EMBRYOLOGY OF PIC A GLAUCA (MOENCH) VOSS

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INTRODUCTION

In a tree improvement program, it is important to understand the morphological and physiological development of reproductive systems of the species involved. One of the first requirements is a detailed description of the male and female structures -- usually referred to as the staminate and ovulate strobili, respectively. Reproduction involves four major steps; namely formation of gametes, pollination, fertilization, and embryo development. The male and female gametes are formed in the strobili by reduction-division of diploid nuclei to haploid nuclei. Pollination is the transfer of pollen produced by the staminate strobili to the ovules in the ovulate strobili. There is a variable time lag from pollination to fertilization; the time being dependent upon the species. Fertilization is the union of the male and female nuclei within the ovule. The resulting zygote, the first diploid cell of the sporophyte generation, develops into the embryo. Considerable work in this field has been done in other genera, but a limited amount of information is available for Picea.

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The genus Pinus, which has been studied by many investigators, is often used as the representative for the conifers. There are many contributor's to the pines, but few for the spruces. Among the most notable workers in spruce are Miyake (1903), and Mergen, Burley, and Furnival (1965). Many of the features exhibited by the pines are seen in the spruces, but there are some distinct differences which separate the two genera.

FIELD PROCEDURE

Four white spruce trees from the Southern Research Station, Maple, Ontario, were selected for this study. Collections for microscopic analysis were made at various intervals during the fall of 1966; and the spring and summer of 1967. Collections to determine differences among male, female, and vegetative buds, and to determine the abundance of male and female strobili were taken in September 1966, February 1967, and early May 1967. Female Strobili were also collected on May 25, at the time of pollination. Cones were collected bi-weekly for the first month after pollination and weekly thereafter 'until seed maturity.

When the female buds had swollen, and prior to their emergence from the bud: scales, the twigs were bagged. After the bud scales were shed, and the cone scales separated, the strobili were pollinated with a mixed white spruce pollen collected the previous week. As all of the females are not receptive simultaneously, the bags were pollinated twice, once on May 25, and once on May 29, 1967.

The strobili were killed and fixed, in F.A.A. (10:5:50:35 by volume of formaldehyde, acetic acid, alcohol, water).

CYTOLOGICAL PROCEDURE

When the samples were brought into the laboratory, they were aspirated, dehydrated, infiltrated, and embedded in paraplast. Fifteen to twenty-five micron serial sections were cut on the rotary microtome. The sections were stained in safranin-fast green as they were taken through an alcohol-xylene series. The slides were mounted in Permount (Sass, 1951; Steedman, 1960)

A total of 2700 slides were studied to observe the reproductive development of this species.

RESULTS AND DISCUSSION

Bud Features

At the time of the first. collection in September 1966, the vegetative, female, and male buds could readily be distinguished(pl. I,1_3). In the vegetative bud, the needle primordia were easily Seen; in the female bud, the bract and ovule-scale complex were identified; and in the male bud, the microsporophylls with their microsporangia were evident. In the Pinus genus, the reproductive strobili are contained in a mixed bud which also has vegetative properties,, but in the Picea genus, the male, female, and vegetative buds, are distinct entities. _In all, spruce buds of this collection, the pith, procambium, and peripheral layers of tissue could be seen (pl. 1,4), but no actively dividing ,cells were found in this collection .

Very little change was observed in the collection for February 1967.. -All buds- had increased slightly in size, but this, was mainly due to cell expansion rather than cell division. Again, no actively dividing cells were found within the buds. In the female bud, the outline of the ovules could be seen, but there was no differentiation of tissue within this area. After fertilization, .these ovules will develop

into mature seeds. In the male bud, the archespores within the microsporangium were present. The archespores will differentiate into the microspore mother cells and each mother cell will produce four pollen grains.

Ovulate Strobili and Ovules

Three weeks prior to pollinations, on May 4, 1967, a collection was made of ovulate strobili. Many differences were apparent in comparison with the February collection. Cells had been actively dividing and several tissues differentiated (pl.I, 5). The pith was almost completely composed of large, darkly stained cells, whereas earlier only a small number of these cells were scattered throughout the area. Similar cells in ovulate cones of ponderosa pine were filled with tannin (Gifford and Mirov, 1960). The procambium, or provascular tissue, had developed into the primary phloem and primary xylem. Traces could easily be followed from the vascular cylinder to the bract and to the scale. The scale had grown larger than the bract, whereas in earlier collections the bract was larger than the scale (pl. 1,2, 5).

Several different tissues could be identified in the ovule. The three layers of the integument were recognizable, but still not well defined (pl. I, 5; pl. III, 20). The middle layer, which becomes hard and stony, is the sclerotesta; the outer fleshy area, the sarcotesta; and the inner fleshy area, the inner sclerotesta (Sporne, 1965). The end of the ovule closest to the central axis is the micropylar end of the ovule. At this end, the integument extended beyond the nucellus top to form the stigmatic flap. The cavity within the flap is the micropylar canal (pl. I, 5,6). At this stage in the development, the integument was fused to most of the nucellus (pl. I, 5). At the end of May, the integument was mainly free from the nucellus due to a difference in the rate of growth within the nucellus as the micropylar end grew more rapidly than the chalazal end (pl. I, 6). In most species of pines, the integument remains fused throughout the development for the chalazal end grows faster than the micropylar end (Sporne, 1965). One example of an exception is in Pinus roxburghii in which the integument is free (Konar, 1960).

The nucellus is enclosed by the integument. The inner sclerotesta cells of the integument resembled the cells of the nucellus, but the latter appeared more darkly stained as they had a higher cytoplasmic content (pl. I,6). Within the nucellus, the megaspore mother cell had already undergone meiosis. The resulting megaspore was surrounded by a spongy layer of cells (pl. I, 6, 10). These cells were densely cytoplasmic and are derived from the nucellus. Pressure exerted by a colorless fluid within the cavity of the megaspore cell distorted the cells of this spongy layer (Chamberlain, 1957).

Staminate Strobili and Microspores

The staminate strobili in early May were considerably different from the strobili observed in February. Microspore mother cells within the sporangium were derived from the archespores which were present in February. Each microspore mother cell underwent reduction-division to form four microspores. The microspore, which was seen in the early May collection, is the first cell of the male gametophyte generation. Each microspore had two coats; the outer layer, the exine; and the inner layer, the intine. Two wings were formed by a separating of the exine layer from the intine layer (Chamberlain, 1957). After formation of the wings, the microspore developed into a multi-celled structure, the pollen grain (pl. I, 7).

The nucleus of this microspore divided twice to form two prothallial cells and a central cell. After the third miotic division, the central cell produced a tube cell and a generative cell. The generative cell lies between the tube cell and the

prothallial cells. The generative cell then divided to form a sterile cell and a spermatogenous cell. Until recently, the sterile cell was known as the stalk cell, and the spermatogenous as the body cell (Miyake, 1903; Sporne, 1965). Four of these five cells are shown in pl. 1,7.

Pollination

When the pollen grain matured, a longitudinal split occurred in the sporangia which allowed the pollen grains to escape from the staminate strobili. As controlled pollinations were used in this experiment, a mixed white spruce pollen was collected on May 18, 1967, from several white spruce trees in the area. When the ovulate strobili were receptive, the pollen was directly applied to the strobili. Pollinations were made on May 25, and May29, 1967. After the pollen grains land on the ovulate scales, a drop mechanism draws the pollen grain into the micropylar canal (McWilliam, 1958). Here the pollen grain is deposited on the nucellus top where it germinates. Prior to the arrival of the pollen grain, the nucellus top deteriorated as the cell walls had broken down and the cell contents dissolved (pl. I, 6).

On June 5, seven to ten days after pollination, the pollen grains were seen germinating on the nucellus top. As the pollen tube starts to grow, the tube nucleus is the first cell to enter, followed by the spermatogenous cell and the sterile cell (pl. 1,8). Shortly after entering the pollen tube, the sterile cell passes the spermatogenous cell (pl. I, 9). The latter then divides to form two unequally sized gametes. The sterile cell is lightly stained and highly vacuolate, whereas the spermatogenous cell is darkly stained and highly cytoplasmic (Miyake, 1903). The rest of the pollen grain, including the degenerate prothallial cells, remains on top of the nucellus (Chamberlain, 1957). Since the male gamete is a short lived cell, fertilization must occur soon after its formation. The pollen tube continues to work its way through the nucellus, and when it reaches the egg, the bottom of the tube ruptures. The pressure within the tube ejects the stalk, tube, and two sperm nuclei into the egg (Chamberlain, 1957).

Female Gametophyte

The megaspore is the first cell of the female gametophyte. It originates from the megaspore mother cell, a diploid cell, which undergoes reduction-division to produce four haploid cells in a linear arrangement. Only the lowermost cell functions and becomes the megaspore. The nuclei produced were contained within the megaspore wall (pl. I, 10; pl. II, 11). This wall is pushed outward by the fluid in the central vacuole forming a sphere lined with free nuclei. The free nuclear stage was first seen in the May 4 collection. This same stage was still present in the May 29 collection. The free nuclear stage continued until several hundred nuclei were produced. The initial divisions occur very rapidly, but the more nuclei present, the longer the period between divisions. As the period lengthens, walls are laid down starting at the peripheral edge and progress inwards (Chamberlain, 1957). The first walls were seen on May 29 (pl. II, 12). The spongy tissue surrounding the female gametophyte was gradually absorbed (Konar, 1960).

At the top of the female gametophyte (the end closest to the micropylar opening) several archegonia initials had formed by June 5. Each initial was separated by a layer of vegetative cells of the gametophyte which divided and developed into the archegonial jacket (pl, 11,13,15). The nuceleus of the initial migrated to the top of the cell. By June 12, it divided to produce the primary neck cell and the central cell. The primary neck cell divides until it produces a minimum of eight cells and a maximum of thirty-two cells. These are arranged in plates of four cells from two to four tiers thick (Miyake, 1903). Figure 14 (pl. II)shows four neck cells of the eight neck cell stage. The layer of gametophyte cells which surrounded the central

cell of the archegonium was very pronounced as these cells are rich in protoplasm and contain large nuclei (pl. II, 13, 16). The central cell enlarged rapidly, became vacuolate, and appeared as an amorphous mass. The nucleus was still located close to the neck cells. According to Chamberlain, the central cell of pines does not divide for nearly a month after division of the primary neck cell, but in white spruce the central cell divided within a few days after formation of the primary neck cell. When the central cell divided, it produced the ventral canal cell and the egg cell (pl. 11,14,16) . The ventral canal cell was minute in comparison with the egg cell, and the former disintegrated soon after its formation (Miyake, 1903).

Fertilization

Another difference between pine and spruce involves the time span from pollination to fertilization. Actual fertilization of white spruce occurred between June 12 and June 19, approximately three weeks after pollination. Thirteen months elapse between pollination and fertilization of pines.

Division of the central cell of the female gametophyte is nearly simultaneous with the division of the spermatogenous cell of the male gametophyte. Usually the apex of the megaspore wall is weak, and the neck cells of the archegonium disorganize, so the pollen tube has little difficulty in reaching the egg (Chamberlain, 1957).

When the pollen tube ruptures, the male nucleus is pushed out towards the egg nucleus. When the two initially come in contact with each other, their membranes remain intact. This is well illustrated by Mergen, Burley, and Furnival (1965). Only when the male nucleus has penetrated the female nucleus do the adjoining membranes disappear so that the two nuclei are surrounded by common cytoplasm and one common membrane. Syngamy occurs shortly thereafter and the zygote is formed (pl. II, 18). The haploid generation ends when the chromosomes are arranged on the metaphase plate.

Embryo Development

The fertilized egg which gives rise to the zygote is the first cell of the diploid, sporophyte generation. All of the cells produced from the initial divisions will not form the embryo as three of the four tiers produced will disintegrate before the embryo matures (Chamberlain, 1957). The zygote was no longer circular as was the unfertilized egg nucleus, but oval with its long axis the same as that of the archegonium. Instead of the uniform mass of material found in the egg nucleus, this cell was very coarsely granular (p1.II, 17, 18). The zygote underwent two divisions to produce four free nuclei in the center of the archegonium. They then migrated to the base of the archegonium where they underwent a third mitosis. This was the end of the free nuclear stage; cell walls were laid down at the end of this division.

The eight cells produced were arranged in two tiers of four cells each. The top group of cells divided to form two sister tiers (p1.II, 19; p1. III, 21). The cells in the upper tier were walled on three sides but open on the fourth side which faced the archegonium. This tier is termed the upper tier or open tier. The other set of cells produced by this division is termed the rosette tier. These cells deteriorate almost as soon as they are formed; but occasionally in pines the rosette cells produce embryos, but these seldom reach maturity (Chamberlain, 1957).

The lower group of cells also divided to form two sister tiers 19). The lowermost, the embryonal tier, is the only one whose cells develop into the mature embryo. The other layer produced is the primary suspensor tier. These cells did not divide again, but elongated to push the embryonal tier into the gametophyte tissue where it could receive nourishment from the surrounding cells (p1. III, 22).

Since the free nuclear stage is very short, different phases could be observed in the same collection on June 19. Though most archegonia still contained the zygote some had progressed to the free nuclear stage, while others showed elongating suspensors. Figure 19 (pl. II)illustrates the four tiered stage, while figure 23 (pl. III) shows the archegonium on the left with a zygote, the one on the right with elongating suspensors. The embryo seen in figure 19 will develop no further as the primary suspensor cells have failed to develop.

Only cells of the embryonal tier developed into the embryo. These cells were. in the proembryonal stage or early embryogeny until the suspensors started to elongate and the embryonal tier was pushed through the archegonium. Late embryogeny begins when the suspensors elongate. Often, more than one archegonium was fertilize to produce more than one embryo (pl. III,24). This is termed simple polyembryony. According to Dogra (1967), this is the only type that occurs in the spruces, although Mergen, Burley, and Furnival (1965) state that cleavage does occur in white spruce. In pine, cleavage polyembryony is common; here the suspensors, each one wit an embryonal cell, split apart to form individual embryos (Dogra, 1967).

Usually only the embryo which is first formed in the ovule reaches maturity. cells of the primary suspensors elongated, other cells were cut off by the embryonal tier. The first ones produced formed the secondary suspensors. Their function is identical to that of the primary suspensors, but their origin is different. The primary suspensors were formed from the lower tier at the eight nucleate stage, whereas the secondary suspensors were formed from the embryonal tier (Chamberlain, 1957). When the zygote appeared, cells in the female gametophyte broke down to form the corrosion cavity (pl. 111,23,24). It is into this cavity that the suspensors pushed the growing embryo (pl. III, 24, 25, 26). The gametophyte, rich in starch and foodstuffs, nourishes this developing tissue (Chamberlain, 1957). As the embryo grew, regions which could be identified were the basal meristematic zone, the rib meristem, the apical meristematic zone, and the cotyledon primordia (pl. III, 27, 28). The procambium, which did not appear until the cotyledon primordia were well developed, could be traced from the main stem to each cotyledon (pl. III, 28, 29). At this point in the development, a definite layer of epidermal cells could be seen surrounding the embryo and cotyledons (pl. III, 29). The gametophyte which encloses this mature embryo is extremely rich in starch and fats which will nourish the embryo until it able to sustain itself (Singh and Chatterjee, 1963).

SUMMARY

The follwoing table summarizes the stages of development which were seen at various collection dates.

Collection date	Structure observed	Observations made
Sept. 1966	ovulate strobili	-bract and ovule-scale complex present -bract larger than scale -pith, procambium, and peripheral layers visible
	staminate strobili	-sporangium and sporophyll present -pith, procambium, and peripheral layers visible
	vegetative structure	-needle primordia present -pith, procambium, and peripheral layers visible
Feb. 1967	All observations same as above, except that all structures have increase slightly in size.	
May 4, 1967	ovulate strobili	-primary xylem and primary phloem present -scale larger than bract

- continued -

Collection date	Structure observed	Observations made
May 4, 1967	ovule	-cells within ovule actively dividing -integument with 3 layers present, but layers not well defined -micropylar canal present -nucellus is fused to the integument -megagametophyte in free nuclear stage
	staminate strobili	-microspores present, but still one-celled
May 25, 1967	ovule	-integument with 3 layers well defined -micropylar canal open -nucellus top deteriorated -nucellus mainly free from integument -megagametophyte still in free nuclear stage
May 29, 1967	ovule	-same as May 25 except -some cell walls seen in megagametophyte -pollen grains in micropylar canal
Jun. 1, 1967	ovule	-pollen grains on nucellus top -pollen grains have not yet germinated -megagametophyte has ended free nuclear stage
Jun. 5, 1967	ovule	-pollen grains germinating on nucellus top -sterile and spermatogenous cells seen in some pollen tubes -two male gametes seen in other pollen tubes -immature archegonium present
Jun. 12, 1967	ovule	-immature and mature archegonia seen -neck cells visible -some archegonia have only central cell, others have ventral canal cell and egg cell
Jun. 19, 1967	archegonium	-zygote present in some archegonia -some archegonia in early embryogeny, i.e., four and eight nucleate stage -other archegonia in late embryogeny, i.e., suspensors have elongated
Jun. 26, 1967	ovule	-embryonal mass developing in corrosion cavity
Jul. 4, 1967	ovule	-embryo enlarging
Jul. 10, 1967	embryo	-apical and basal meristems visible -cotyledon primordia visible below the apical point
Jul. 17, 1967	ovule	-suspensors crowded to apex
	embryo	-apical and basal meristems well defined -cotyledon primordia extend beyond apical point
Jul. 24, 1967	embryo	-cotyledon primordia well formed
Jul. 31, 1967	embryo	-vascular system well defined from main axis to cotyledons -epidermal layer present surrounding entire embryo and cotyledon primordia, except for base of embryo attached to the suspensors
	ovule	-embryo extends from apex to base of ovule -embryo is laterally surrounded by storage tissue of gametophyte
Aug. to Sept. 1967	ovule	-little change observed from first of August to seed maturity in mid-September

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EXPLANATION OF PLATES

Plate I

Fig. 1. Sept. 1966. Vegetative bud showing (A) needle primordia, (B) procambium, (C) pith. (x 100).
Fig. 2. Sept. 1966. Female bud (ovulate strobilus) showing (A) ovule-scale complex, (B) procambium, (C) pith. (x 75).
Fig. 3. Sept. 1966. Male bud (staminate strobilus) showing (A) microsporophyll with microsporangium, (B) procambium, (C) pith. (x 75).
Fig. 4. Sept. 1966. Female bud showing (A) pith, (B) procambium, (C) peripheral layer. (x 750).
Fig. 5. May 4, 1967. Ovulate strobilus showing (A) pith, (B) vascular system, (C) scale, (D) bract, (E) ovule. (x 75).
Fig. 6. May 29, 1967. Ovule showing (A) pollen grain, (B) stigmatic flap, (C) integument, (D) nucellus, (E) developing megaspore. (x 300).
Fig. 7. May 29, 1967. Pollen grain showing (A) prothallial cell, (B) sterile cell, (C) spermatogenous cell, (D) tube cell. (x 1900).
Fig. 8. June 5, 1967. Germinating pollen grain showing (A) spermatogenous cell, (B) tube cell. (x 1200).
Fig. 9. June 5, 1967. Germinating pollen grain showing (A) sterile cell, (B) spermatogenous cell, (C) tube cell. (x 1200).
Fig. 10. May 4, 1967. Free nuclear stage of megaspore showing (A) spongy layer, (B) free nuclei within megaspore wall. (x 1200).

PLATE I

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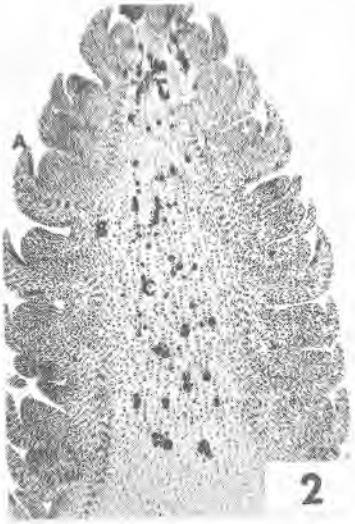

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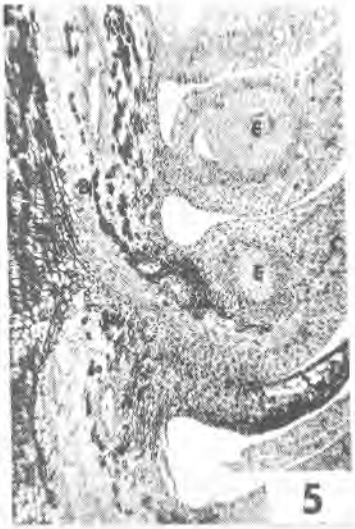

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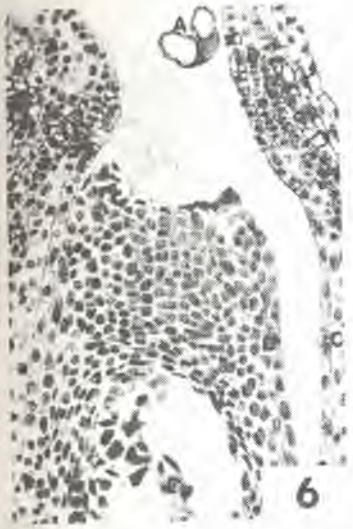

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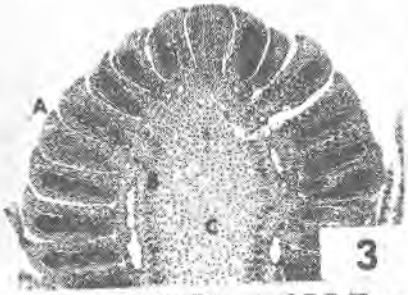

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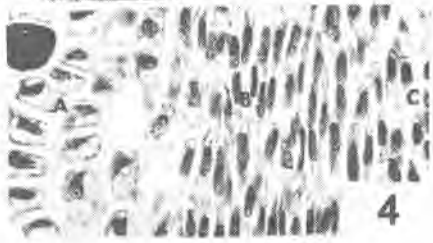

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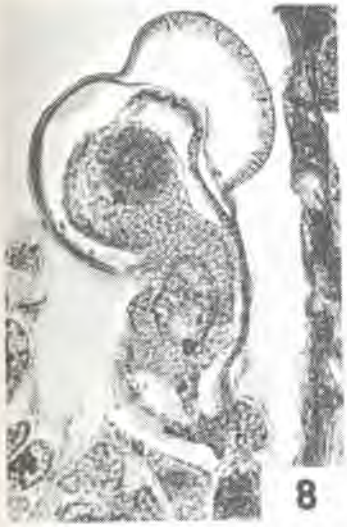
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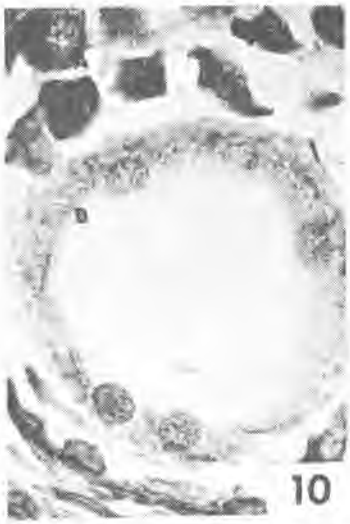

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Plate II

Fig. 11. May 4, 1967. Free nuclear division of megaspore showing simultaneous division of chromosomes. (x 1200).

Fig. 12. May 29, 1967. Developing gametophyte showing (A) cell walls between nuclei. (x 1200).

Fig. 13. June 12, 1967. Developing archegonium showing (A) archegonium initial, (B) nucleus, (C) archegonial jacket, (D) megagametophyte, (E) nucellus. (x 300).

Fig. 14. June 12, 1967. Archegonium showing (A) neck cells, (B) ventral canal cell, (C) egg cell. (x 1200).

Fig. 15. June 12, 1967. Megagametophyte showing (A) archegonium, (B) jacket cells dividing, (C) megagametophyte tissue. (x 190).

Fig. 16. June 12, 1967. Mature archegonium showing (A) neck cells, (B) ventral canal cell, (C) egg cell, (D) egg nucleus. (x 300).

Fig. 17. June 12, 1967. Egg cell showing (A) egg cell cytoplasm, (B) egg nucleus, (C) nucleolus. (x 1200).

Fig. 18. June 19, 1967. Egg cell after fertilization showing (A) cytoplasm, (B) zygote, (C) chromatic material from male and female gametes. (x 1200).

Fig. 19. June 19, 1967. Four tiers of proembryo showing (A) upper tier, (B) rosette tier, (C) primary suspensor tier, (D) embryonal tier. (x 850).

Plate III

Fig. 20. June 26, 1967. Layers of tissue within ovule showing (A) megagametophyte, (B) megaspore wall, (C) nucellus, (D) inner sclerotesta (inner seed coat), (E) sclerotesta (seed coat), (F) sarcotesta. (x 300).

Fig. 21. June 19, 1967. Upper and rosette tiers shortly after elongation. (x 1200)

Fig. 22. June 19, 1967. Suspensor and embryonal tiers in corrosion cavity. (x 1200).

Fig. 23. June 19, 1967. Megagametophyte showing (A) archegonium, (B) zygote, (C) megagametophyte tissue, (D) corrosion cavity, (E) elongating suspensors. (x 300).

Fig. 24. June 26, 1967. Developing seed showing (A) remains of suspensors in corrosion cavity, (B) two developing embryos. (x 75).

Fig. 25. June 26, 1967. Developing embryo. (x 1200).

Fig. 26. July 10, 1967. Developing embryo showing (A) basal meristematic zone, (B) apical meristematic zone, (C) cotyledon primordia. (x 300).

Fig. 27. July 17, 1967. Developing seed showing (A) basal meristematic zone, (B) rib meristem, (C) apical meristematic zone, (D) cotyledon primordia, (E) megagametophyte, (F) remains of suspensors. (x 100).

Fig. 28. August 8, 1967. Embryo showing (A) rib meristem, (B) apical meristem, (C) cotyledon primordia, (D) vascular system, (E) epidermal layer. (x 190).

Fig. 29. August 8, 1967. Cross section of embryo showing (A) cotyledon primordia, (B) vascular system, (C) epidermal layer. (x 300).

PLATE II


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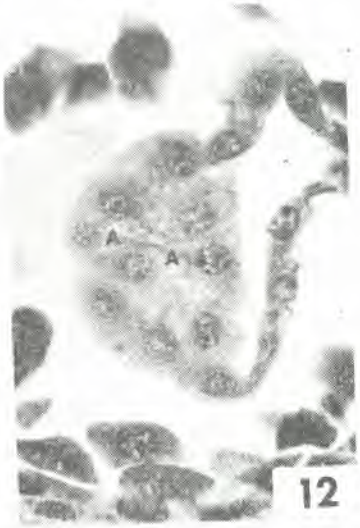

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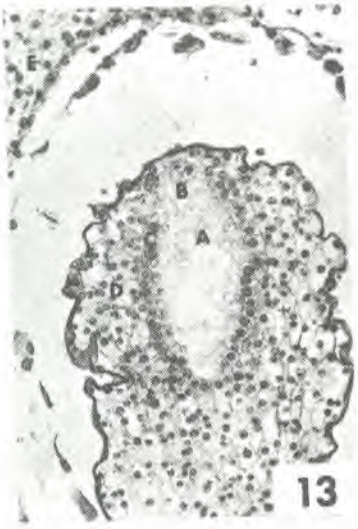

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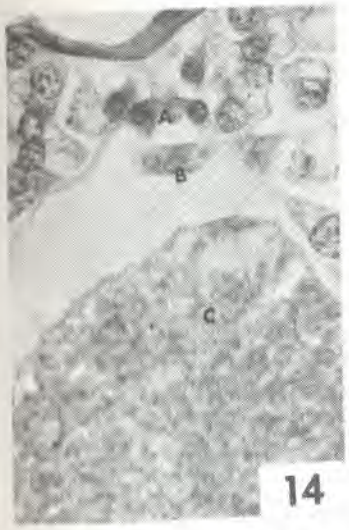

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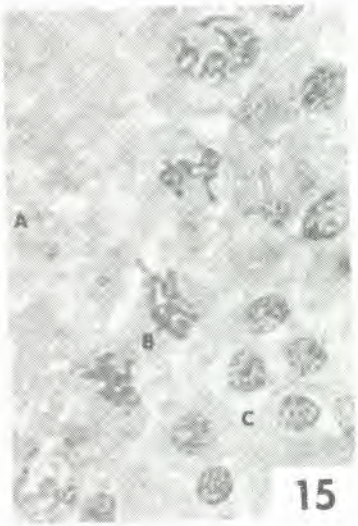

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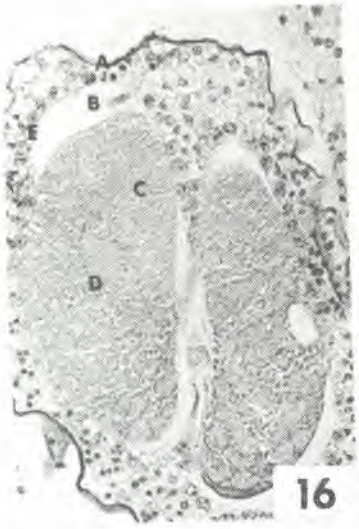

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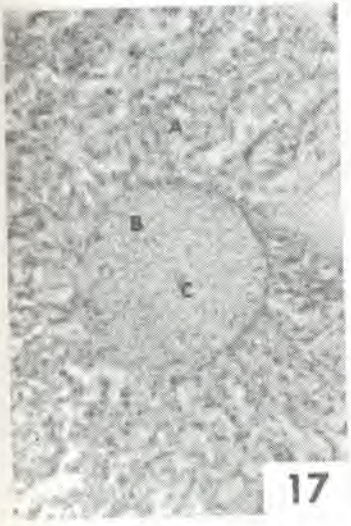

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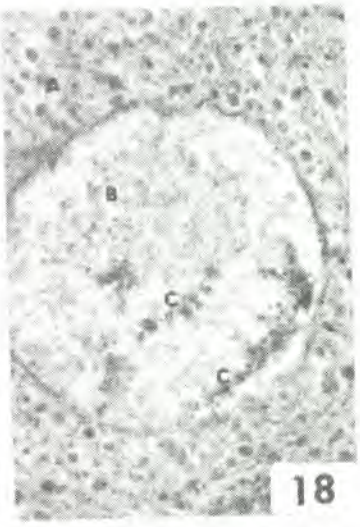

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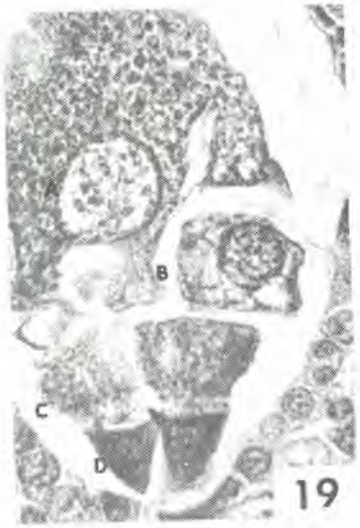

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PLATE III