
embryos
nucellus
seed coat

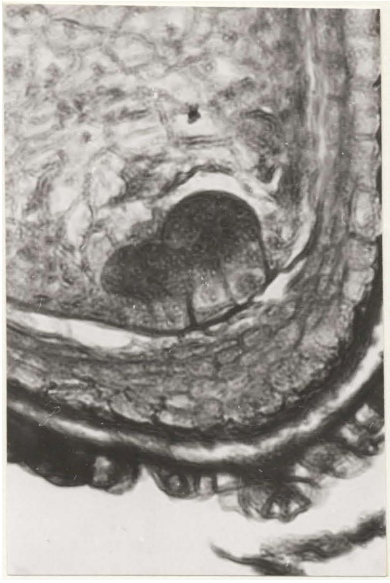


Figure 20a

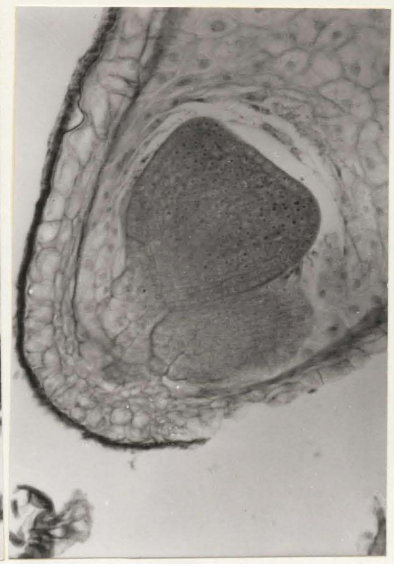


Figure 21a

Figure 22a

except for size; thus it is not possible to conclude which one may have been formed from the synergid.

The suspensor cells shown on Figure 21 both appear to be somewhat abnormal. Therefore, it is not possible to determine which one may have been the synergid-formed embryo.

Figure 22 shows a well-developed embryo with only part of the second embryo beside it. The other half of the embryo on the right was lost during sectioning. Both embryos, however, appear to be "normal" suspensor cells.

Figure 23 has two very well-developed embryos growing next to each other. Figure 24 has two embryos developing together, with one embryo almost surrounded by the other embryo. Figure 25 gives a close-up view of the smaller embryo and the suspensor cells.

As was stated previously, it is not known for certain how these double embryos are formed, but because of the many occurrences of the large egg-like cell beside an embryo, as well as the side-by-side position

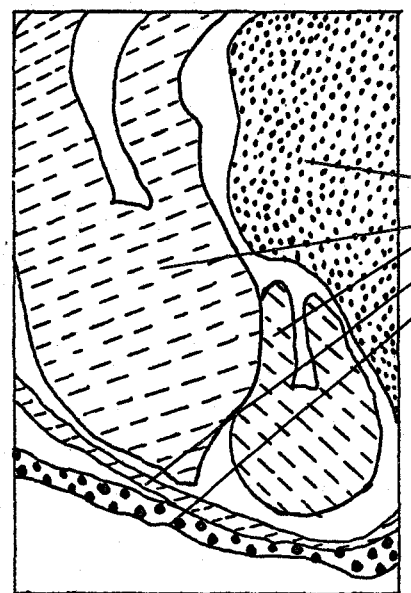


Figure 23b

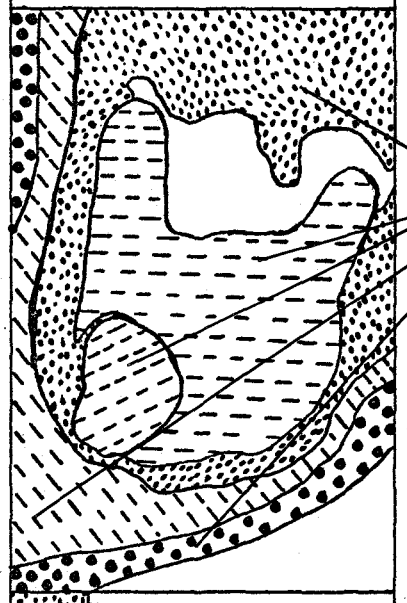


Figure 24b

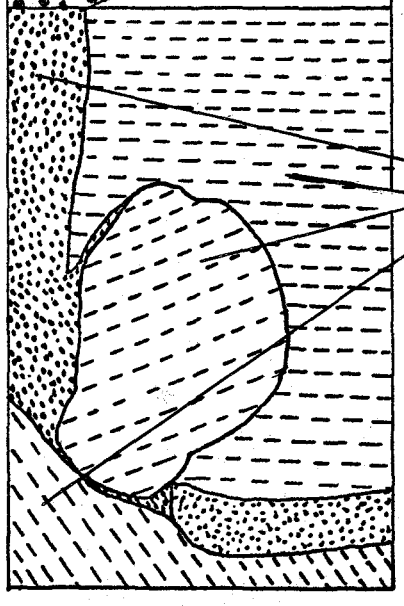
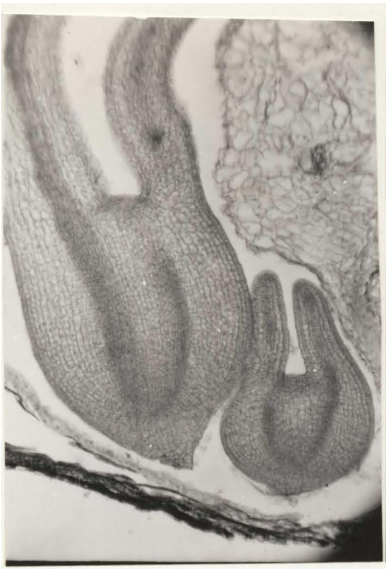
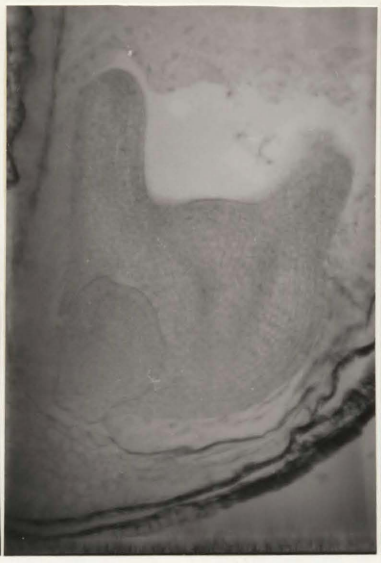


Figure 25b





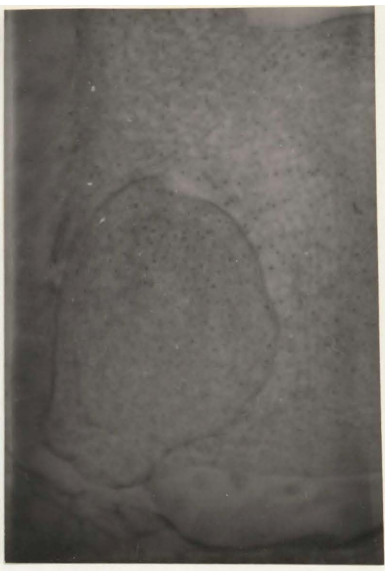


Figure 23a Figure 24a Figure 25a

of the embryos, it is concluded by the author that the second embryo, in the previously discussed figures (Figures 4-25), is formed from a synergid.

Splitting of the Embryo

Indications of embryo splitting were found on only two slides, but evidence for embryo splitting in C. reticulata is supported in the seed germination studies by Christensen. As was previously stated, Christensen found many instances of double epicotyls on a single hypocotyl showing partial embryo splitting. He also found instances of four cotyledons on a single embryo.

This method of development is in agreement with Johansen's discussion of true polyembryony where the egg gives rise to a multiple cellular body and in turn this multiple cellular body gives rise to several embryos. It is also in agreement with Webber's discussion of true polyembryony which he calls cleavage polyembryony or the splitting of the embryo.

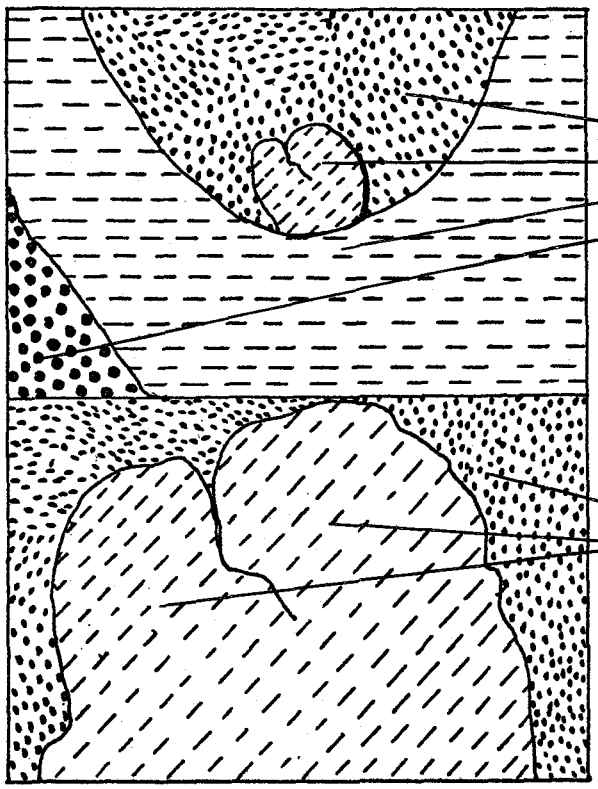


Figure 26b

Figure 27b

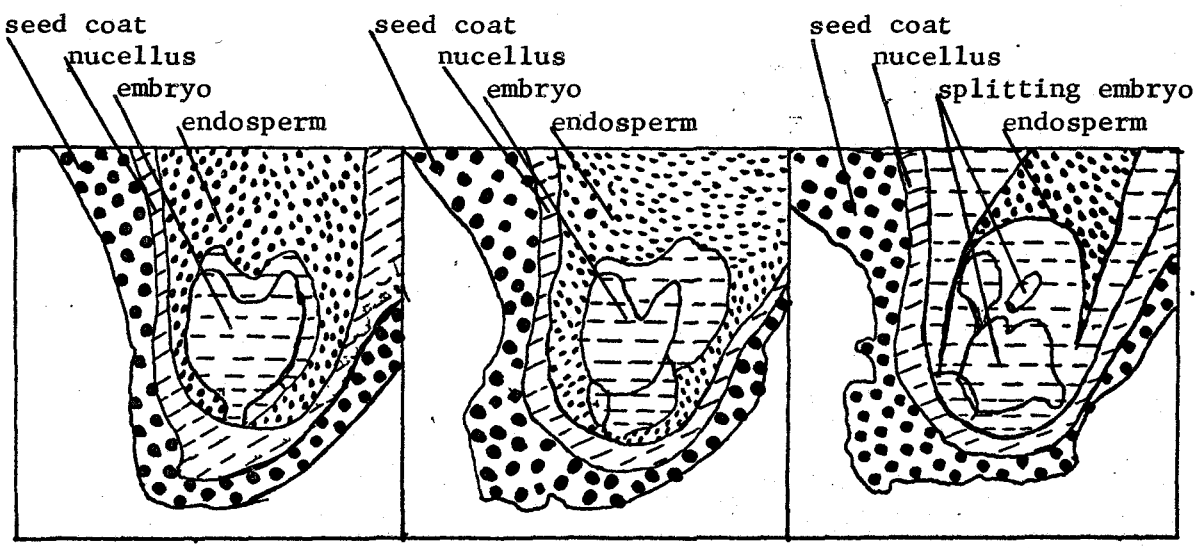


Figure 28b

Figure 29b

Figure 30b

Figure 26 shows definite embryo splitting. The cleavage line is not the formation of cotyledons, but rather it is quite definite evidence of embryo splitting. Figure 27 is an enlargement of Figure 26.

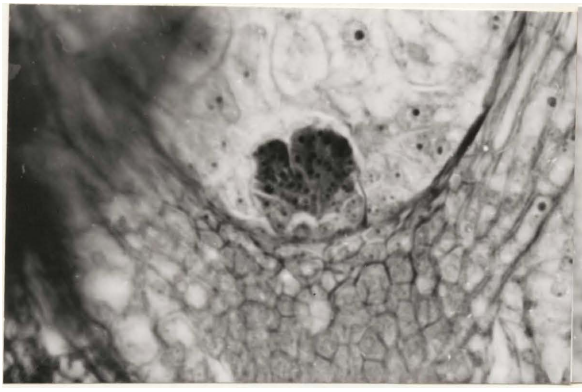


Figure 26a

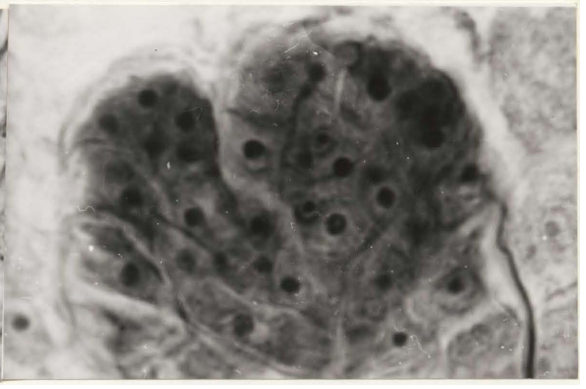


Figure 27a

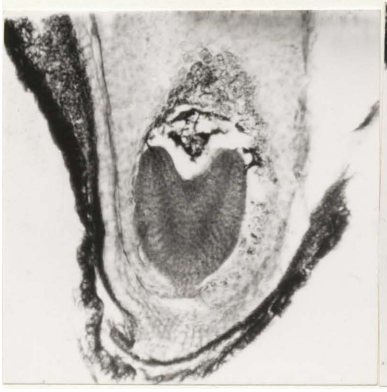


Figure 28a

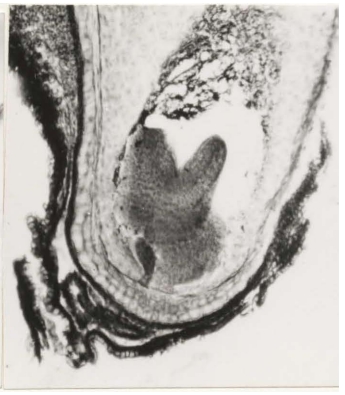


Figure 29a

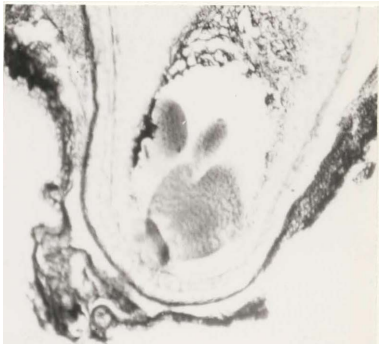


Figure 30a

Figures 28-30 are serial sections through what appears to be the splitting of an embryo. The upper part of the embryo (Figure 28) appears normal; but Figures 29 and 30 show splitting of the embryo. Figure 31 is an enlargement of Figure 30 and shows the splitting of the embryo in greater detail. It should be noted that while these figures appear to show embryo splitting, it could be a result of cutting through two closely associated embryos; the buds could be the intertwined cotyledons sectioned so as to appear to come from one embryo.

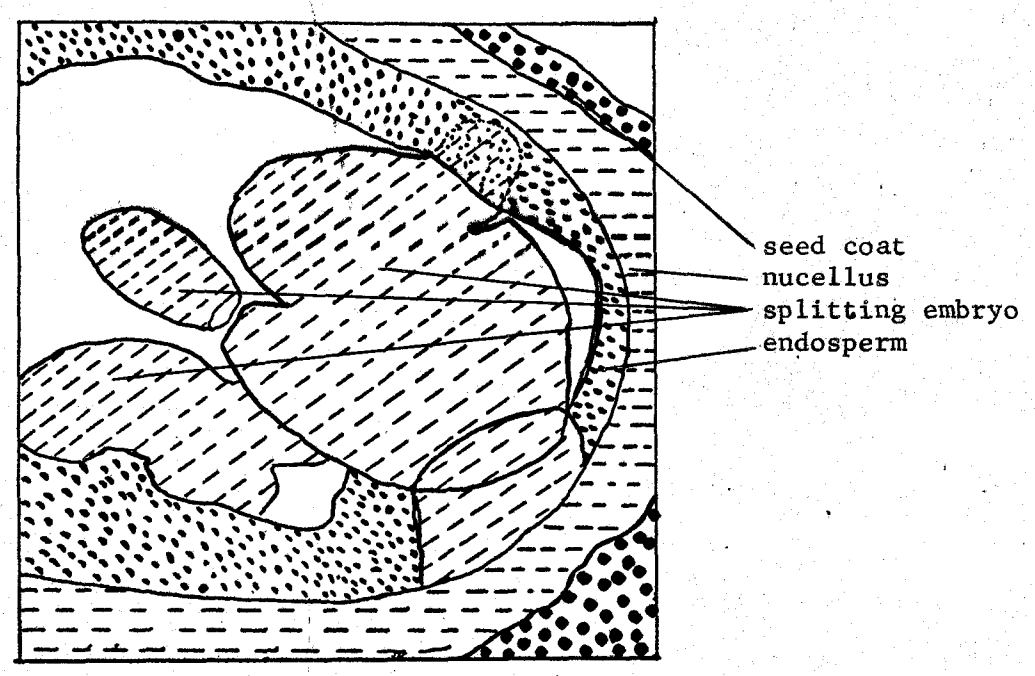


Figure 31b

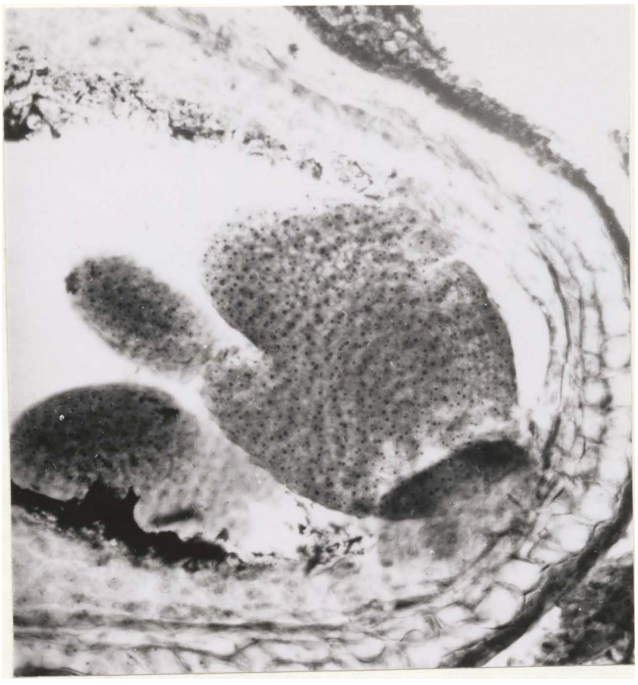


Figure 31a

Budding of the Suspensor Cells

Another possible method of multiple embryo development observed in this study is the development of an embryo or embryos from the suspensor cells. This method is illustrated by Figures 32-35. In this instance, the additional embryos could actually be termed as having arisen from the original embryo, but the author believes that a definite distinction between the formation of the embryo formed from the suspensor cells and those formed by the embryo itself should be made. This method of development is in agreement with Crete's statement that polyembryony occurs when the suspensor cells give rise to a second embryo.

In Figure 32, the larger embryo appears to be quite normal in structure, except that it is not connected to the suspensor cells. From the appearance of the embryo sac, it is very doubtful that the larger embryo was torn from the basal cells during sectioning. The larger embryo could, however, be curved and the portion connecting the embryo and the

Figure 32b

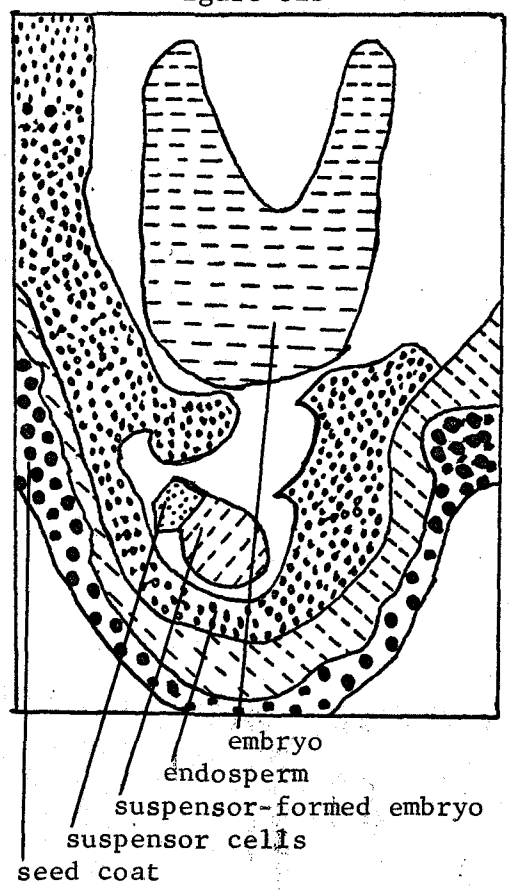
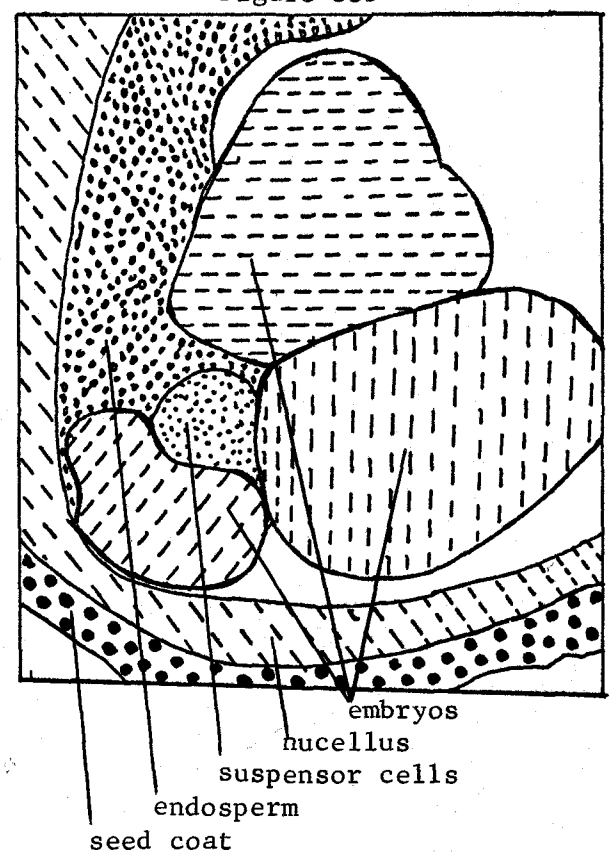


Figure 33b



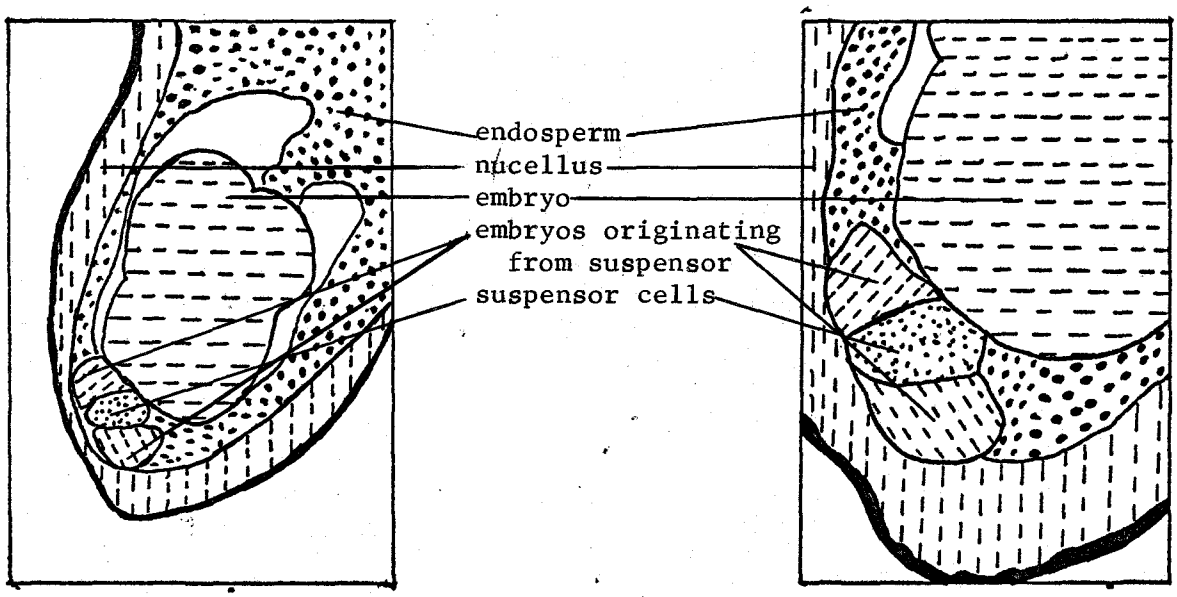


Figure 34b

Figure 35b

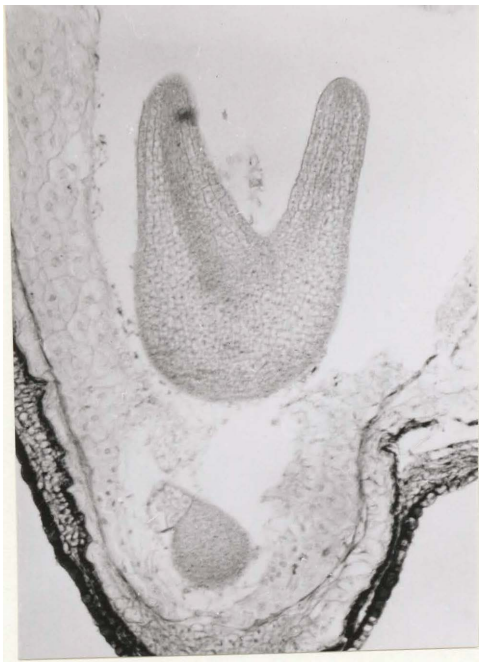


Figure 32a

Figure 33a

suspensor cells would then have appeared on other "lost" sections.

In Figure 33 there are three embryos apparently all growing from the same suspensor cells. Figures 34 and 35 show the original large embryo along with two smaller masses of embryonic tissue. Figure 35 is

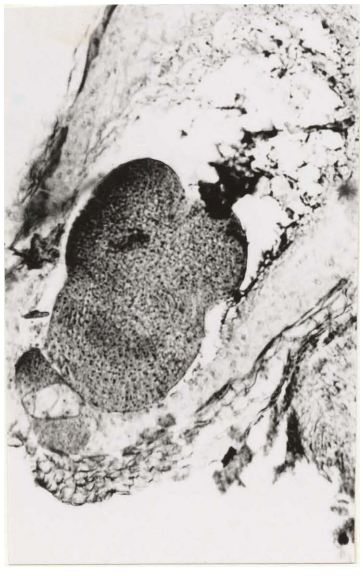


Figure 34a

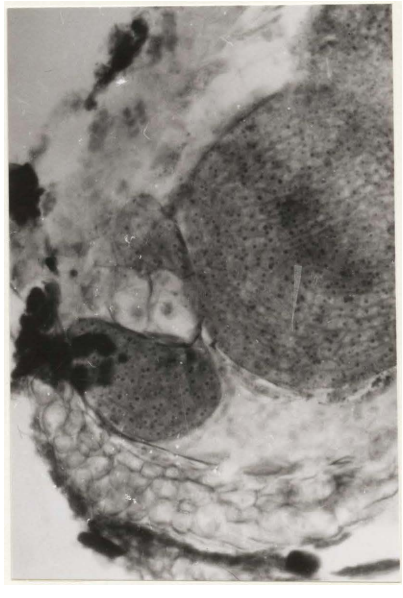


Figure 35a

an enlargement of part of Figure 34. Neither of the two embryonic masses shown in these figures have much differentiation, and both appear to have arisen from the suspensor cells. The large embryo in this case is not developing as a normal embryo should; the cotyledons have not developed to the extent they normally do at this stage. Possibly the malformed, original embryo has stimulated the production of secondary embryos, or possibly the secondary embryos have caused the malformed primary embryo.

In all of the instances where secondary embryos appear to be forming from the suspensor cells, there appears to be some malfunction of the original embryo. In two instances, Figures 33 and 34, the larger, original embryo appears to be developing abnormally. This abnormal appearance could, however, be due to the manner of sectioning of these embryos. It should also be pointed out that the apparent suspensor budding could be due to the way in which the embryo was sectioned.

Nucellar Budding

Another method of multiple embryo development occurring in C. reticulata is nucellar budding. This type of development is expressed in Johansen's discussion of false polyembryony wherein nucellar embryos are developed during the ontogeny of the ovule. It is also in agreement with Webber's discussion of true polyembryony which he calls sporophytic polyembryony or embryos formed by budding of either the nucellus or integument tissue. This method of development is evidenced by three separate slides, pictured on Figures 36-42.

Figures 36-38 show nucellar budding occurring with at least three buds being formed. The three embryos pictured in these figures all occur within the same embryo sac, but they were not close enough to one another

Figure 36b

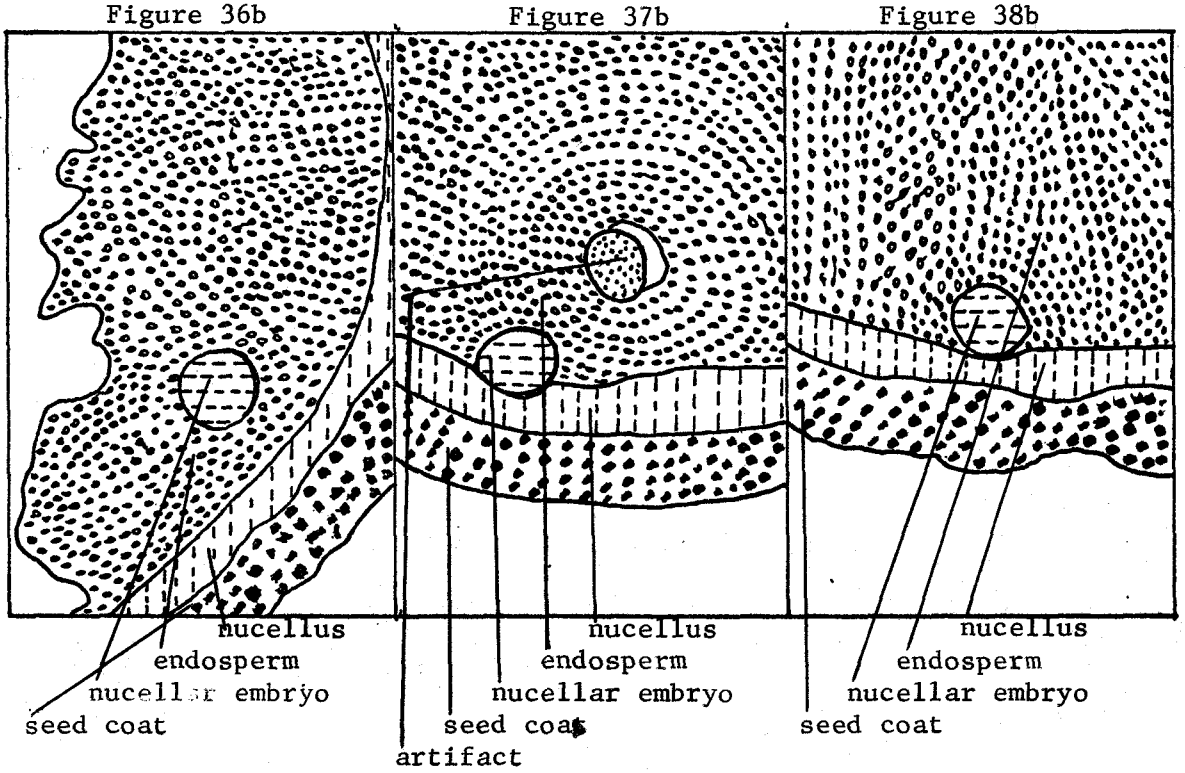


Figure 37b

Figure 38b

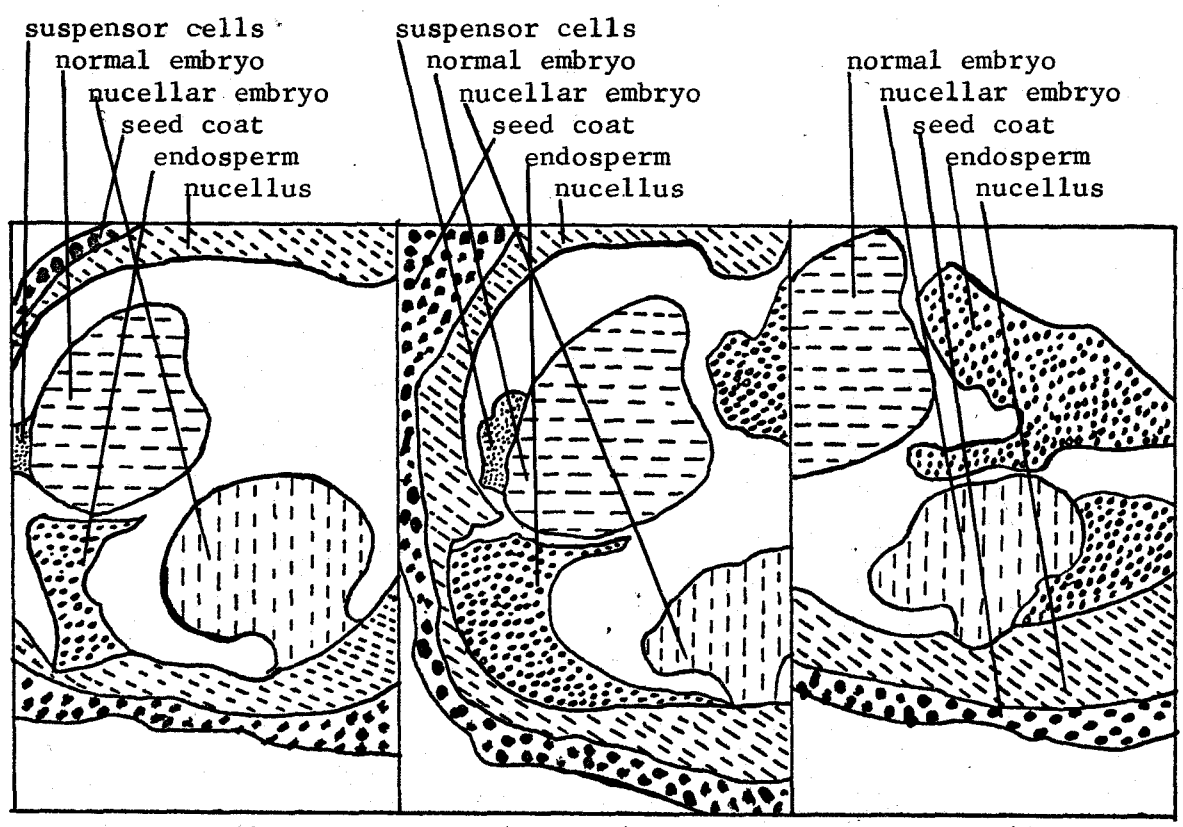
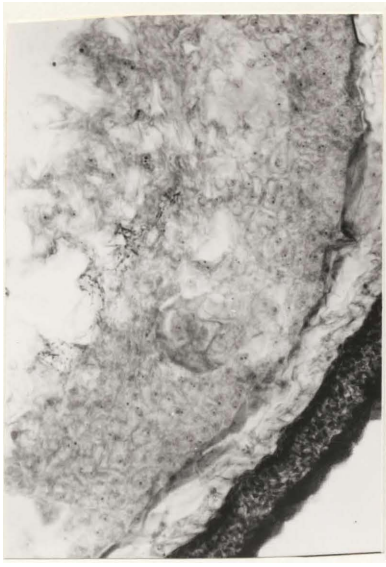
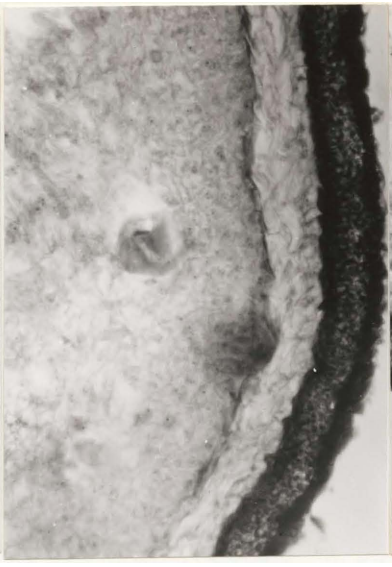


Figure 39b

Figure 40b

Figure 41b





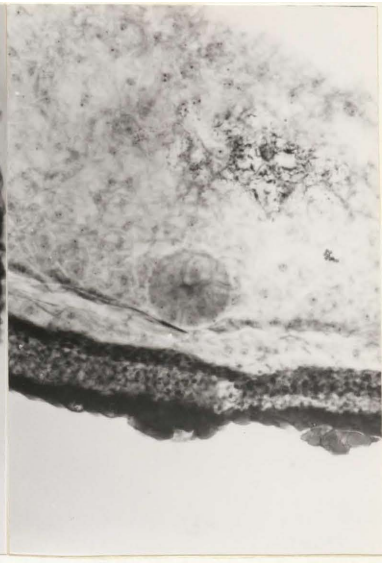
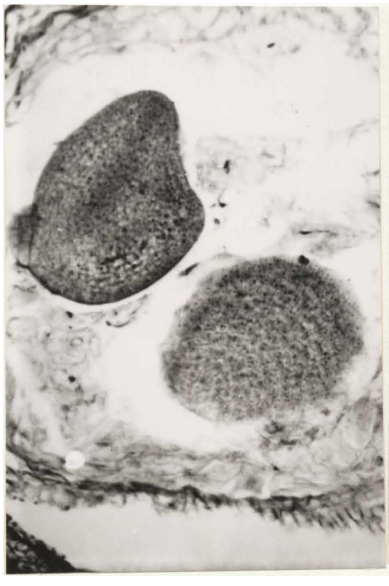
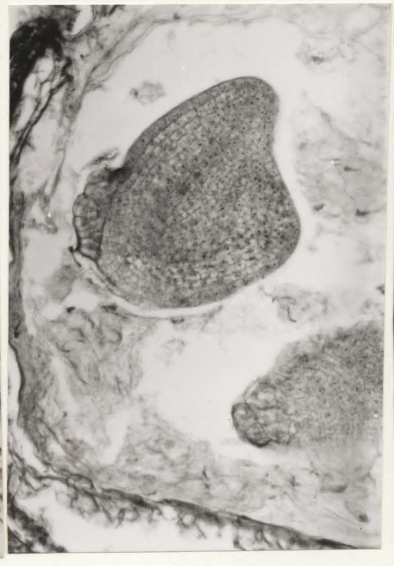


Figure 36a Figure 37a Figure 38a

to get them all in one picture.





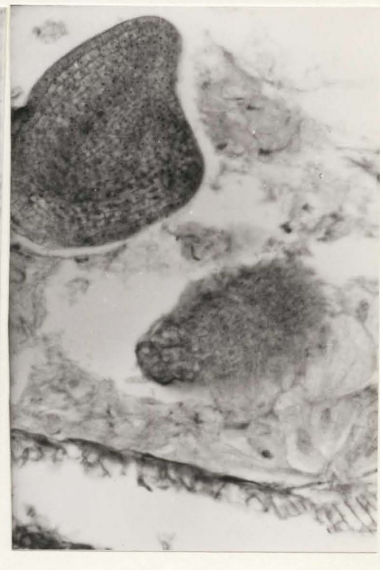


Figure 39a Figure 40a Figure 41a

Figures 39-41 show another instance where a multiple-embryoed seed has been formed through nucellar budding. Figures 39-40 are two consecutive sections showing the same two embryos. These figures show one normal embryo with a second embryo being developed from the nucellar

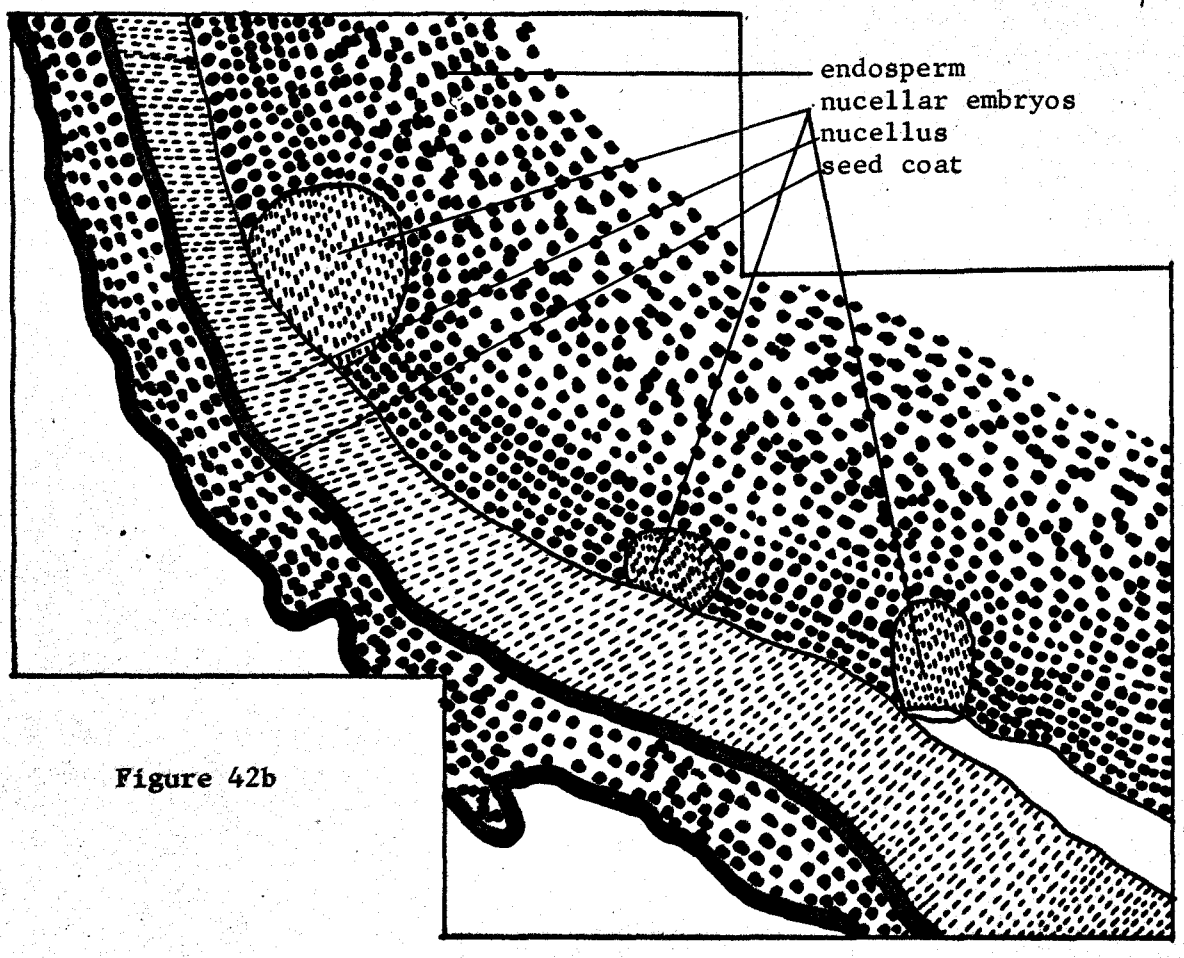


Figure 42b

tissue. Figure 40 shows the normal embryo in the upper left and the embryo formed by nucellar budding in the lower right of the picture. Figure 41 shows the nucellar embryo more clearly.

It is the author's belief that the extra embryo in Figures 39 and 40 are formed from nucellar budding rather than from an extra egg-like cell. The author bases his conclusion on the position of the two embryos; they are somewhat removed from each other. The possibility does exist, however, that the extra embryo could have arisen from an extra egg cell.

The normal embryo discussed in Figure 40 is the embryo believed to have arisen from the union of egg and sperm. The embryo on the upper left is believed to be normal because of the suspensor cells which it has and because of its position in the embryo sac.

Figure 42a

Figure 42 shows three embryos being developed from nucellar budding,

and all three occur along the nucellus. From this, it is concluded that in this particular seed, nucellar budding has occurred. None of these three embryos appears to have been formed from the union of the egg and sperm nuclei. Their general appearance is different from a normal embryo in that they lack a suspensor and they are sphere-shaped.* Also, they are not in the part of the embryo sac where the embryo is normally found; that part of the embryo sac is found past the top of the picture. There was no evidence of a normal embryo in this embryo sac.

Seed Germination

Of the 450 seeds planted in flats, only about 35 germinated the first year, and only one seed germinated the second year. The low germination rate could be a result of collecting the mature seeds too late in the year; therefore, they may have dried out. Of the 36 which germinated, 7 were twin seedlings. In most cases one seedling was much larger than the other, but in one instance both seedlings were almost identical in size. Most of the time the larger seedling continued to grow, while, after approximately one month, the smaller seedling died.

Figures 43 and 44 show twin seedlings which germinated. Figure 43 shows the much larger seedling with the smaller seedling just barely out of the ground. This smaller seedling is one which continued to grow (Figure 45). Figure 44 shows the two seedlings which were very nearly the same size. Both of these seedlings were still growing after one year (Figure 46). Figure 47 shows twin seedlings after one year's growth. Early pictures of these seedlings were not taken.

The planting of the scarified, Giberellic acid-soaked seeds

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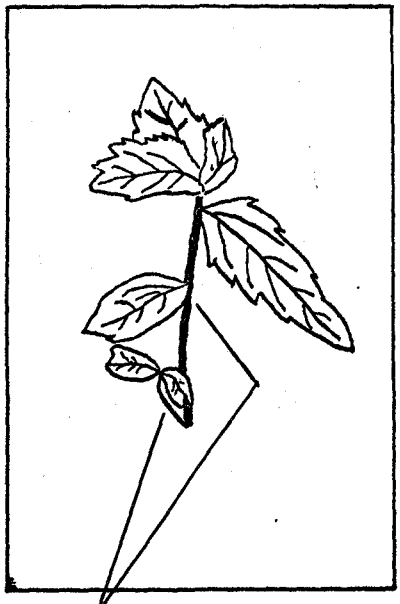
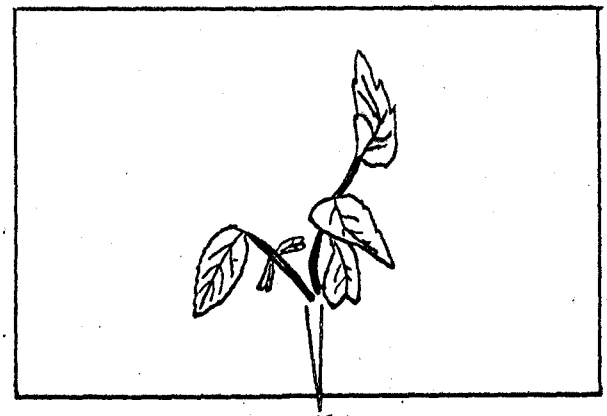
*Johansen gives this account about nucellar budding in Euphorbia: "A nucellar cell becomes enlarged and divides . . . divisions are decidedly irregular; either a globular mass of cells or a cylindrical 'proembryo' . . . may be formed."

Figure 43b



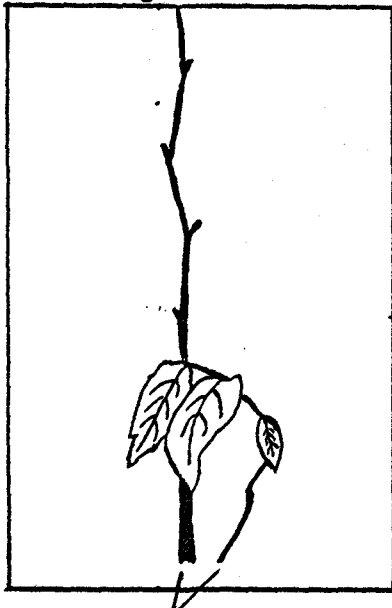
twin seedlings

Figure 44b



twin seedlings

Figure 45b

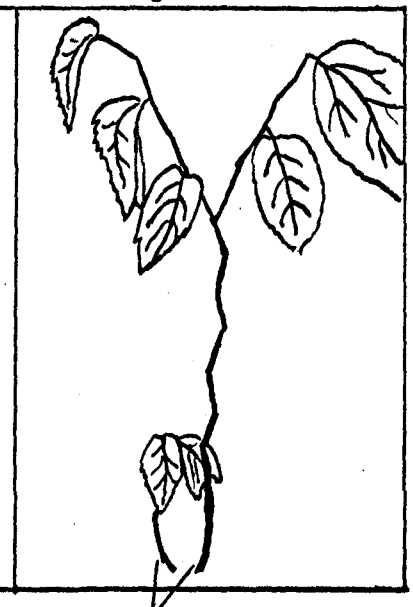


twin seedlings

Figure 46b

twin seedlings

Figure 47b



twin seedlings

produced no seedlings. Why these seeds did not germinate is not known.

Figure 43a

Figure 44a

Figure 45a

Figure 46a

Figure 47a

Abortion of Young Seeds

It was noted by the author that a few weeks after pollination (see Appendix I for pollination dates) many of the young drupes wither and die. Some of the abortive fruits appear to contain an embryo.

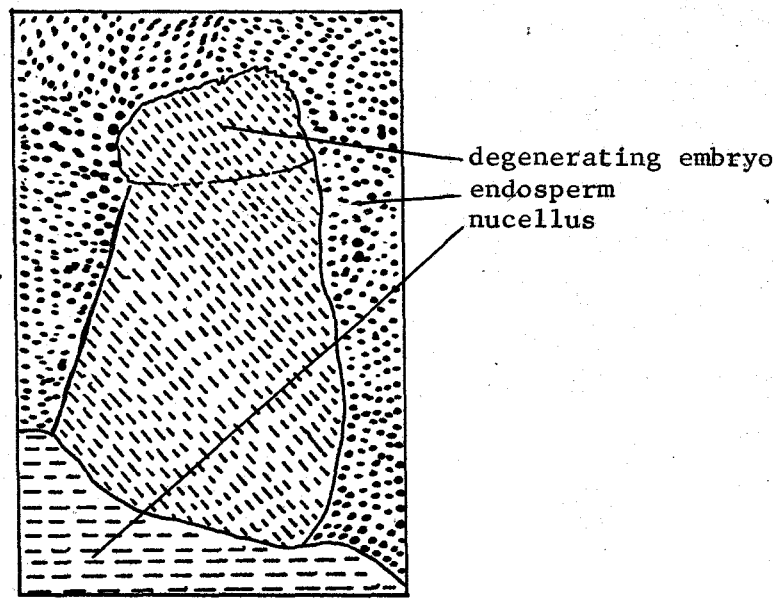


Figure 48b

Figure 48 shows what the author believes to be an embryo which is dying. The upper portion of the embryo appears to lack organization. If this is a degenerating embryo, then this could explain the reason for the seed's withering and dying. The cause of such degeneration is unknown by the author.

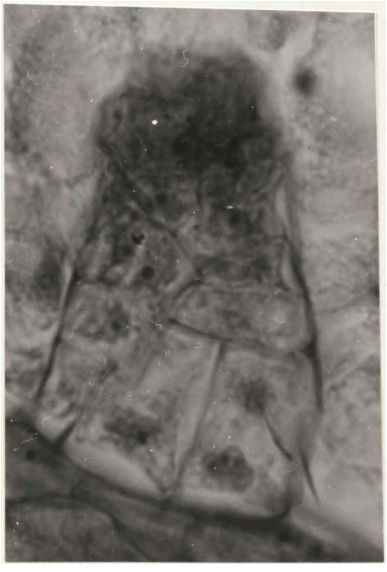


Figure 48a

Psyllid Infestations

With the methods of polyembryony development so varied, the exact cause, if there is an exact cause, cannot be determined. However, it is speculated by the author that the Psyllid which attacks this plant may inject a substance into the seed area which causes the increased instances of meristematic activity. Appendix IV gives a more detailed account of the Psyllids' attack on the hackberry. Examination of the seeds of Psyllid-free plants would tend to verify or disprove this theory.

CONCLUSION

It is the conclusion of this study that polyembryony in *Celtis reticulata* occurs in at least two, and possibly four, ways.

The author believes that strong evidence has been given in this study to support the theory that the occurrence of an extra egg-like cell within the ovule is a major means by which second embryos are formed.

Embryo splitting is also very much in evidence as an important means by which second embryos are formed.

Suspensor budding is a possible means of multiple embryo formation in *C. reticulata*, but there is a possibility of faulty interpretation of the sections.

Nucellar budding is a fourth possible method of multiple embryo formation in *C. reticulata*.

The possibility of a growth-stimulating substance being injected into the plant by the Psyllid which parasitizes *C. reticulata* is, the author believes, a theory warranting further investigation.

SUMMARY

1. Drupes of netleaf hackberry, Celtis reticulata, exhibit multiple embryos commonly.

2. Microscope slides of C. reticulata seeds were prepared, from pollination through maturation. It was determined that multiple embryos were formed in at least two, and possibly four, ways:

a. Numerous slides showed evidence of an extra egg-like cell--probably a synergid--in the embryo sac. It is assumed that this cell is fertilized to form the extra embryo. This appears to be the most common method by which multiple embryos in C. reticulata are formed.

b. The splitting of the zygotic embryo is evidenced by slides and supported by germination studies in which double epicotyls were found on a single hypocotyl.

c. Budding of the suspensor cells was observed to be a possible means of multiple embryo development.

d. Nucellar budding was found to be the fourth observable method of multiple embryo development.

3. The possibility of the Psyllid which attacks C. reticulata injecting a growth-stimulating substance into the plant is conjectured by the author.

4. Of the 36 seeds which germinated, 7 produced twin seedlings.

5. Many young fruits abort after a few weeks of development. The cause is not known.

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APPENDIXES

APPENDIX I

Collection of Material

Collections were made from trees found on the Brigham Young University campus, Provo, Utah, and from trees found on the foothills northeast of Springville, Utah.

Collection Dates	Materials Collected
1965	
February 24	early buds and twigs
March 3	early buds and twigs
April 1	early buds and twigs
April 8	early buds and twigs
April 15	buds
April 21	buds and twigs
April 28	*buds and twigs
May 17	young seeds
May 24	young seeds
June 4	young seeds
June 17	young seeds
June 28	ovules
July 28	ovules
August 12	fully developed seeds
1966	
April 27	*flowering spikes

APPENDIX I (continued)

Collection Dates	Materials Collected
1967	
April 5	buds
April 10	buds
April 14	buds
April 19	early flowering buds
April 25	early flowering spikes
April 28	early flowering spikes
May 1	*flowering spikes
May 5	*flowering spikes
May 11	*flowering spikes and early seeds
May 16	seeds
May 19	seeds
May 23	seeds
May 26	seeds
June 2	seeds
June 7	seeds
June 13	seeds
June 21	ovules and seeds
June 22	ovules and seeds
June 26	ovules
June 30	ovules
July 5	ovules
July 10	ovules

Collections of ripe, mature seeds were made the late fall of 1965 and 1966. These seeds were those used in the seed germination studies.

*Pollination was occurring at this time.

APPENDIX II

Preparation of FAA Solution

The following is the method for preparing FAA solution per 100 ml of solution (after Johansen, 1940):

ethyl alcohol (70%)	90 ml
glacial acetic acid	5 ml
formalin	5 ml

APPENDIX III

Preparation of Embedding Wax

The following is the method for preparing embedding wax (after Gray, 1952):

paraffin	100 grams
stock rubber solution	4 grams
bayberry wax	7 grams
beeswax	1 gram

The stock rubber solution is prepared in the following manner: Heat 100 grams of paraffin to the point at which it begins to smoke. While stirring constantly, add 20 grams of crude rubber cut into small pieces. Stir until the rubber is dissolved. Cool, pour into a block, and use as needed (Johansen, 1940).

APPENDIX IV

Leaf Galls of Hackberry

The life histories of species of Pachypsylla which cause hackberry leaf galls is given by Sterling (1952), Wells (1920), and Lewis and Walton (1964). The adults escape from the gall in the fall of the year and hibernate throughout the winter under fallen leaves and other debris. As soon as the hackberry leaves begin to develop, the insect is ready and lays its eggs on the immature leaf. The point of attachment on the leaf is killed, later causing a hole in the matured leaf. The eggs hatch in two to three days and all larvae move to the upper side of the leaf, if they are not on the surface to begin with. It is on the upper side of the leaf where the insect inserts its proboscis and initiates gall development. The cells located 15 cells from the point of attachment show the greatest amount of activity in gall development. The larva remains in the initial spot, allowing the developing gall to engulf it. The insect, therefore, becomes a prisoner within the gall, until it escapes in the fall as a mature nymph.

It has been shown by Lewis and Walton (1964) that as the Psyllid inserts its proboscis, it injects a virus-like substance called cecidogen. It is this substance, cecidogen, that causes the cells around the insect to begin dividing and thus form the gall. Cecidogen is considered to be a virus because it occurs in crystalline form and is Feulgen-positive.

The occurrence of "giant" multinucleate cells has been noted to also occur in connection with insect-caused galls. These "giant" cells

APPENDIX IV (continued)

occur in the vicinity of where the proboscis is inserted, the largest cell being the one in which the proboscis terminates. They have been known to contain as high as eight nuclei (Wells, 1920).

A STUDY OF MULTIPLE EMBRYO DEVELOPMENT IN THE NETLEAF HACKBERRY (CELTIS RETICULATA)

An Abstract of a Thesis

Presented to the

Department of Botany

Brigham Young University

Provo, Utah

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Gary Porter Lawrence

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ABSTRACT

Previous research has shown that polyembryony occurs in many species of the Ulmaceae. The purpose of this study was to determine the method of multiple embryo formation in netleaf hackberry, Celtis reticulata Torr.

Collections of specimens were made throughout a two-year period. Collections were begun in early spring while the trees were still in the bud state. Collections were continued regularly throughout the growing season.

The collections were preserved in FAA solution and were prepared for microscopic examination as follows: (1) The tertiary butyl paraffin method was used for dehydrating and embedding. (2) Sectioning was done on a rotary microtome at 7-20 microns. (3) Staining of the slides was accomplished by Conant's Quadruple.

Examination of the slides reveals at least two, and possibly four, ways by which polyembryony may occur in C. reticulata. The most frequent method of multiple embryo development appears to be the development of an extra egg-like cell--probably a synergid--in the embryo sac. Some slides show the egg-like cell after it has begun to develop into an embryo.

The splitting of the zygotic embryo was observed on the slides and supported by germination studies in which double epicotyls were found on a single hypocotyl.

Suspensor budding appears to be a means of multiple embryo formation, but the possibility of faulty interpreation of the sections, due

to the plane in which they were cut, could discount this theory.

Nucellar budding is also a possible means of multiple embryo development. However, here again the possibility exists that faulty sectioning of the material occurred. While the slides appear to show embryos being formed along the nucellar layer, these embryos could be of synergid origin. If nucellar budding does actually occur, it would have great genetic and evolutionary significance.

Seed germination studies reveal a high percentage (20%) of multiple seedlings. It is also noted that frequently a seed will abort a few weeks after pollination. The reason for this abortion is unknown, but the author speculates that this is due to embryo degeneration.

The possibility of a growth-stimulating substance being injected into the plant by the Psyllid which parasitizes C. reticulata is, the author believes, a plausible theory worthy of further study.