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# Otogeny of the embryo in Cornus florida L.

Joseph T. Dietrich

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I am submitting herewith a thesis written by Joseph T. Dietrich entitled "Otogeny of the embryo in Cornus florida L.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Landscape Architecture.

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# ONTOGENY OF THE EMBRYO IN *CORNUS FLORIDA* L.

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Joseph T. Dietrich

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## ABSTRACT

The zygotic embryo development in Cornus florida L. was documented during two growing seasons. Beginning at anthesis ovules of C. florida were sampled twice weekly. Anthesis was considered to be the time when one-half of the flower buds had opened and occurred near April 21 both years. The ovules were fixed, embedded, sectioned, and stained according to standard histological procedures for paraffin sections. Although several tissues sampled from four-to-five weeks post-anthesis revealed suspensor-like cells, the first tissues which contained recognizable embryos were collected six weeks post-anthesis. The globular embryo stage was completed three weeks later and most embryos had reached the torpedo stage by ten weeks post-anthesis. The embryos were anatomically fully developed and were accumulating storage material by July 30, fourteen weeks post-anthesis. At that time, fruits remaining on the tree were beginning to develop red coloration. Further embryo development was limited to continued storage substance accumulation and slight elongation with a final average length of 6.0 mm being reached by embryos collected in early October.

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# CHAPTER I

## INTRODUCTION

*Cornus florida* L., the flowering dogwood, is a native understory tree in forests of the eastern United States. It has been adapted to nursery production and is specified frequently where small trees are needed in designed landscapes. Wyman (1986) stated that the flowering dogwood "is considered to be the best ornamental of all the trees native to the northern U.S." Dirr (1983) described *C. florida* as "the aristocrat of native flowering trees." He also noted the seasonal interest that flowering dogwood provides with its excellent flower display in the spring, high quality leaf character in the summer and fall, abundant red fruits in autumn, and attractive winter habit.

*C. florida*'s value as a commercial commodity in the nursery industry is reflected in production figures from Tennessee nurseries. Sixteen percent of nursery production in Tennessee is devoted to flowering dogwoods (Badenhop et al, 1985). Due to its stature as an important native species and its high economic value, *C. florida* is an excellent candidate for scientific research, including *in vitro* culture methods.

The natural development of a plant needs to be understood whenever a researcher is working with that plant *in vitro*. This includes the natural zygotic embryo development. This information can be used to understand the developmental stage of the tissue explant (the part of the plant introduced into culture). If, in a tissue culture system, post-fertilization ovules are used as explants, it is important to know the stage of development that the enclosed zygotic embryo has reached.

The natural zygotic embryo developmental sequence also needs to be understood if an *in vitro* embryogenic system has been developed. According to Ammirato (1987), it is important to understand *in vitro* somatic embryo development and it is particularly important to compare *in vitro* embryogenesis with the plant's normal zygotic embryo development. He noted that many abnormal embryo forms can be produced by an *in vitro* embryogenic system. Any difference from the normal course of embryo development has the potential to affect the ability of the somatic embryos to function as embryos in practical applications (Ammirato, 1987). The zygotic embryo development in *C. florida*, however, is not well documented.

*C. florida* is a valuable and important woody plant and is deserving of *in vitro* research methods. The natural

zygotic embryo development is important information which needs to be determined. Therefore the objective of this study was to document the natural histological development of the zygotic embryo in C. florida as a data base for in vitro research. An attempt was made to provide a frame of reference for the embryo development using internal and external signs on the fruit. This will allow an estimation of the stage of embryo development at a particular time without undertaking a histological investigation.

## CHAPTER II

## LITERATURE REVIEW

Megasporogenesis and megagametogenesis has been documented for C. florida (Smith, 1973, 1975) and several other species of Cornus (Chopra and Kaur, 1965), but specific information on embryogenesis in the genus has been limited. Erdelska (1986) cited the hard lignified layer of the pericarp in fruits of the genus Cornus, which leads to difficult embryo removal and histological procedures, as the primary reason for the lack of information about dogwood embryogenesis.

In spite of these difficulties, embryo development has been reported for C. mas (Erdelska, 1986). Zygotic embryo development appeared to follow the solanad type, although the cell division patterns were not always absolute. With this type of development, the first two divisions following the two-celled stage are transverse, forming a linear four-celled embryo. The basal cell continues to divide transversely to form a uniseriate suspensor (Maheshwari, 1950). Embryo development in C. mas occurred over a four month period. The linear stage was present in late May, while in June the embryo developed through the globular and heart-shaped stages and into the torpedo stage, which

measured 1-to-2 mm. By the end of July, the embryos measured 7-to-8 mm. At this time, embryo development stopped and accumulation of storage materials began primarily in the cotyledons and the cortex of the radicle. The accumulation continued into August.

Endosperm formation in the family Cornaceae is of the cellular type (Davis, 1966). In this type of development, the initial and most of the subsequent nuclear divisions are accompanied by cell wall formation. The embryo sac appears to contain many chambers, some of which may have more than one nucleus (Maheshwari, 1950). Cellular endosperm development was confirmed for C. mas (Erdelska, 1986). In mid-May endosperm development began and continued until mid-June when cell divisions ceased. During later development, the endosperm cells enlarged and were filled with proteinaceous storage material. The endosperm remained in the developing seeds and appeared as a few cell layers filled with storage substances.

Embryology studies on C. mas provide useful information but may not apply to C. florida. Several embryological features of flowering dogwood are very different than those of other members of the genus (Smith, 1973). These include the origin of the megaspore mother cell, the number of megaspore mother cells per nucellus, and the location of the

megagametophytes in relation to the nucellus. There are also differences between C. florida and other members of the genus in the inflorescence and the distribution of the plant (Smith, 1973). Due to these differences, Smith (1973) concluded that C. florida should be removed from the genus Cornus. Thus, to predict the pattern of embryo development for flowering dogwood based on the develpoment of C. mas or any other species of the genus would be erroneous.

There is some information available on C. florida embryo development from a study done by Morse (1907). By collecting tissue samples for a year, beginning in September of 1905, and performing the necessary histological techniques, he was able to gather much information on several aspects of development, including flower bud development, micro- and megagametogenesis, and endosperm and embryo development. However, his information is somewhat limited and the camera lucida drawings lack clarity.

Embryo sac samples collected by Morse June 12, 1906 revealed that the micropylar end of the embryo sac was filled with endosperm tissue. Within two weeks, the endosperm had reached an arrangement which Morse considered to be characteristic for the species. Endosperm cells at the micropylar end formed a "cap-like" structure. Material collected June 12, 20, and 29 showed a short chain of irreg-

ular cells with thicker cell walls than the surrounding endosperm. However, he was not able to determine if these cells were the young embryo.

The first tissue that definitely contained an embryo was collected July 9. This tissue contained a small globular embryo attached by a suspensor to the "cap" of endosperm cells. The endosperm cells did not completely fill the embryo sac at that point. Twelve days later, tissue samples revealed rapid embryo development. At that time, cotyledons were present and some tissue and cell differentiation had occurred in the vascular cylinder. The endosperm had completely filled the embryo sac. By July 28, his final sample date, the embryo had greatly enlarged, and the cotyledon, hypocotyl, root tip, root cap, and embryonic tissues were well differentiated (Morse, 1907).

Morse's study provides some information, but with only three sample dates showing an embryo, information about the embryo development incomplete. A more complete histological study, using modern methods of tissue processing and documentation, can reveal much more useful information about the development of the zygotic embryo in C. florida.

# CHAPTER III

# MATERIALS AND METHODS

## Sampling

Tissue samples for the study were collected from one specimen of flowering dogwood located on the Agricultural campus of the University of Tennessee, Knoxville. Samples were collected twice weekly beginning at the time of anthesis and continuing until mid-October. Anthesis was considered to have occured when half the flower buds had opened. This was determined by counting the number of open flower buds and the total number of buds on ten randomly selected inflorescences twice weekly. When the ratio of open flower buds to closed buds reached 1:1, random sampling of inflorescences was initiated.

The piece of tissue collected depended on the fruit development. Initially all ovaries were the same size, therefore all ovaries on an individual inflorescence were collected. Within two weeks, a few ovaries on each receptacle began to enlarge. Thereafter, enlarged ovaries and ovules from enlarged ovaries were sampled.

About five weeks after anthesis, a long slender embryo sac was visible and easily excised. At this time, ovules and embryo sacs from enlarged ovaries were fixed. This type

of sampling continued for three weeks until the endocarps became hard and prevented histological processing and sectioning. Samples then consisted of excised embryo sacs until the time the embryo itself could be removed. Samples for the duration of the study consisted of excised embryos and embryo sacs.

A minimum of ten pieces of each tissue type were fixed at each sampling date. At each sampling, notes were made describing ovule and ovary development which corresponded to the stage of the embryo present in the sampled tissue. For example, red coloration of the fruit is a visual sign that may indicate a particular stage of embryo development.

## Fixation

Tissues will respond differently to different fixatives, therefore three killing/fixing solutions were used in this study. Satisfactory results were obtained with each fixative, but often one fixative gave superior results at a particular stage of plant development. The three fixatives which were used are briefly described below.

Formalin-propionic acid-alcohol (FPA) is a modified formalin-acetic acid-alcohol (FAA) fixative. It consists of 5% formalin, 5% propionic acid, and the balance 50% ethanol (Johansen, 1940). The second fixative used was a modified Navashin fluid, also known as CrAF. When using this

fixative, two solutions are mixed immediately before use. One solution is a mixture of 1% chromic acid and 10% acetic acid, whereas the other contains 20% formalin and water. The modification that was used is known as CrAF III (Sass, 1958). The third killing/fixing solution was a 3% formaldehyde-3% gluaraldehyde (F-G) solution in an acetate buffer (O'Brien and McCulley, 1981).

After placing the tissue in the killing/fixing solutions, all samples were aspirated in a vacuum chamber to allow for more rapid penetration of the fixatives and removal of gases (Jensen, 1962). Aspiration time varied with the type of tissue sampled, but in all cases it continued until all the tissues had sunk in the fluids and there was no sign of gases escaping from the tissue. The plant tissue was stored in the fixative solutions in capped 2 ounce glass bottles until needed for processing.

Dehydration

Ten pieces of each tissue type were dehydrated using a standard series of graded alcohol solutions (Sass, 1958). The entry point of the tissue into the dehydration series depended on the type of fixative used for that particular tissue. In all cases, the tissue remained in each alcohol solution a minimum of one-half hour to allow for complete

water and alcohol exchange before the old solution was decanted off and the next solution added.

Tissue fixed in F-G and CrAF III were initially dehydrated through a series of ethanol solutions that contained 5%, 15%, 25%, and 40% ethanol. FPA contains a high percentage of 50% ethanol (90% by volume), so tissues in this fixative entered the dehydration series at the 50% level (Sass, 1958). The tertiary butyl alcohol (TBA)/ethanol series (Johansen, 1940) was used to facilitate the transition from the dehydrating solution to paraffin. Concentrations of TBA/ethanol solutions used were 50%, 70%, 85%, 95%, and 100% (percent of total alcohol). The last solution listed also contained a small amount of the dye erythrosin B which temporarily stained the tissue and made it visible throughout the embedding and sectioning process (Johansen, 1940). From the 100% alcohol solution, the tissue was transferred through two changes of a 9:1 mixture of TBA and 2-propanol. After sufficient time had elapsed, the tissue was transferred to a small volume of the same solution.

## Paraffin Infiltration and Embedding

Eight-to-ten paraffin pellets (Tissue-Prep, melting point 56-57°C, Fisher Scientific Co.) were placed into the

bottles which contained the plant tissue and the TBA/2-propanol mixture. These were placed in an oven at 60°C, until the paraffin melted. Pellets were added several times to increase the paraffin volume to approximately six-to-eight times the volume of the alcohol solution. When the final pellets had melted, the alcohol/molten paraffin mixture was carefully decanted and replaced with fresh, molten paraffin. The bottles were returned to the oven uncapped. This process was repeated four-to-five times to evaporate off the alcohol until there was no scent of TBA in the vials. The tissues were cast into paraffin blocks using stainless steel molds and plastic block rings. A paper label recording the date collected, fixative, and tissue type was attached to each specimen block.

Sectioning

Initially four of the ten tissue samples were randomly chosen for sectioning. The tissues were sectioned 10 μm thick on a rotary microtome using a resharpenable steel microtome knife. In some instances it was necessary to section additional material to obtain suitable slides.

The resulting paraffin section ribbons were cut into pieces and transferred to pools of water on washed glass slides without adhesive. The slides were placed on a

warming tray at 45-50° C to decompress and flatten the sections. When decompressed, excess water was carefully removed and the slides returned to the warming tray for complete drying.

Staining

To prepare for staining, the slides were passed through four changes of a proprietary paraffin and clearing solvent (nonspecified essential oil) in coplin jars to remove the paraffin. They were then passed through a descending ethanol series consisting of three changes of absolute ethanol to remove the paraffin solvent, then 95%, 85%, 70%, 50% and 30% ethanol, and finally to water, to rehydrate the tissue, allowing five minutes in each solution.

A combination stain composed of the Periodic Acid-Schiff (PAS) reaction (Jensen, 1962) and Heidenhain's iron hematoxylin (Johansen, 1940) proved superior to other staining procedures. The PAS reaction stains polysaccharides, such as cellulose cell walls, a pink or magenta color. The Heidenhain's iron hematoxylin stains nuclear material gray or black. The entire staining procedure was performed by transferring the slides through the solutions in coplin jars.

The PAS reaction was performed first. Staining was initiated by placing the slides in a 0.5% periodic acid solution for 20 minutes followed by four water rinses. Next, the slides were placed for five minutes in Schiff reagent to produce the stain, then passed through four water rinses. A two minute period in 2% sodium metabisulfite followed by four water rinses completed the PAS reaction staining.

To begin the Heidenhain's iron hematoxylin stain, slides were placed in a 4% Lang's mordant solution (Lang, 1936) for five minutes. The mordant solution was then rinsed off with four changes of water. Following the mordant application, slides were placed in a 0.5% aqueous Heidenhain's iron hematoxylin solution for five minutes. The slides were then passed through four changes of water to rinse off excess stain.

Each slide was then observed individually and quickly under a low power objective lens to check for hematoxylin overstaining. Tissues fixed in CrAF III were generally not overstained, but those fixed with FPA or F-G were often very dark. The stain was extracted with a 2% Lang's extractant solution (Lang, 1936). The time needed for destaining varied by tissue and fixative and ranged from five to thirty seconds. Following the stain extraction, the slides were

rinsed in four changes of water and observed under the microscope a second time to check for satisfactory staining. In a few instances, the extraction was repeated to produce an adequate stain.

Once an optimum stain was attained, the slides were passed through another graded ethyl alcohol series which was the reverse of the rehydrating series. Slides were placed in each solution for five minutes. The 95% ethanol solution was followed by three changes of absolute ethanol to remove all trace of water. The slides were then passed through four coplin jars of essential oil clearing solution to remove the ethanol. After a sufficient time in the clearing solvent, the slides were removed from the coplin jars and #1 1/2 coverglasses were mounted with resin. The finished slides were placed on a slide warmer (45-50° C) to cure the resin and adhere the coverslip to the slide.

Interpretation

Completed slides were observed at x10, x20, x50, x100 and x300. Globular embryos were measured, when possible, with a stereo microscope and a micrometer slide with a 2 mm scale. The slides with the embryo sections were set on top of the micrometer slide, with the edge of the embryo lined up with the end of the scale. In this way it was

possible, by adjusting the focus and varying the magnification, to measure even very small embryos. Embryos in the torpedo stage were large enough to measure with a clear plastic ruler under the stereo microscope. The mean embryo length was calculated for each sampling date that was measured. Photomicrographs were taken on 35 mm Kodak Technical Pan film and on Poloroid positive/negative 4x5 black and white film with electronically controlled exposures.

Notes were taken for the stained slides and a generalized developmental scheme was compiled. These results were compared with the visible ovary development to produce a useful zygotic developmental scheme and a simple frame of reference for estimation of embryo development.

# CHAPTER IV

## RESULTS

Well developed ovaries and ovules were observed in tissue sampled in late March (Plate 1, Figure 1). From this point, the embryo sac began to enlarge.

Anthesis occurred about the third week of April (April 22, 1987 and April 19, 1988). Sections through the gynoecium at this time revealed a row of distinctive stylar canal cells through the center of the style, extending from the stigma into the ovary tissue (Plate 1, Figure 2). Pollen was seen among the hairs on the surface of the stigma at this time. Pollination, however, may occur before anthesis. Dry, dehisced anthers were commonly seen in unopened flower buds. The pollen released from these anthers was often seen on the stigma of the same flower. Ovule sections at this time reveal a large embryo sac, with very little internal cellular development (Plate 2, Figure 3).

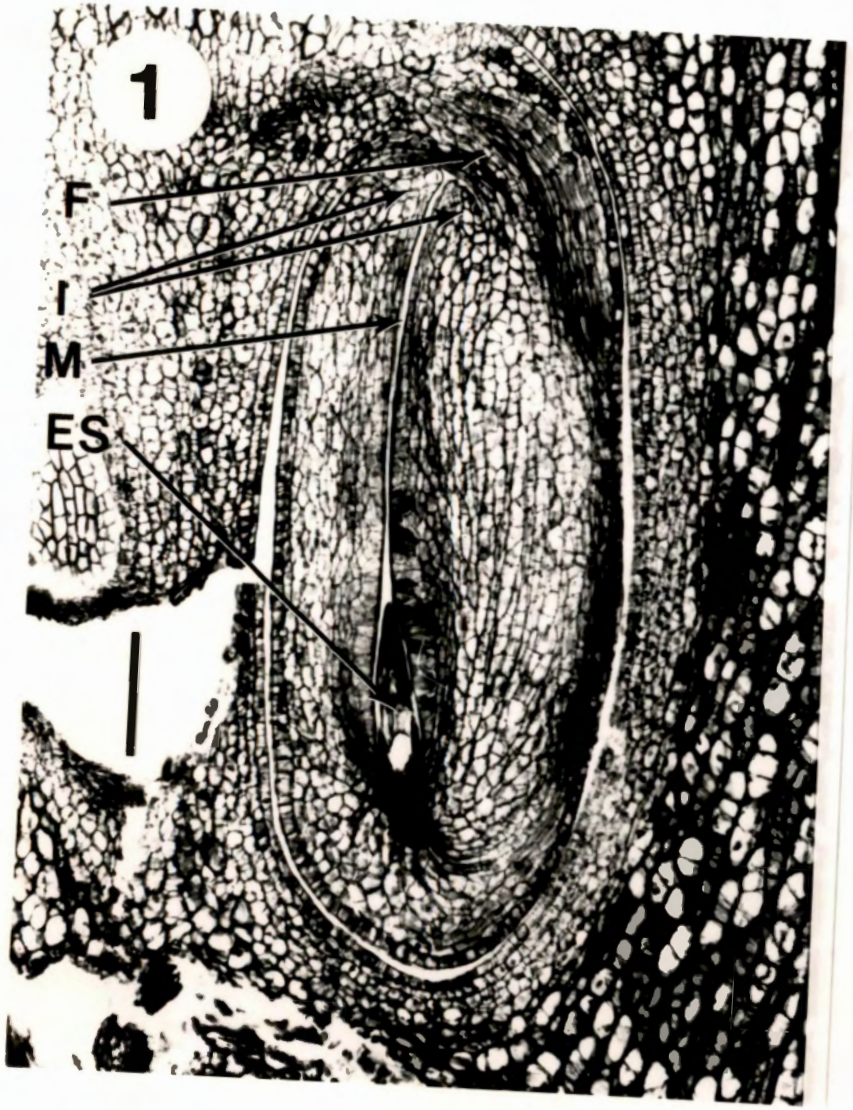
Within ten days following anthesis, the first signs of endosperm development were observed. This coincided with the abscission of the large showy bracts from the receptacle. After another ten days, or three weeks post-anthesis, all samples collected contained large, irregular

Plate 1. Preliminary Anatomical Observations.

Figure 1. Longitudinal section of an ovule sampled in late March. X 130 Line = 100 μm

Figure 2. Longitudinal section of a style showing prominent stylar canal cells. X 40 Line = 500 μm

cc = canal cells, es = embryo sac, f = funiculus, i = integuments, m = micropyle,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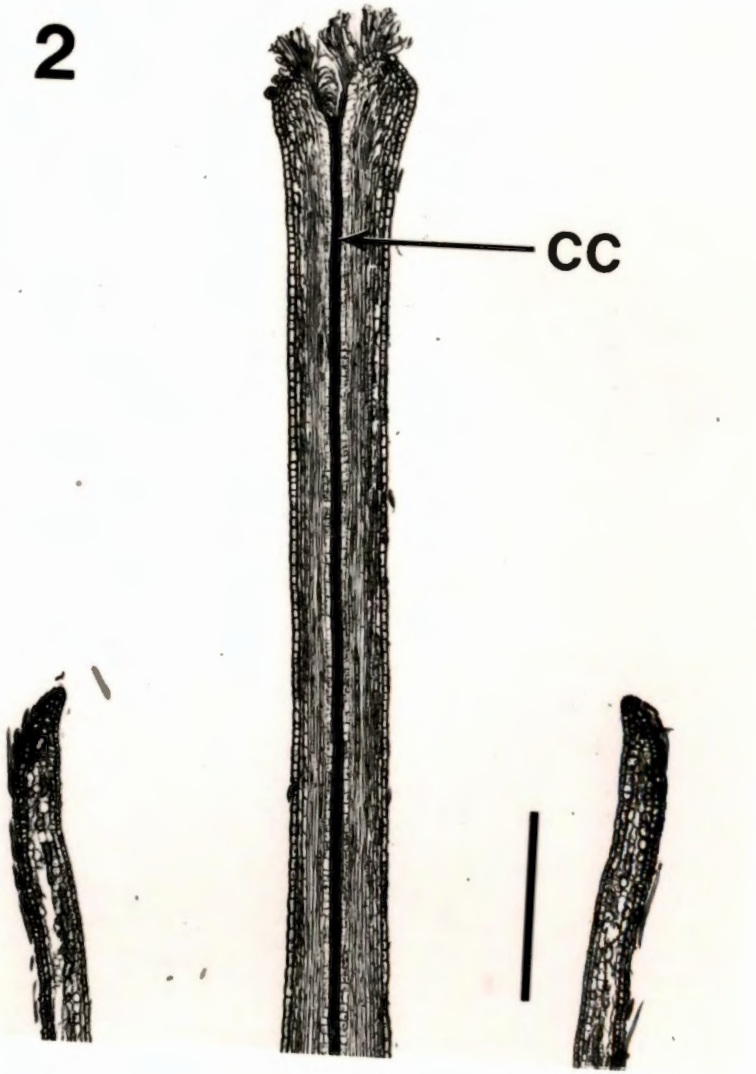
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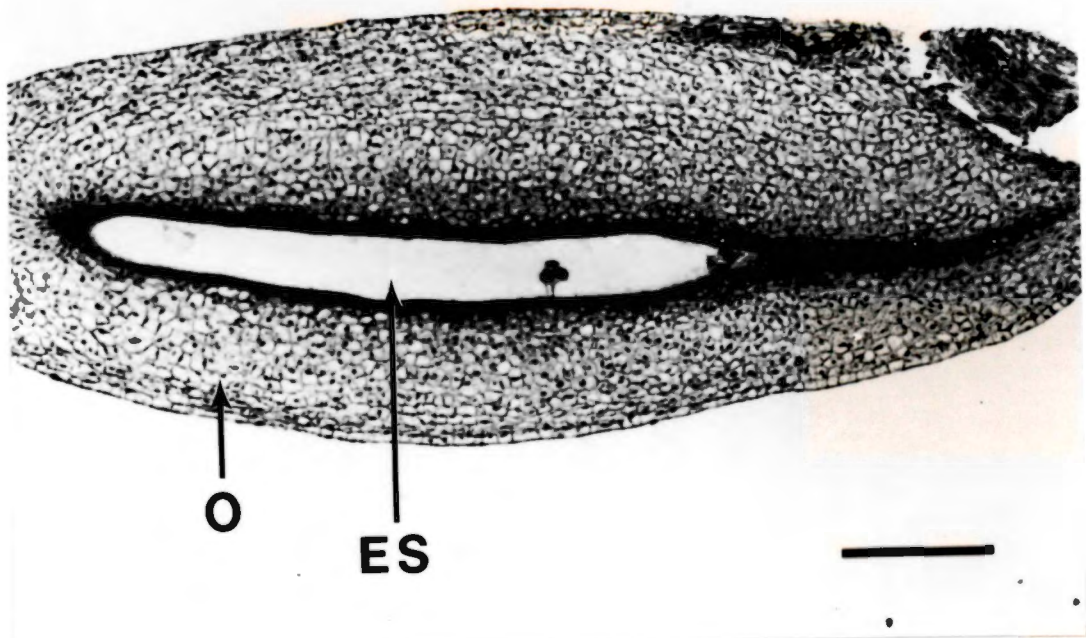
Plate 2. Early Post-anthesis Embryo Sac and Endosperm Development.

Figure 3. Longitudinal ovule section showing an embryo sac before endosperm development. X 80 Line = 200 μm

Figure 4. Longitudinal ovule section showing an embryo sac with cellular endosperm formed. Note the formation of the "cap" of smaller, densely staining endosperm cells at the micropylar end of the embryo sac. X 40 Line = 200 μm

c = "cap" of endosperm cells, e = endosperm, es = embryo sac, o = ovule

3

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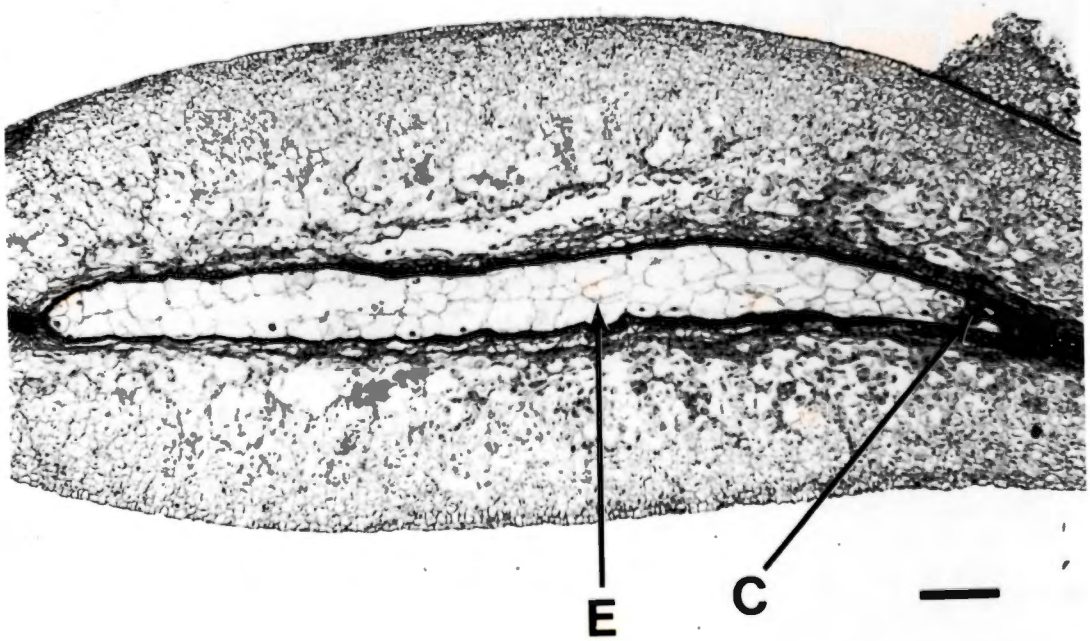


endosperm cells throughout the embryo sac (Plate 2, Figure 4). At this time, it was apparent which ovaries on each floral receptacle were enlarging and contained developing ovules. The location of the enlarged ovaries on the receptacle appeared random.

Samples collected throughout the following three weeks revealed a lengthening embryo sac and an increase in the number of endosperm cells, but a proembryo was not seen. By four weeks post-anthesis, the "cap" of dense endosperm cells at the micropylar end of the embryo sac was present in all tissue samples examined (Figure 4). At five weeks post-anthesis, the embryo sac was visible to the naked eye as a long, slender, translucent, fragile structure and was excisable.

Many samples during a two week period, from four-to-six weeks after anthesis, contained cells that suggested embryo development, but none clearly contained proembryo tissue. Seventy-two percent (49-of-68) of the tissue samples collected during this time contained linear chains of suspensor-like cells. These cells were larger than the surrounding cells and were stained pink by the PAS reaction. These contrasted sharply with the gray staining, irregular shaped endosperm cells that encased them (Plate 3, Figure

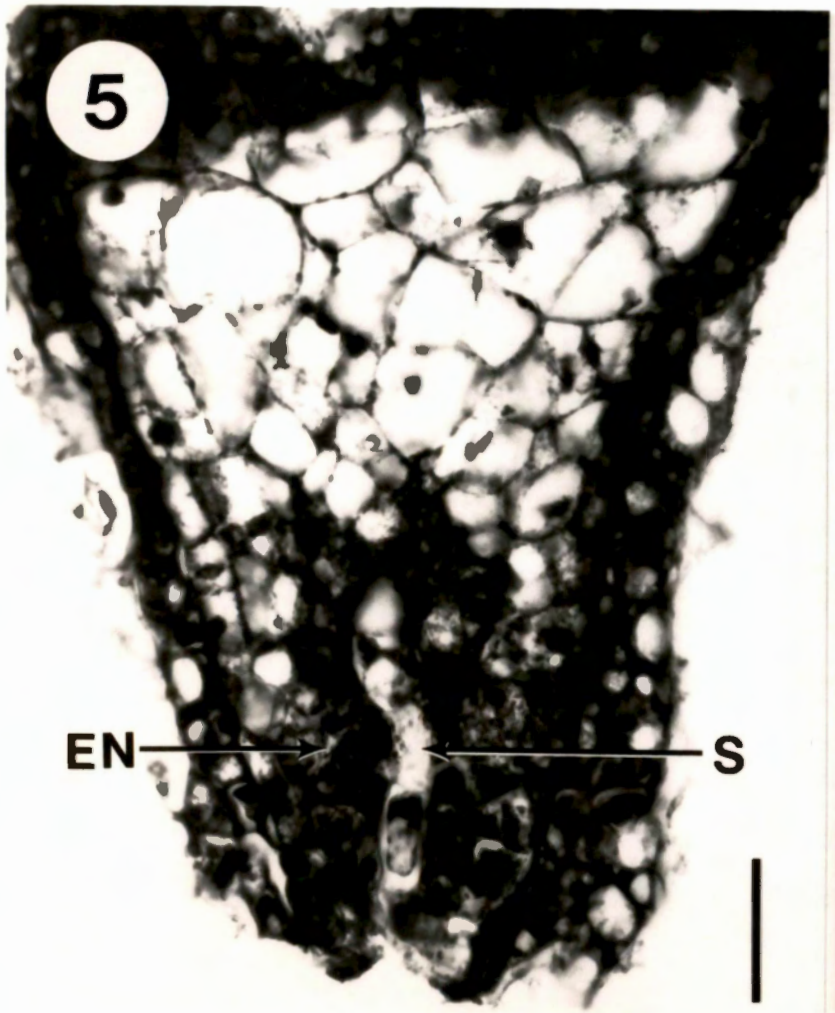
Plate 3. Initial Embryo Detection.

Figure 5. An embryo sac section showing the large suspensor cells surrounded by the darker staining endosperm. X 300 Line = 50 μm

Figure 6. A young embryo section from embryo sac tissue collected during the period of the first definite embryo development, six weeks post-anthesis. X 600 Line = 50 μm

pe = proembryo, en = endosperm, s = suspensor

Note: The micropylar end of the embryo sac is at the lower end of these and subsequent photomicrographs.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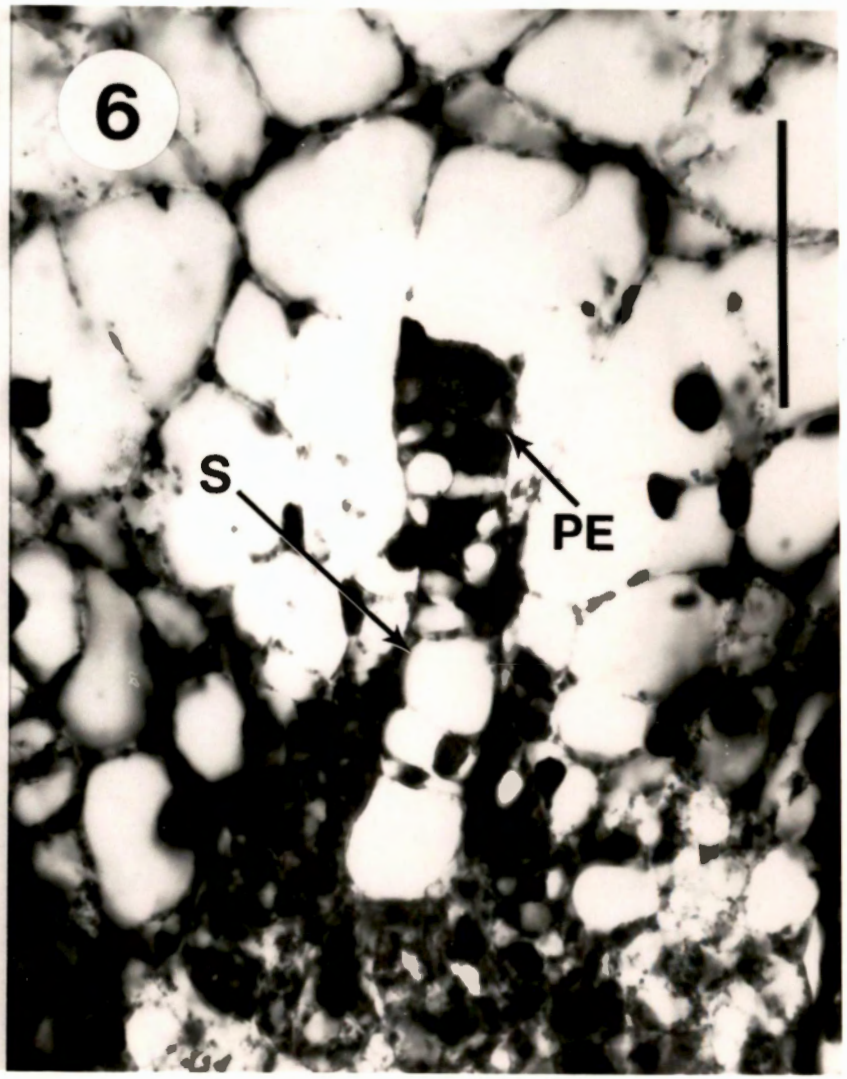
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5). Proembryo development, although suggested, was not confirmed by the samples collected during this time period.

The first tissues which clearly contained embryos were collected six weeks post-anthesis. Embryos were a small group of densly staining cells attached to a multicelled, linear suspensor, surrounded by the endosperm cap (Plate 3, Figure 6). At this time, the ovule was becoming difficult to remove from the ovary.

Early globular embryos of approximatly 10-to-16 cells had developed within a week. These were attached to rather long uniserate suspensors (Plate 4, Figure 7). The endosperm cells throughout the embryo sac were becoming more numerous, making the endoperm tissue appear more dense. The endocarp was beginning to harden, making ovule excision increasingly more difficult.

From eight-to-nine weeks post-anthesis, or two-to-three weeks following the first embryo detection, the embryos continued the globular phase of their development (Plate 4, Figure 8). By nine weeks post-anthesis the embryos had reached a measurable length. The mean embryo lengths from the entire study are summarized in Table 1. At nine weeks post-anthesis, there were a variety of sizes represented on an inflorescence at any one time. The embryos averaged 0.1 mm in length, and ranged from 0.05-to-

Plate 4. Embryo Development Through the Cordate Stage.

Figure 7. An early globular embryo showing a portion of the prominent suspensor, seven weeks post-anthesis. X 290 Line = 50 μm

Figure 8. A globular embryo eight weeks post-anthesis. X 355 Line = 50 μm

Figure 9. A late globular embryo which is beginning to flatten at the area of the future shoot apex, nine weeks post-anthesis. X 275 Line = 50 μm

Figure 10. A section of an embryo at the cordate stage of development, ten weeks post-anthesis. X 115 Line = 100 μm

c = cotyledons, e = embryo, en = endosperm, s = suspensor

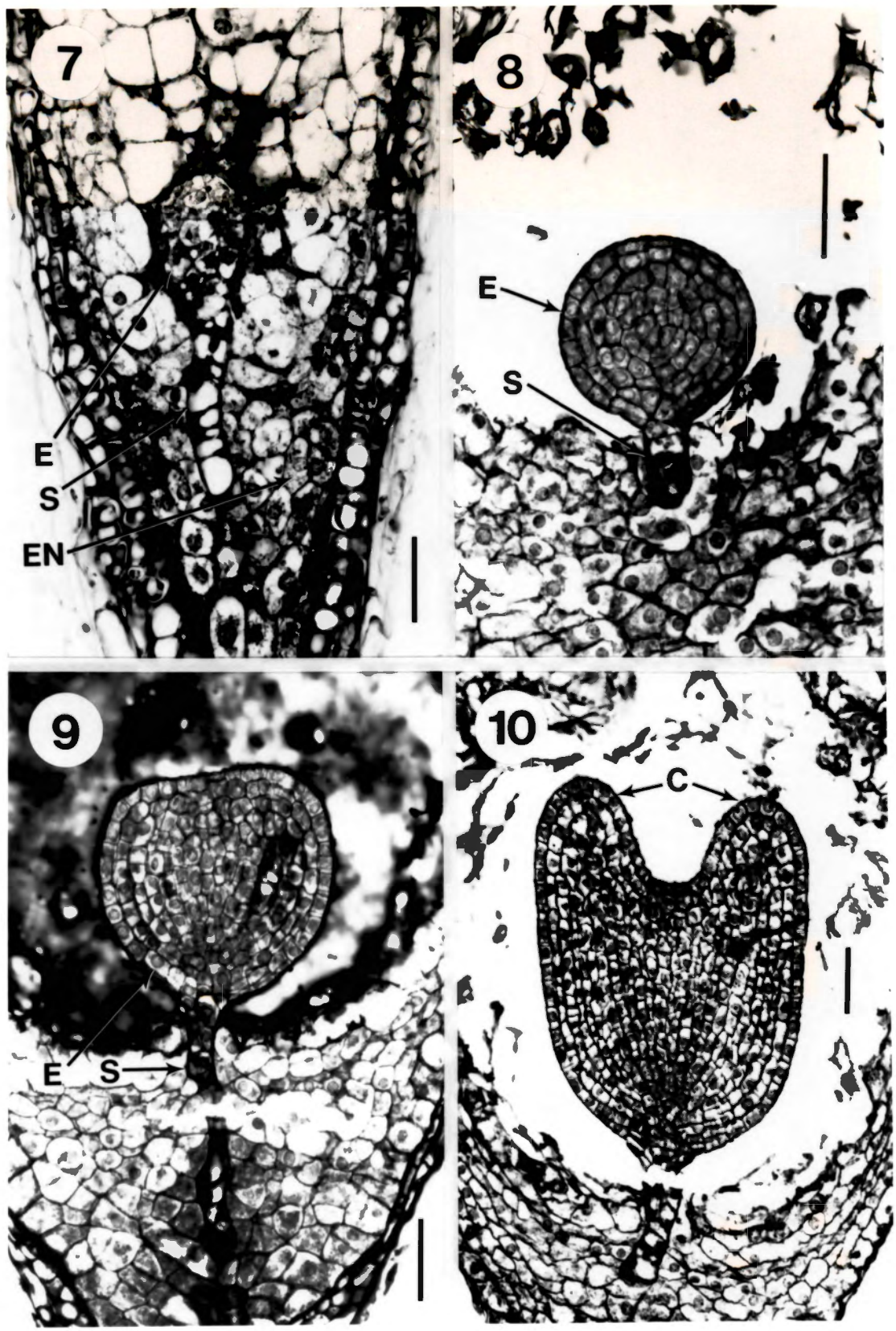

7
E
S
EN
8
E
S
9
E
S
10
C

Table 1. Physical Development of Embryos in *Cornus florida*.

| Weeks Post-anthesis | Weeks Post First Embryo" | Mean Embryo Length (mm) | Range (mm) | Standard Deviation |
|---|---|---|---|---|
| 9 | 3 | 0.1 | 0.05-0.15 | 0.05 |
| 10 | 4 | 0.3 | 0.1-0.4 | 0.14 |
| 10.5 | 4.5 | 0.6 | 0.5-0.9 | 0.20 |
| 11.5 | 5.5 | 1.2 | 0.75-1.75 | 0.51 |
| 12.5 | 6.5 | 3.6 | 3.0-4.5 | 0.64 |
| 13.5 | 7.5 | 4.5 | 3.5-5.5 | 0.72 |
| 14.5 | 8.5 | 5.1 | 4.25-6.0 | 0.61 |
| 15.5 | 9.5 | 5.3 | 4.0-6.0 | 0.50 |
| 16.5 | 10.5 | 5.6 | 5.0-6.25 | 0.43 |
| 20.5 | 14.5 | 5.8 | 5.0-6.75 | 0.55 |
| 24.5 | 20.5 | 6.0 | 5.5-7.0 | 0.71 |

0.15 mm. By the end of nine weeks post-anthesis, the embryos were beginning to flatten at the area which would become the shoot apex (Plate 4, Figure 9). The sepals on many of the developing fruits had turned red by this time and the endocarps had become very hard.

During the next week, embryos at a late cordate/early torpedo stage were observed (Plate 4, Figure 10). This stage corresponded to the time the embryo sac was becoming broad or flat, and the endosperm cell walls were becoming very irregular. At ten weeks post-anthesis (four weeks following the first embryo detection) torpedo stage embryos were observed. These showed the characteristic bipolar nature, prominent cotyledons, and a provascular system (Plate 5, Figure 11). Embryos at this time averaged 0.3 mm, with a range of 0.1-to-0.4 mm.

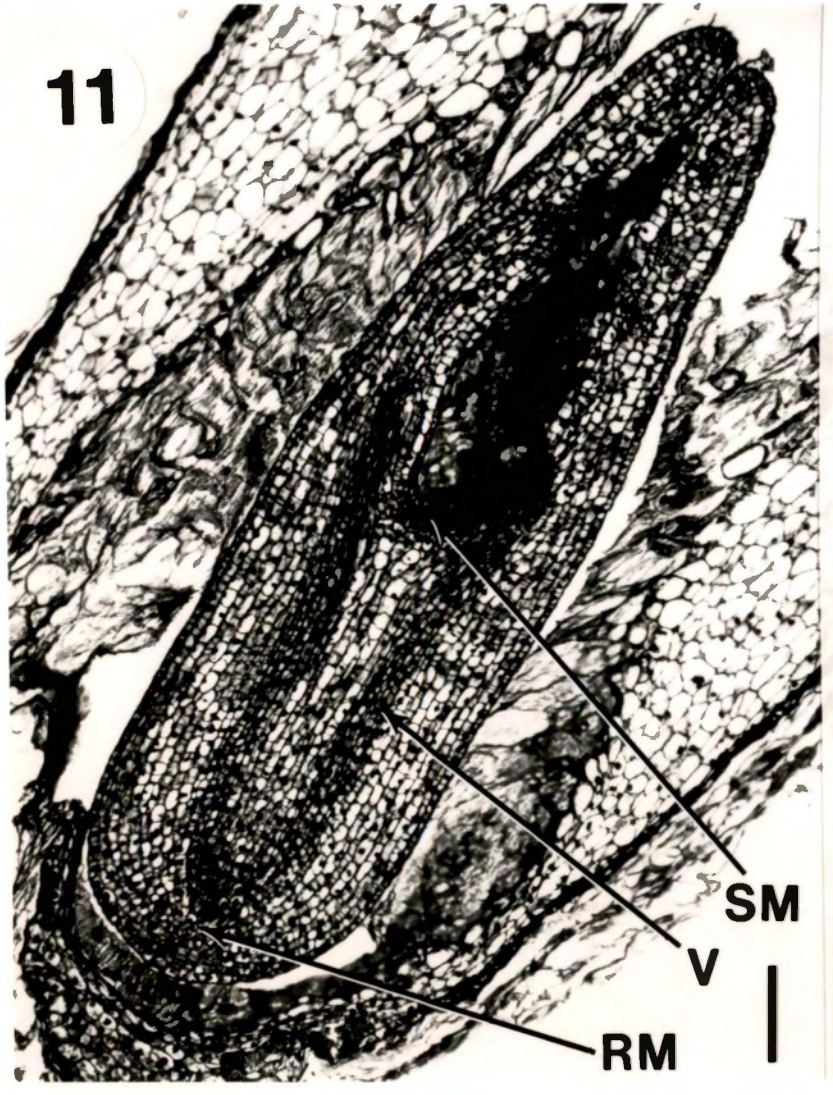
Samples collected throughout the next two weeks revealed lengthening embryos. Embryo samples collected 10 1/2 weeks post-anthesis averaged 0.7 mm, and ranged from 0.5-to-0.9 mm. Embryos at this time were visible and excisable. The sepals on the enlarged ovaries had changed from red to a deep burgundy color. At 11 1/2 weeks post-anthesis, the embryos had lengthened to an average of 1.2 mm long with a range of 0.75-to-1.75 mm. At this stage, the endosperm in the center of the embryo sac had begun to

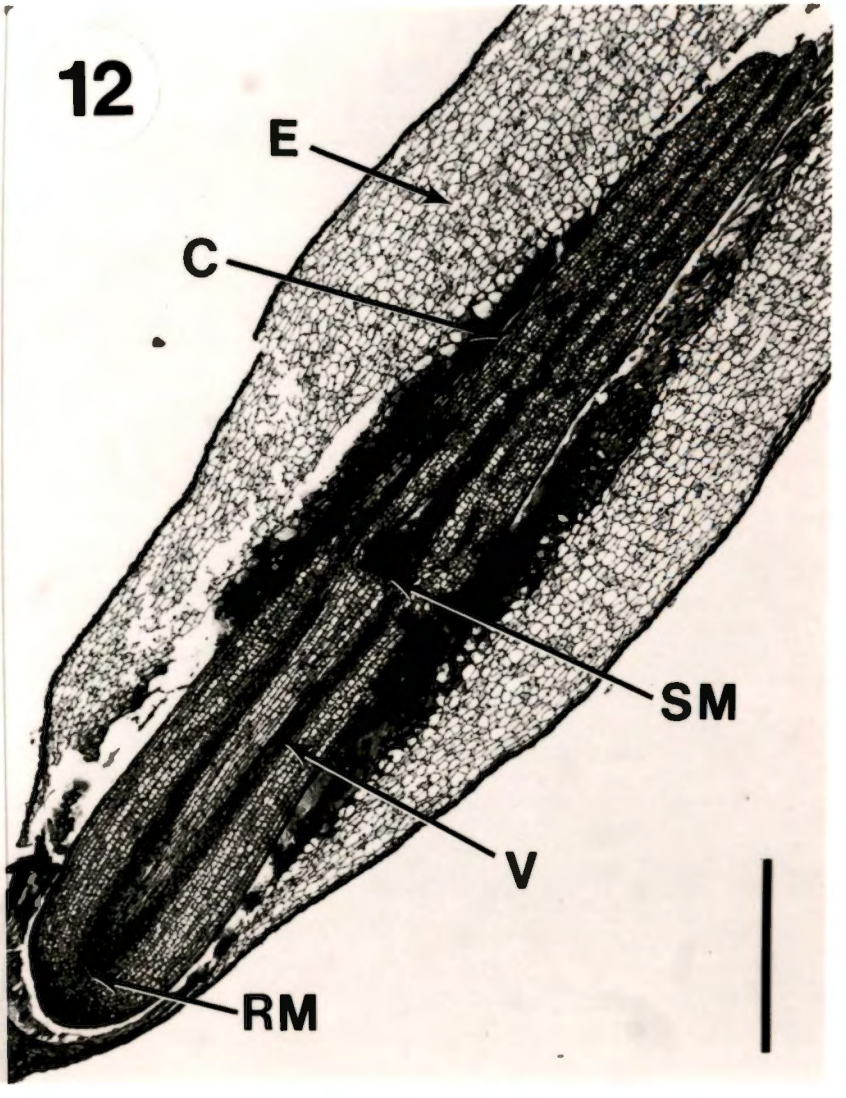
Plate 5. Developing Embryos in the Torpedo Stage.

Figure 11. A young torpedo embryo 11 weeks post-anthesis. Note the open area at the upper right where the endosperm has broken down. The cotyledons move through this open area as the embryo lengthens. X 100 Line = 100 μm

Figure 12. A torpedo embryo 12 1/2 weeks post-anthesis which has extended over half the length of the ovule. X 40 Line = 500 μm

c = cotyledons, e = endosperm, rm = root meristem, sm = shoot meristem, v = vascular tissue


11
SM
V
RM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deteriorate. The cotyledons pushed through this opening as the embryo lengthened.

One week later, 6 1/2 weeks after the initial embryo detection, the embryos measured an average of 3.6 mm (range of 3.0-to-4.5 mm) and extended over half the length of the embryo sac (Plate 5, Figure 12). A well developed shoot meristem had formed by this stage (Plate 6, Figure 13). Tissue fixed in CrAF III preserved the apical dome well, however F-G caused the apical cells to shrink which gave the apex a sunken or depressed appearance. The sepals, styles and distal end of the ovaries had become a deep red by this time. At 13 1/2 weeks post-anthesis, the sampled embryos averaged 4.5 mm in length with a range of 3.5-to-5.5 mm. A few of the embryos had begun to accumulate storage material in the mesophyll of the cotyledons.

One week later, at 14 1/2 weeks after anthesis and 8 1/2 weeks after the first embryo detection, the embryos appeared anatomically fully developed. They averaged 5.1 mm (range of 4.25-to-6.0 mm), had a well developed root apex (Plate 6, Figure 14) as well as a well formed shoot meristem, and were accumulating storage material in the mesophyll of the cotyledons (Plate 6, Figure 15). The form and staining of the storage deposits suggested that they were primarily protein as distinguished from lipid or starch

Plate 6. Anatomically Fully Developed Embryo Tissue.

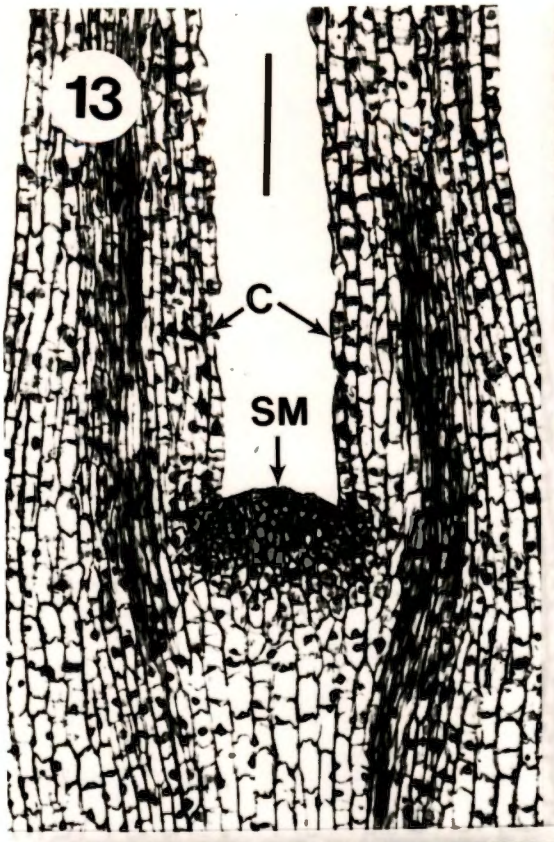
Figure 13. A section of a shoot apex from tissue fixed in CrAF III, 12 1/2 weeks post-anthesis. X 190 Line = 100 μm

Figure 14. A section of a well developed root apex, 14 1/2 weeks post-anthesis. X 100 Line = 100 μm

Figure 15. Longitudinal section of cotyledons showing the storage substance accumulation in the mesophyll cells, 14 1/2 weeks post-anthesis. X 200 Line = 100 μm

Figure 16. Longitudinal section of an embryo sac collected in early October, 20 1/2 weeks post-anthesis. Tissue collected late in the season sectioned poorly, and often appeared ragged and torn. X 40 Line = 500 μm

c = cotyledon, rm = root meristem, sm = shoot meristem, st = storage material, v = vascular tissue


13
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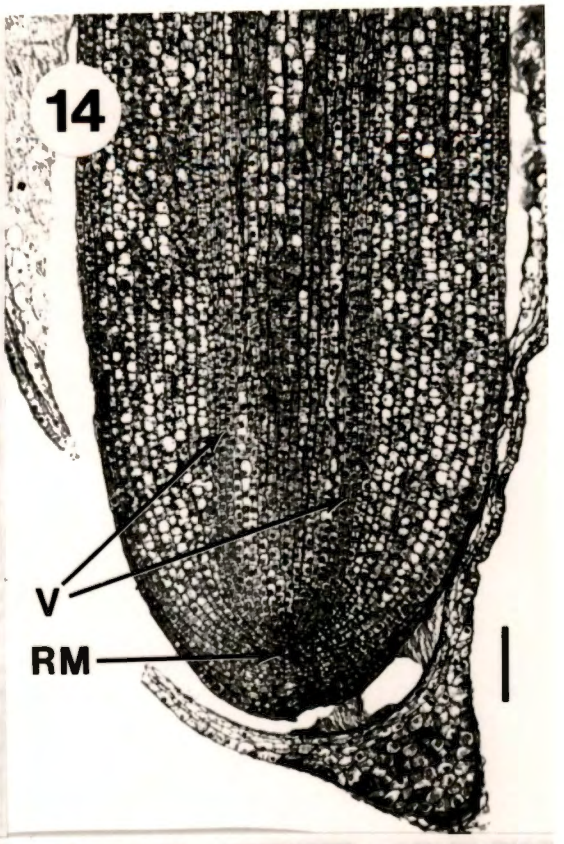

14
V
RM


15
ST


16

although this was not established histochemically. The tissues were not processed in a manner that would preserve lipids, and lacking a hilum the deposits did not appear to be starch. This stage of development corresponded with the onset of the orange/red color of the fruit. Fifty percent of the randomly collected ovaries were developing the coloration, although this was dependant upon the locaton on the plant. Fruits in full sun developed the red/orange color first, whereas those that were shaded followed.

Further embryo development appeared to be limited to elongation and storage substance accumulation. Tissue collected 15 1/2 weeks post-anthesis showed extensive storage substance throughout the embryo and endosperm. Embryos at that time averaged 5.3 mm long, with a range of 4.0-to-6.0 mm. One week later, collected embryos averaged 5.6 mm (range of 5.0-to-6.25 mm). Samples collected 20 1/2 weeks following anthesis averaged 5.8 mm long (range of 5.0-to-6.75 mm). Four weeks later the collected embryos averaged 6.0 mm (range of 5.5-to-7.0 mm). The tissue at this age sectioned poorly, and was often ragged and torn (Plate 6, Figure 16). Embryo development may be considered completed at this time, as the seeds are capable of germination, provided the proper stratification period and cultural conditions are met.

# CHAPTER V

## DISCUSSION

This study provides many of the details not reported for C. florida (Morse, 1907). With increased sampling and more advanced techniques and equipment, a more detailed and complete picture of the zygotic embryo development can now be presented.

Results of previous studies on development in the genus Cornus have been confirmed for C. florida. Endosperm development in C. florida follows the cellular type, as reported for the Cornaceae (Davis, 1966). The appearance of linear chains of cells four-to-six weeks post-anthesis, although not confirming proembryo development, does suggest a solanad type of embryo development as reported for C. mas (Erdelska, 1986).

An important point which needs to be made in a study such as this is that embryo development is not synchronous within ovules on an individual inflorescence. Samples collected at any one time, especially during early stages of development will contain embryos of varying sizes. Samples taken during any given week may contain embryos which are further developed than some taken the following week. By collecting, processing and observing many samples, a general

developmental scheme can be determined, as was done in this study.

Although anthesis occurred the end of the third week of April in both years of this study, enviromental conditions and other factors would cause variation from year to year. Anthesis is easily determined, however, and can be ascertained for any location in any year to provide a point of reference to estimate embryo development.

The time of pollination or fertilization was not determined in this study. The presence of pollen on the stigma of unopened flower buds suggests a mechanism of self pollination, but it is unknown if the stigmatic surface is receptive to the pollen at that time.

A plot of mean embryo length in millimeters (mm) plotted against time (the number of weeks post-anthesis and the number of weeks following the first embryo detection) produces a typical sigmoid growth curve (Figure 17). The lag phase of the curve represents the embryo growth through the globular stage and into early torpedo growth. Although the globular and cordate embryos are small, it takes approximately four and a half weeks following the first embryo detection to complete those stages.

The exponential growth phase occurs once the torpedo stage is reached, at ten weeks post-anthesis. The embryos

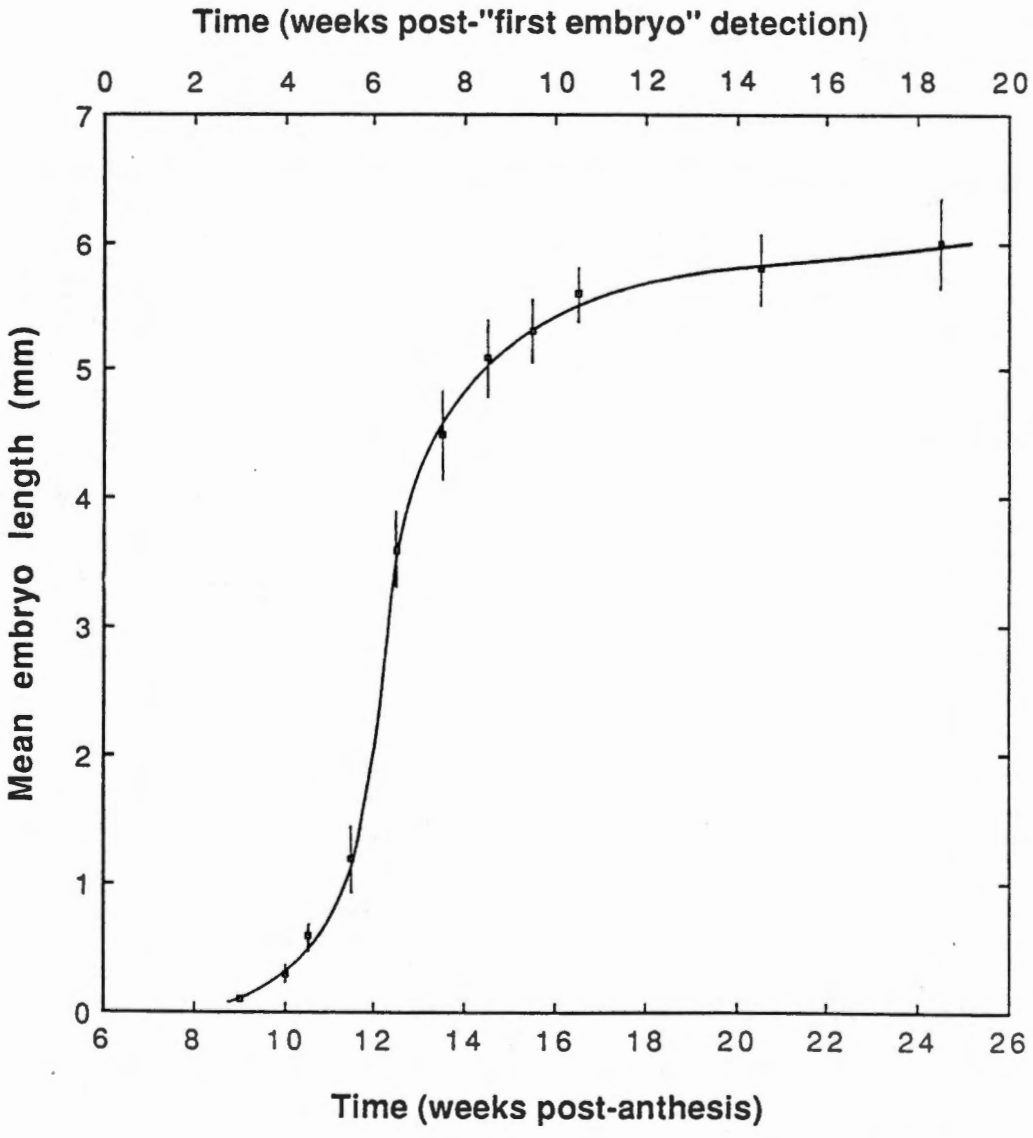
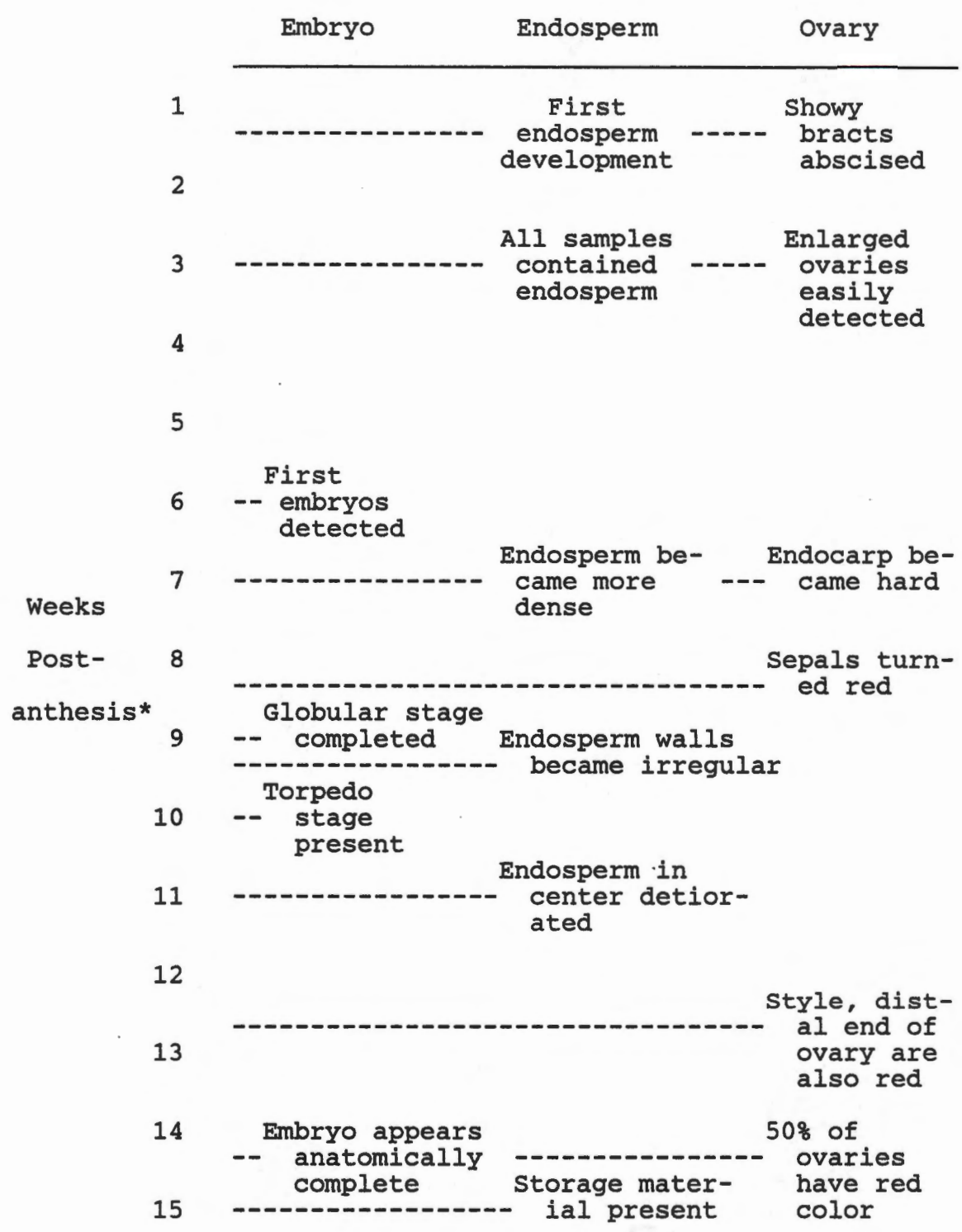



Figure 17. Growth curve formed from a plot of mean embryo length and standard deviation against time.

increase over fifteen times in length and show a marked increase in tissue complexity in four weeks. In the same (or less) time required to complete the globular stage, the embryo developed from a small undifferentiated structure averaging 0.6 mm long to one that appears anatomically fully developed and averaged 5.1 mm long. This very rapid development is apparently under hormonal control. The exponential growth phase slows at about 15 weeks post-anthesis to a period of decelerating growth, or a plateau, which is characterized by some embryo elongation and an increase in protein storage.

Figure 18 summarizes the information for embryo and endosperm development, as well as the ovary stages which correspond to certain stages of development. The first embryos detected occurred at the time it became difficult to remove ovary tissue from the ovule. This can be determined from a dissection, as would be done if ovules are being excised as a tissue culture explant. The globular stage corresponded to the time the sepals on the developing fruits turned red. When the style and distal end of the ovary also turned red, the embryo was well into the torpedo stage. By the time one-half of the fruits had developed red color, embryos appeared anatomically fully developed, with well developed shoot and root apices, hypocotyl and cotyledons, a




*Anthesis occurred April 21.

Figure 18. Schedule of events showing simultaneous embryo, endosperm, and ovary development.

provascular system, and an accumulation of storage substances, which were probably proteins. Although the embryos appeared fully developed, a stratification requirement may prevent germination at that time. Further development is limited to a slight increase in embryo length and an increase in protein storage in the endosperm.

By documenting the zygotic embryo development in flowering dogwood, and by providing reference points to estimate developmental stages, this study has increased our knowledge of this important native woody plant and provides researchers useful information for future work.

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# VITA

Joseph T. Dietrich was born on February 16, 1958 in Madison, Wisconsin. He attended school in the Mount Horeb, Wisconsin school system, and was graduated from Mount Horeb High School in May, 1976. The following August he entered the University of Wisconsin-Madison, and received the Bachelor of Science degree in Agriculture with a major in Horticulture in May, 1980 from that school.

After spending six years in the landscape construction and maintenance industry, he entered the Graduate School of the University of Tennessee-Knoxville in September, 1986. While working toward the Master of Science degree in Ornamental Horticulture and Landscape Design, he was employed as a Graduate Research Assistant for the Department by the University of Tennessee Agricultural Experiment Station. He was granted the degree in December, 1988.

The author is a member of Alpha Zeta, Gamma Sigma Delta, and Pi Alpha Xi. He is married to the former Jean Williams and has one daughter, Sara Elizabeth.