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SUSPENSOR AND EARLY EMBRYO OF PINUS

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(WITH PLATES VI–X AND THREE FIGURES)

It has long been recognized that the embryos of plants furnish trustworthy morphological features for comparison in the study of phylogeny, but the surprising variations found in the proembryos of various gymnosperms have always been more or less of a stumbling block. This work was undertaken with the hope that a more critical study of the suspensor and early embryo of *Pinus* and of the phenomenon of polyembryony might prove of value in properly interpreting the rather flexible program that has been ascribed to this genus. Here it is, also, that we find a striking parallel to some of the early cleavage phenomena involved in the biology of twins in animals, a subject of some current interest to zoologists.

This paper will limit itself largely to such phases of the embryogeny of *Pinus* as were most effectively studied by means of a special technique for dissection, developed by the writer, and to a discussion of the relation of the early *Pinus* embryo to other conifer types. Certain phases of the later embryo will also be described, but the development of the internal features of the late embryo will be treated in another paper.

Historical

A summary of views in regard to the embryogeny of conifers is given by COULTER and CHAMBERLAIN (10), so that it will suffice to note those features in the historical development of the subject which concern our own investigation.

ROBERT BROWN (2), in discussing the similarities of the ovulate structures of cycads and conifers, mentioned his own observations of occasional polyembryony in conifers, which was known to be a constant feature in cycads. In a later treatise (3) he announced polyembryony as a constant feature among several genera of the Pinaceae and felt convinced that this feature is common to the entire family. He noted the origin of the embryos from "corpuscula" or "areolae," 3–6 in number, at the upper extremity of the "aminos" (endosperm), and pointed out that this provision of several "corpuscula" was like that in cycads, where it also made possible the development of several embryos. He called the suspensors "funiculi," finding that these frequently branch to form still other embryos.

MIRBEL and SPACH (27) announced their results from a careful study of several pines and also *Thuja* and *Taxus*, confirming the work of BROWN and extending our knowledge to other forms. In this account these workers were the first to use the terms "suspensor" and "rosette," although in their otherwise excellent figures they show 5 cells in each tier of the early embryo, 5 rosette cells, and 5 vertical rows of cells coming from the base of the corpusculum.

SCHLEIDEN (36) gave the first accurate general description of the development of the early embryo, beginning with the "embryonal globule" on the end of the suspensor. His views regarding the earlier stages of the embryo were confused by his erroneous conception that the pollen tube formed the embryo. He pointed out the correct order of appearance of the stem tip meristem and cotyledons, and gave a good account of the formation of the suspensor in its late stages after it becomes massive, describing it as an elongation of cells from the radical portion of the embryo.

HARTIG (15) was possibly the first to point out that the upper end of the suspensor is a single cell, but he regarded this cell as

being of vegetative origin. He described a "nest of cells" from which individual cells elongate, and thought that the embryonal tip cell was cut off some time after elongation.

SCHACHT (35) agreed with SCHLEIDEN that the pollen tube enters the corpusculum and produces the embryo at its base. He described the rosette correctly as consisting of 4 cells instead of the 5 shown by MIRBEL and SPACH. SCHACHT described the 4 tiers of 4 cells each, known to us as the end product of the pro-embryo stage. He announced definitely that the 4 rows of cells in *Pinus Pumilio* always separate into 4 embryos and believed that they would split up further. In *Taxus baccata* and *Abies* he reported no splitting of the product of the corpusculum into several embryos.

GOTTSCHE (14) gives a critical review and confirmation of the facts known at the time and a more accurate description of the corpusculum, which he found to originate in some unpollinated cycads, and is therefore independent of the pollen.

HOFMEISTER (17) made a careful study of all stages in the development of the ovule and confirmed the facts then known. He pointed out how wonderfully simultaneous fertilization occurs in all the plants of the same species and how rapidly the pro-embryo stages are passed through. He was the first to regard the terminal cell of the early embryo as an apical cell. He thought also that the later embryo and seedling grow by means of an apical cell, and even believed he could demonstrate it in the adult stem tip of conifers.

PFITZER (32) denied the existence of an apical cell in the stem tip of conifers, but confirmed HOFMEISTER's work in regard to the existence of an apical cell in the early embryo, although he assigned to it only about 5 segments as a maximum for *Thuja*, and in Pinaceae he stated that the apical cell stage was even shorter. He calls attention in his conclusion to the fact that this may be taken as a case of embryonic recapitulation of the pteridophyte manner of development. He published no figures.

STRASBURGER (38) made a very careful study of the embryogeny of 8 or more genera of gymnosperms. In many particulars he corroborated the former accounts. His many excellent figures

are accurate and most of them still useful. He denies the existence of an apical cell in all but the Cupressineae, where he found a definitely organized apical cell in the early embryo. In *Pinus* and other Abietineae he finds this stage omitted or not constantly present, an indication that these are less primitive than the Cupressineae. In the further differentiation of the embryo he goes into greater detail than any previous worker. In *Pinus* and *Picea* the plerome tip of the root is set off about 0.15 mm. from the apex of the cylindrical mass of cells which is now about 0.5 mm. long, measured to the point where the cells form suspensors. In the account, which he says is practically the same for all the conifers, the stem tip meristem is next in appearance, followed by the cotyledonary primordia which arise in a circle about this point. His description of the cotyledon and stem tip development is substantially the same as that of SCHLEIDEN (36). At this stage of development the embryo reaches the lower end of the endosperm, and further growth and elongation bring the radical end of the embryo back to the place of origin of the suspensor.

STRASBURGER states that the number of embryos beginning development may be as high as 20, all but one of which abort in various early stages of development. In *Picea vulgaris* he finds that the 4 rows of cells of the proembryo do not separate, but all 4 of the embryonal cells at the tip of the suspensor contribute to the formation of 1 embryo.

The accounts, by the early workers, of the proembryo stages differ widely. SCHACHT (35) shows correctly the completed proembryo when it consists of 4 tiers of 4 cells each with the upper tier open to the egg. STRASBURGER (38, 39) attempted to explain the stages between fertilization and this completed proembryo, but, like other early workers, he failed to recognize the nature and extent of the free nuclear divisions. CHAMBERLAIN (5) described some details in the development of the proembryo, and later COULTER and CHAMBERLAIN (9) figured a more complete series of these stages. FERGUSON (13) added still more, working on 6 genera of *Pinus*, and found, as did MIYAKE (28) in *Picea*, that the upper tier of 4 cells, in the 8-celled proembryo, divide before the lower. Later, KILDAHL (19) found both orders of division in

Pinus Laricio, between the upper and lower tier of this stage, and also made a detailed study of the order and manner of development of walls in the proembryo, a thing which had confused many previous investigators.

COULTER (8) and COULTER and CHAMBERLAIN (9) described some of the early stages in the developing embryo, and, like STRASBURGER, denied the existence of a true apical cell stage. They also stated that the lower tier of the proembryo may develop into a single embryo, or that the vertical rows of cells frequently become separated to form 4 embryos. One of these may even divide by a vertical wall and the 2 daughter embryonal cells become organically separated (8), developing subsequently as 2 separate embryos on the end of the same suspensor. This would give us a very fluctuating program of possibilities in the development of the early embryo of *Pinus*.

SAXTON (33), in a study of the embryo of *Pinus pinaster*, gives some of the stages in the development of the embryo between the proembryo and the ripe seed. He concludes that an apical cell stage exists, which develops several segments, and in one case shows an embryo which he estimates as one of 30 cells, which still has an apical cell. He describes as anomalous some of the ordinary stages, and his account is rather incomplete, in many respects less adequate than that of STRASBURGER (38), to which he does not refer. SAXTON also finds that "the cotyledon primordia are exactly equal and equivalent in their origin."

Investigation

MATERIAL AND METHOD

The cones of *Pinus Banksiana* were collected from the dunes near Miller, Indiana, during the summers of 1914 and 1916, at weekly intervals during the latter part of June, July, and August. Cones of *P. Laricio* were secured from the parks in Chicago in 1914 and 1916, and from Richmond, Indiana, in 1915. *P. sylvestris* was also secured with the Richmond collections, and *P. echinata* was collected at Conway, Arkansas, during the summers of 1914 and 1915.

The embryos were removed from the ovules in the living condition by dissection under water, and these embryos with their suspensor systems were stained and mounted as permanent preparations. Studies were also made from serial sections cut in paraffin, but most of the drawings accompanying this paper were made from the dissected preparations mounted in Venetian turpentine. The latter were found to be superior to anything else for a study of the coiled suspensors and the further development of the rosette.

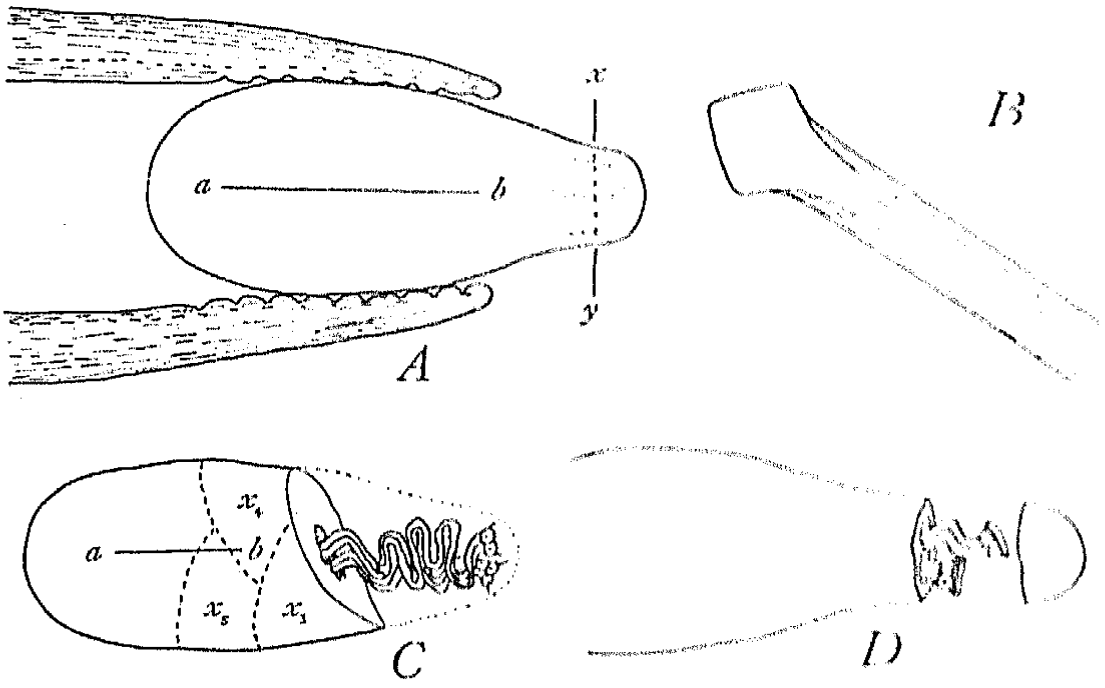

FIG. 1.—Illustrating methods of holding and dissecting pine ovules

DISSECTION.—The dissection must be done with living material under a dissection microscope, or preferably under a binocular microscope with magnification of about 20. The gametophytes are removed from the testa and placed in water in a watchglass. A very useful tool for the dissection, which must be executed under water, is a needle whose point has been flattened and ground to form 2 cutting edges, as shown in *B*, text fig. 1. The naked gametophytes, after being removed from the ovule, are held with forceps in the position shown in text fig. 1, *A*. Frequently the nucellus may be found, resembling a thin cap over the end of the gametophyte, and must first be removed, and sometimes the gametophyte may still be surrounded by the thin inner testa. The

forceps with which the gametophytes are held should be small and have weak springs, in order to avoid crushing the tender tissue. With the dissecting tool *B* the end of the gametophyte is removed along the line *xy*. By teasing a little deeper into the tissue around the edges of the archegonia it is possible to loosen the embryos at the bases of the archegonia, allowing the rosettes to be pushed out by the suspensor as in *D*, text fig. 1.

Usually a little gentle stroking with slight pressure in the direction *a* to *b* with the dissecting instrument held nearly horizontal (to avoid crushing the tissue) is sufficient to loosen the embryo and gradually force it out. A slight pressure with the forceps on the sides of the gametophyte at the proper moment may help. Sometimes gametophytes must be split vertically along the line *ab* before the older embryos can be removed.

When the embryos are imbedded more firmly, it may be impossible to dislodge them by these methods. Sometimes it has been found possible to remove embryos with the complete suspensor system by chipping away pieces of the gametophyte, first from one side and then from the other. This is accomplished most easily by rolling the gametophyte over after each chip has been removed, cutting off pieces x_3 x_4 x_5 (text fig. 1, *C*) alternately, until the embryos are sufficiently loosened. Any method of pulling the embryos out by taking hold of the upper part of the suspensors without first loosening the embryos below results in an incomplete embryo and suspensor system. In spite of the greatest care and perseverance it is often impossible to remove the suspensors and embryos without some of the latter breaking off. Which of the preceding methods is to be used will depend somewhat upon the condition and stage of development of the embryos.

In the earlier studies, which were carried out in this manner, many embryos were found abnormal, in which the protoplasts had escaped from the cells and could be found as dark staining masses near the empty cells. Careful study revealed the fact that this was an osmotic phenomenon, due to the fact that the dissection was executed under water. The cells have a high osmotic pressure, and when placed in water they swell and break in a short time. This may be avoided by dissecting the embryos out under

a 0.3 gm. molecular sugar solution. This strength of solution is still low enough to allow the cells to become fully turgid, and was found satisfactory for a number of species. Doubtless the strength of solution required will vary somewhat with the species and with the condition of the material.

KILLING AND STAINING.—After removal the embryos may be transferred to the killing fluid by means of a pipette with a 2 mm. opening. A good fixing agent is 6 per cent formalin in 50 per cent alcohol, and it is at the same time an excellent preservative in which they may be kept indefinitely, but aqueous formalin alone is not satisfactory. The embryos should be washed through several changes of water before staining, and may be transferred directly to water from the solution. The staining was done in saltcellar watchglasses with Delafield's haematoxylin or safranin. The haematoxylin was used for most of the preparations and was diluted to one-half of its usual strength. The water is removed with a pipette and a few drops of the stain applied for 5–10 minutes, which will stain them very deeply. At this point one of the most difficult steps is encountered, namely, to prevent losing the material while the stain is being removed. It was found best to dilute the stain with water until the watchglass is full. The upper layers of the solution may now be removed without disturbing the embryos at the bottom, but great care must be exercised to prevent losing the embryos, and the material should be watched as the pipette is filled by holding it over an illuminated white surface, as on the stage of a block dissecting microscope. More water is added and the operation repeated until the liquid is clear.

The overstained embryos are now de-stained with acidulated water (about one drop of HCl per 200 cc. of water). The stain is extracted slowly and must be watched over a low power microscope. The de-staining should be continued until the cytoplasm is well differentiated from the nucleus in the embryonal cells at the tip, and the suspensor cells should still be slightly blue. Very thorough washing is necessary to remove all traces of the acid or the preparations will fade. If safranin is used, it is advisable to overstain and then extract the stain to the desired point.

MOUNTING.—After the last washing 10 per cent glycerine is added and the material set aside to evaporate in a place protected from dust. When the concentrated glycerine is washed out with 95 per cent alcohol, great care must be exercised to prevent injury to the preparation. Several changes of alcohol will be necessary to remove all the glycerine, and after washing twice in absolute alcohol the 10 per cent Venetian turpentine is added and the watchglass placed in a desiccator. It is not desirable to allow the Venetian turpentine to get too stiff, as the specimens will be broken in mounting. If more of the 10 per cent Venetian turpentine is added to thin it down, as is frequently done, it causes the cells to swell, the cell walls separating from the protoplasts, leaving a permanent clear space between. If the Venetian turpentine must be thinned down, it should be done by adding about 85 per cent Venetian turpentine. The preparations may be picked up for mounting by means of a needle with a curved point, or a spear point. In handling them they should be picked up in a drop of Venetian turpentine and not by attempting to pull them out.

Preparations were also made by changing the embryos from concentrated glycerine into glycerine jelly. These mounts were not very satisfactory and compare very unfavorably with those prepared in Venetian turpentine.

METHODS FOR SERIAL SECTIONS.—The ovules were prepared for the fixing agent by removing the testa completely from the gametophytes. This can be done without crushing the latter by slicing away one side of the ovule down to the gametophyte with a sharp scalpel, then slicing away the edge, whereupon the gametophyte may be pried out without injury by inserting the point of the scalpel under it. For the early proembryo stages it is not necessary to remove the gametophytes from the testa, but a slice should be cut from one side, or better from opposite sides, to permit good fixation. The older testa cuts with difficulty, and it was not possible to get good sections of *P. Banksiana* when the coat had been left on in stages after the early elongating suspensor.

The naked gametophytes were removed and placed for 20–30 hours in the killing fluid, consisting of a chromic-acetic mixture ($\frac{2}{3}$ per cent chromic, 1 per cent acetic). After washing overnight

in running water they were dehydrated through a close series of graded alcohols, as follows: 5, 12, 20, 35, 50, 70, 85, 95, and 100 per cent. The xylols were also graded, but less closely: 15, 30, 50, 70, 85, and 100 per cent. The material was infiltrated with paraffin by adding the latter a little at a time, and preventing actual contact of the paraffin with the material by means of a perforated cardboard shelf fitted into the vial, a centimeter above the material.

Longitudinal and cross sections were cut serially 10 μ thick and stained by the usual methods employed for iron-alum haematoxylin. A counterstain of gold orange was found very effective in bringing out the otherwise transparent walls. The gold orange is dissolved in the clove oil to saturation. This is then decanted off and about one-fourth the volume of fresh clove oil added. This solution is poured on the slide after it has been stained and cleared in xylol. Only about a minute is necessary to stain the walls; if continued longer it colors the cytoplasm also. The gold orange has a great tendency to crystallize out as the oil evaporates, especially if the stain is too highly saturated. It is therefore advisable to rinse the slide with clove oil, followed by xylol.

In more recent work it was found that very brilliant preparations may be stained with safranin and light green as follows: the safranin must be a concentrated solution in 50 per cent alcohol (a full strength stock solution was used), with the sections left in it 1–3 days. After a rapid washing in 50, in 80, and then in 95 per cent alcohol the sections were placed in light green (about 1 per cent in 95 per cent alcohol) 2–5 minutes. The time for the action of the light green varies with the age of the material, the strength of the stain, and the length of time the sections were stained in safranin. It is desirable, therefore, to stain all the sections of one collection at the same time, and not to mix several collections in one staining. One or two trials will enable one to determine how long to leave the sections in light green, and the remaining slides may be carried through by this time schedule. If left in light green too long, the safranin will be washed out of the nuclei, and if taken out too soon the light green is not impregnated in the cell walls sufficiently to give the desired brilliant contrast. From light

green the slides must be transferred rapidly through 95 per cent alcohol, absolute alcohol, alcohol-xylol, into xylol. The de-staining process is not checked until the sections have reached the pure xylol solution; thus only a short dip should be given into each solution. Although safranin with gentian violet, Delafield's haematoxylin, and iron alum haematoxylin with light green were all tested, they were found much less satisfactory than the iron-alum haematoxylin with gold orange or the safranin with light green.

FORMATION OF CORROSION CAVITY WITHIN GAMETOPHYTE

The first change that is noticeable in the tissue below the archegonia is a starch deposit, which appears in the cells of this region about the time of fertilization, or a few days later. In the living gametophyte this deposit makes the tissue appear opaque, and it gradually spreads down into the central part of the gametophyte until this white opaque region comes to occupy a funnel-shaped region extending downward from the archegonia. About the time the embryos break through the bases of the archegonia the cells at the center of this opaque region break down, at first in the large part of the funnel nearest the archegonia. This forms the beginning of the corrosion cavity, an opening which, as it enlarges, assumes the shape of a slightly flattened trumpet. At the same time the starch-containing zone enlarges and becomes more conspicuous.

Sections like that shown in fig. 1 indicate clearly that the digestive action of an enzyme on the endosperm doubtless precedes the elongation of the suspensor. The embryo is soon pushed so far into this cavity by the elongation of the latter that further elongation can only bring about its well known coiled and twisted condition. The importance of this mechanical action of the suspensor in keeping the embryo pressed into the bottom of the corrosion cavity is better realized when one tries to dislodge some of these embryos by dissection.

Many ovules were examined in which the gametophytes had well developed corrosion cavities, yet no traces of embryos could be found in them, indicating that the archegonia may secrete the digestive enzymes to form the cavity even though the eggs have not been fertilized. Many sections of this kind may be found in

the collections of ovules made from one to two weeks after fertilization. These soon dry up and wither away within the hardening testa, so that one would not include them in the later collections of material if the testa is first removed.

The subsequent enlargement of the corrosion cavity to accommodate the growing embryo is unquestionably due to digestive enzymes secreted by the embryo itself. The archegonia disappear as recognizable structures soon after the primary suspensor has fully elongated. The rosettes are usually found pressed against the top of the cavity, which now includes the space occupied by the archegonia after the latter have broken down. An unfertilized archegonium withers away soon after the formation of the corrosion cavity, its place being marked by a shrunken chip of hardened protoplasm which is often molded into the shape of the lower portion and side of this organ. Later this disappears also.

EMBRYO DEVELOPMENT

This investigation takes up the development of the embryo beginning with the 16-celled stage, which has generally been recognized as the end stage of the proembryo. It is necessary, however, to consider some of the well known earlier stages, and for these facts we will depend upon the results of previous workers which have been reviewed in the historical discussion.

Of the 4 tiers of 4 cells each, the lowest constitutes the embryonal group, each of which is an apical cell of one cutting face; the next tier above constitutes the suspensor group, each of which elongates to form a primary suspensor cell; the third tier has been called the rosette, and its further development has never before been followed out; and the uppermost tier of cells, which have incomplete walls and are in open communication with the egg, sooner or later disintegrate. Fig. 1 shows a longitudinal section through the base of an archegonium after the suspensor cells have begun to elongate and before any of the cells of the embryonal tier have undergone further division. In fig. 38 the embryonal tier has given rise to a tier of cells (e_1) between it and the suspensor, and at the left in fig. 37 an embryonal cell may be seen in anaphase of division.

SUSPENSOR.—The tier of suspensor cells elongates and pushes the tier of embryonal cells into the cavity below. When the suspensor cells have elongated slightly more than in fig. 1, the embryonal cells give rise to the first embryonal tube initials (e_1), and by the time the suspensor cells have elongated to the stage shown in figs. 39 and 40 another transverse wall has appeared in the apical cell below, giving rise to e_2, the second embryonal tube initials. This is soon followed by the elongation of the first embryonal tube initials (fig. 6, e_1) to form tubes like the suspensor cells, the first embryonal tubes. This added part of the suspensor is the secondary suspensor.

Separation of the vertical rows of cells soon follows the division of the embryonal cells, although it may occur earlier, as is the case in fig. 37 at the left. In none of the species of pines studied was a single case found in which the 4 vertical rows of cells did not separate to form 4 embryos. It will be seen from a study of figs. 39, 40, 41, and 44 that the elongating first embryonal tubes are no longer in an even tier, and one of the embryos has already gained the lead in penetrating the endosperm. The struggle for supremacy between the 4 primary embryos of an archegonium is well shown in figs. 40, 41, and 44, while in figs. 43 and 45 two archegonia are concerned.

Since the primary embryos have now separated, we shall regard one of these 4 as the unit for discussion. One of the 4 suspensor cells and all of the cells formed below it by the embryonal cell constitute one primary embryo, while all the embryos produced by an egg will be spoken of as an embryo system.

It is evident from a study of the development of the early part of the suspensor that the primary suspensor tubes never divide to form other tubes or cells. Likewise, an embryonal tube never undergoes division after it has begun to elongate, but an embryonal tube initial cell may divide by a vertical wall before elongation, as e_2 in figs. 6, 8, 14, and 20, or e_3 in figs. 10 and 16. When the embryonal tube initial divides and gives rise to 2 or more cells in a tier, these elongate together into a collateral group of embryonal tubes (figs. 47–50), forming a suspensor division. These suspensor divisions are all parts of the secondary suspensor, but when they

consist of 4 tubes, as in fig. 50, they look very much like the lower part of a group of primary suspensors. For example, if the e_3 group of fig. 50 were studied from sections only, with the upper part of the suspensors confused as they are in fig. 45 (making it impossible to trace any of the tubes back to the rosette), it would be natural to mistake this perfect suspensor division as the group of primary suspensors. It is quite possible that a study of such sections has given rise to the statement that in *Pinus* all 4 of the embryonal cells may contribute to the formation of 1 embryo, or they may form 4 embryos.

The initial cell for the second embryonal tubes (e_2) and for the third and subsequent embryonal tubes are cut off as segments of the apical cell, first by transverse walls, and later as oblique segments. The initial cell of an early embryonal tube may elongate into a 1-celled suspensor division, resembling a primary suspensor cell, or it may first divide by a vertical wall as e_2 in figs. 6 and 8. Fig. 16 shows e_2 as a single elongated cell and e_3 with 3 cells, while fig. 20 shows e_2 of 4 cells. There is considerable variation in the number of cells found in the embryonal tube groups of corresponding suspensor divisions, and variations are frequently found among the individuals of the same embryo system.

After the initial cells of the embryonal tubes begin to divide by vertical walls and elongate to form the suspensor divisions, each succeeding bundle of embryonal tubes consists of more cells than the tier above it (figs. 46–52). Only one exception to this has been found among the 500 or more dissected preparations of various pines, and this one was *P. Laricio*, shown in fig. 24. Here s and e_1 (not shown) are single-celled, e_2 is of 2 cells, and e_3 again 1-celled, while e_4 and e_5 will undergo other divisions before beginning to elongate. Careful examination of many preparations indicates that the separation of the 4 primary embryos precedes the division of any of the embryonal tube initial cells by vertical walls.

The primary suspensor, that is, the first suspensor division, is often collapsed and withered by the time 4 or more divisions have formed. The upper parts of collapsed suspensors are shown in figs. 65 and 68, while fig. 46 still has a turgid primary suspensor. The primary suspensors frequently collapse in about the stage shown

in fig. 46 or soon after, and the cells of the older portion of the secondary suspensor also collapse in turn, so that in an older embryo, like that of fig. 51, the upper part of the suspensor cannot be studied.

In order to determine the amount of variation in the early suspensor divisions, several hundred preparations of *P. Banksiana* were examined and the types of suspensor development noted.

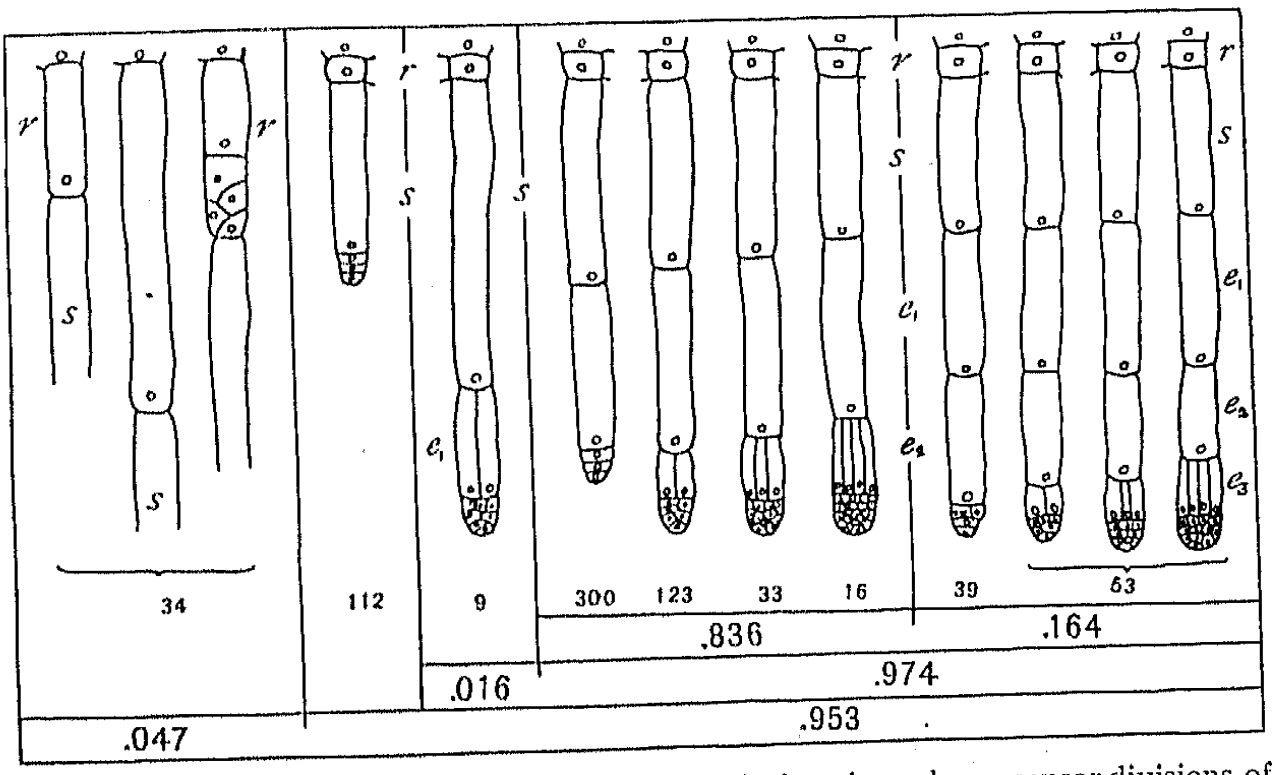

FIG. 2.—Graphic statistical summary of variations in early suspensor divisions of *Pinus Banksiana:* the figures indicate the number of examples of the various types of suspensors observed, and their distribution in percentage.

The results are summarized in the diagrams of text fig. 2. It was common in more than four-fifths of the cases examined to find the single primary suspensor followed by a 1-celled embryonal tube (e_1), this followed by 2 or more cells in the next suspensor division, after which the tubes interlock and elongate irregularly, as in figs. 47, 49, and 51. Less than one-fifth of the cases were found with the primary suspensor followed by 2 successive single-celled suspensor divisions and 2 or more tubes in the fourth suspensor division e_3. Only about 1.6 per cent of the embryos were found to have the first embryonal tubes or second suspensor division of 2 cells.

In nearly 5 per cent of the cases the rosettes were elongated to resemble suspensor cells.

It will be seen that the third embryonal tube group, or fourth suspensor division, always consists of 2 or more cells, and after this division or the one following the tubes begin to elongate and interlock to form the suspensor. The transition from the jointed to the interlocked and more massive portion of the suspensor is well illustrated by figs. 47, 49, 50, and 51.

The suspensor becomes more and more massive as the embryo increases in diameter. The embryo is first pushed as far as possible into the corrosion cavity by the mechanical action of the suspensor; later it remains nearly stationary in the lower end of this cavity, but continues to give off the suspensor by the successive elongation of the cells from the radical end of the embryo; finally, as the embryo develops to its full size, the radical portion again reaches the archegonial end of the cavity. As the root cap becomes differentiated in the embryo, it may be seen that this organ and the suspensor gradually merge into each other; in fact, the late suspensor is formed from the root cap by the successive elongation of layer after layer of cells.

ELONGATED CELLS. The nuclei of the suspensor cells and embryonal tubes always seem to hold a definite size relation to the cells. A large suspensor tube may frequently contain a nucleus larger than an entire cell in the embryonal group at the apex. The position of these nuclei is always at the embryonal end of these tubes. More of the cytoplasm of the cell is usually found here, near the nuclei. The ends of these cells containing the nuclei are frequently enlarged considerably. Often one of the primary suspensor cells breaks loose at the lower end during elongation. Figs. 41 and 45 show such tubes which continued to enlarge at the lower end and formed a balloon, while fig. 42 shows an earlier stage in another tube. These phenomena are not uncommon.

BASAL PLATE.—A thickened plate (*p*) is deposited above the rosette soon after the suspensor begins to elongate. Something similar was found in *Podocarpus*, where COKER (6) calls it a cellulose plug, "a novelty among gymnosperms." It is called a "basal plate" by the writer because it is a plate rather than a plug, and its

chemical nature in *Pinus* was not determined. The word "basal" seems fitting because it is, in a real sense, basal to the embryos in its position. Doubtless careful search will reveal this in many other gymnosperms. Whether the rosette is present as in fig. 41, elongated as in fig. 54, or absent as in *Podocarpus*, the basal plate is always formed in the egg cavity on the walls toward the embryos.

APICAL CELL.—A distinct apical cell stage exists from the time the embryo cells first have walls. In fig. 1 the suspensor cells (*s*) are the first segments of their respective apical cells (*a*). Here the 4 primary embryos are apparently still united; but if they may be looked upon as organizing distinct from each other, the 4 cells which gave rise to the lower 8 cells of the 16-celled embryo are embryo initial cells. The work of COULTER and CHAMBERLAIN (9), FERGUSON (13), and KILDAHL (19) has shown that these which we call embryo initial cells were formed in the mitosis between the 4-nucleate and 8-nucleate proembryo, the place where KILDAHL (19) found that the first walls appeared. FERGUSON (13) and KILDAHL (19) found that the rosette and upper open tier organize next, from the upper 4 nuclei (although KILDAHL found exceptions to this), and therefore this lowest tier of the 12-celled stage is a hold-over since the first appearance of walls.

The second segment of the apical cell is the initial cell of the first embryonal tube. This segment, as well as the third and fourth, are formed by an apical cell of 1 cutting face. Figs. 1–6 all show apical cells of a single cutting face, while in figs. 7, 8, 9, 11, and 12 the first oblique wall of the apical cell has appeared. This wall is sometimes only slightly tilted, as in fig. 9, or it may be nearly vertical, as in figs. 10 and 14.

The stage at which this oblique wall first appears is not always the same. A large number of embryos of *P. Banksiana* were examined in order to determine the average condition in this respect. This study showed that these variations are somewhat similar to those found in the number of tubes in the early suspensor divisions. In nearly two-thirds of the cases the first oblique wall appeared after the primary suspensor and 2 embryonal tube initial cells (3 suspensor divisions) had been formed by the apical cell of one cutting

face; one-fourth after 2 suspensor divisions; and one-tenth after 4 suspensor divisions had been formed.

It is often difficult to determine with certainty in an embryo like fig. 15, for example, at what stage the first oblique wall was formed. Here the last horizontal wall is tilted slightly, so one might think that this was modified by growth after the first oblique wall appeared; but it is also possible that this segment was first formed with a perfectly horizontal wall, and this later enlarged on one side to appear slightly oblique, so that the first real oblique wall is the one which appears nearly vertical. While these two interpretations could be given to fig. 15, in making the study referred to in the foregoing paragraph, the slightly oblique wall was looked upon as though it has been formed in an oblique position by the apical cell.

A stage in which the apical cell has 2 cutting faces does not exist, or it is so shortened that it cannot easily be recognized. Figs. 15 and 16 have only 2 oblique segments cut off, but these are probably the first 2 segments of the apical cell stage with 3 cutting faces. Apical cells with 3 cutting faces are found in embryos only slightly larger, such as figs. 17 and 18. Figs. 17–23 are all from whole mounts in Venetian turpentine and show pyramidal apical cells of 3 cutting faces.

Many irregularities are found in regard to the position of the apical cell. It is frequently so far to one side of the tip of the embryo that it might be overlooked in some serial sections. A section of an embryo like figs. 17, 20, or 28, if cut in another plane, would not show the apical cell so favorably, and might be mistaken for an embryo without an apical cell.

A very puzzling case is shown in fig. 21*a*, *b*. Fig. 21*a* shows the embryo in a high focus, with the shadows of nuclei of a lower focus shown by the dotted lines. Fig. 21*b* shows the nuclei and cell walls of the same as seen in low focus. This looks like an embryo which has no apical cell, and it is on the basis of very similar figures that STRASBURGER (38, 39), and other workers since, have denied the existence of an apical cell as a constant feature. In this particular instance the apical cell is at one corner of the lower tier of 4 cells. It is either the cell to the right in high focus, or the lower

cell to the left. For instance, if it is the upper right cell, then either the cell below it or the one in the same plane of focus beside it is its last segment, while the remaining 2 cells together constitute the next to the last segment. The other cells of the embryo may well have arisen while the apical cell had 1 cutting face.

Fig. 13 shows a case very similar to fig. 21*a*, but somewhat younger. If the apical cell and the last segment shown here should both divide with walls in the plane of the paper, and the next tier of 2 cells above this (e_3) should do the same, it would not differ essentially from fig. 21*a*, *b*. Fig. 14 is in the same stage as fig. 13, but with the e_2 suspensor division elongated.

In longitudinal sections the apical cell and its segmentation may usually be seen (figs. 25–29). Fig. 31 is an embryo of about 200 cells, one of the smallest embryos that could be found without an apical cell, and fig. 30 is a larger embryo of about 275 cells, which apparently still has one. Fig. 32 shows a larger embryo of 750 cells which no longer has an apical cell; and figs. 35*a* and 35*b* show the first 2 sections through the end of an embryo in which the apical cell is replaced by a meristematic group. Figs. 34*a* to 34*d* are consecutive cross-sections through an embryo a little larger than that of fig. 32, in which the apical cell may still be found, probably in an arrested condition, before the meristematic group of cells has become active. Fig. 34*e* is a diagram combining sections 34*a* to 34*c* and showing the relation of the segments to the apical cell.

Figs. 33*a* and 33*b*, sections through the tip of an embryo slightly smaller, show an apical cell and segments as diagrammed in fig. 33*c*. This shows the segments arranged clockwise, while in fig. 34*e* they are counter-clockwise. This difference is easily accounted for, since the serial sections on these 2 slides run in opposite directions through the embryos. In fig. 34 the views of the cross-sections proceed toward the apical cell from the base of the embryo, while in fig. 33 they proceed from the apex inward. The segments thus appear in the same order on the embryo and proceed in the same direction as the thread of a wood screw, beginning at the point which corresponds to the apical cell and passing back along the thread toward the older segments. This is probably the usual

arrangement of the spiral of segments, as no exceptions were found in an examination of several other cases, although no extensive study of this feature was undertaken.

The early apical cell forms a slightly compressed and slightly conical mass of cells. When the apical cell ceases to function, as in fig. 32, the embryo is more uniformly cylindrical, sometimes slightly club-shaped. The apical cell vanishes long before the stem tip, the cotyledons, or any of the body regions are recognizable, and nearly all of the early part of the embryo formed by apical cell growth goes to form the suspensor by the elongation of layer after layer of cells from the basal part of the embryo.

ROSETTE AND ROSETTE EMBRYOS

No investigator seems to have followed the development of the rosette further than through the early stages of elongation of the suspensor. That the open cells of the tier above the rosette disorganize has been stated by various workers. The writer has also been unable to find any traces of these nuclei of the upper open tier after the early stages of suspensor elongation, and doubtless they disintegrate.

The rosette has usually been regarded as a group of cells between the main body of the egg and the suspensor, having no particular function. This view has proved to be erroneous, for the rosette is a group of young embryo initials which will produce embryos. These embryos are bounded by thick walls and are not so free to elongate as the primary embryos below them.

After a little delay, during which the adjoining primary suspensor cells elongate, the rosette cells divide, as shown in one of the rosette cells of fig. 58, also in some of the rosette cells seen in polar view in fig. 59. A wall soon appears in one of the 2 daughter cells, inclined at an angle to the first (fig. 61, and rosette of fig. 40), forming the second segment of the apical cell. The apical cell continues to cut off segments on 2 or more sides, and the later embryo appears to have 3 cutting faces. Fig. 65 is a side view of a group of rosette embryos and shows well the apical cell and its segmentation, and (*s*) the upper portion of the collapsed primary suspensor.

None of the rosette embryos has been found to reach stages much in advance of those shown in figs. 64–68. In some of these the embryonal tubes elongating from the basal portion of the embryo have formed a recognizable suspensor, which often appears freakish, as in fig. 68, modified no doubt by the unfavorable position and the unequal thickness of the walls of the rosette cells.

It will be seen that the orientation of these rosette embryos is variable. In fig. 67 they have begun to elongate in various directions. The direction of the apical portion and the suspensor must be determined by the first few divisions, and figs. 59–64 show that these are likewise quite variable. Before the rosette embryos have developed much beyond the early stages, such as fig. 59, the archegonium breaks down, and these embryos may be found pushed up against the top of the corrosion cavity by the suspensor. Even before the archegonium has completely broken down the rosette is frequently tilted by the twisting suspensor below, and it is quite probable that the orientation of the rosette embryos is related to the position of the rosette when the first divisions occur in these embryo initial cells, a thing that may well account for the lack of uniformity or regularity.

It often happens that some of the rosette cells disorganize early and fail to produce embryos. Rosette cells may be found with no visible nuclei, or with nuclei in various stages of disintegration, while the neighboring rosette cells are producing embryos. While these exceptions occur, it is evident that the normal product of an archegonium is 8 embryos. This makes polyembryony a much more extensive phenomenon than has hitherto been recognized. All of the species of *Pinus* investigated showed this peculiarity, *P. Banksiana*, *P. Laricio*, *P. echinata*, and *P. sylvestris*. Rosette embryos develop less rapidly than the 4 primary embryos, abort in early stages, and it is entirely outside of the range of probability that they may ever contribute the embryo of the seed.

Elongation of the rosette.—Another abnormal phenomenon that was occasionally noted was that of elongated rosette cells resembling the primary suspensors. Fig. 53 shows a rosette in the first stages of elongation; fig. 54 shows another that is well advanced. Elongated rosette cells were found in nearly 5 per cent

of the total number of preparations examined in connection with the study summarized in text fig. 2.

A condition which demonstrates that these rosette cells are potentially embryos, even when they elongate to form suspensors or embryonal tubes, is shown in fig. 54, in which a mitotic figure may be seen in the lower portion of one of these elongated cells. Fig. 55 shows this mitotic figure of fig. 54 enlarged. An ordinary suspensor cell or embryonal tube has never been found to undergo division after elongation. The origin of the cells intermediate between the elongated rosette and the primary suspensor of fig. 56, and of 1 rosette in fig. 57, seemed a puzzle until the case shown in fig. 54 made it apparent that these cells may arise from the rosette tube. They are terminal cells of the rosette embryos that were formed after the rosette cell had begun to elongate. The rosette cell at the left, in fig. 54, has a nucleus in spirem stage, probably preparing for the first mitosis in the formation of an elongated rosette embryo of this kind.

POLYEMBRYONY

In *Pinus* polyembryony is a much more extensive phenomenon than is generally known. Since the rosette produces 4 embryos, and 4 others are always produced by the splitting of the lower primary embryos, 8 embryos may be formed from each fertilized egg. The greatest number of embryos possible is 8 times the number of archegonia, which might reach as high as 48 if all 6 of the archegonia, present in some species, were fertilized. Fertilization must be very nearly simultaneous in all the archegonia, and other conditions very favorable if the maximum number of embryos is to be produced. Fig. 60 shows an embryo complex, which had a delayed start and was stunted from the beginning, a condition which is frequently found where more than 4 archegonia are fertilized, with 1 more or less delayed.

In the various pines studied, 4 is the maximum number of embryo sets that were actually found, each related to one of the 4, 5, or 6 archegonia. Two or 3 archegonia were the usual number fertilized. In *P. Banksiana*, with only 2 or 3 archegonia, as large a number is not possible as in *P. Laricio*. Since the cones of the

material studied were poorly pollinated, as was indicated by the relatively few good ovules and seeds developed per cone, no doubt the maximum possible number of embryos was not to be found in these collections.

The terminal embryo of the group is the successful one in the struggle for supremacy among the embryos. In very exceptional cases the successful embryo has been found to be the second one instead of the terminal. Occasionally an embryo develops with the reversed orientation, and the abortive embryos are frequently found in this reversed position.

Cases were also found where less than 4 primary embryos were produced from an archegonium, where one of the vertical rows of cells was aborted with little or no elongation of its suspensor, or the embryo initial cell itself was aborted. This condition might give the impression that one of the 2 or 3 primary embryos is composed of 2 vertical rows of cells that failed to separate in the normal way, were it not for the fact that when one of the embryos aborts in this way there are less than 4 suspensor tubes or first embryonal tubes.

No embryos have been found to arise from 2 or more vertical rows of cells combined. Such an embryo would have 2 apical cells, and wherever an embryo possesses a single apical cell and looks normal in other respects it is safe to conclude that it has come from one of the 4 embryonal cells. Another simple criterion is that of tracing the suspensor back to the rosette. If an embryo could be found attached to 2 primary suspensor cells, without the possibility that an embryo has been lost in dissection, it would indicate that 2 primary embryos were combined, but in this case the embryo should also have the appearance of being double, and the number of embryos present in the complex should be one less than the usual number. The writer found several cases which he suspected to be double embryos, but when they were more carefully studied they failed to fulfil these conditions.

Twins.—So far as I have been able to find, no embryos arise by a further splitting of one of the 4 primary embryos. Since the terminal cell of the early embryo is an apical cell, an equal splitting could only occur after the formation of a vertical wall, as in figs. 10,

13, 14, and 15, and no cases of "twin embryos" formed by such a vertical splitting could be found. The embryos never even show tendencies to round off at these nearly vertical cleavages, or upon the formation of any wall other than the one which separates the 4 primary embryos. Such "twins" would be easily recognized in dissected preparations, since they would be found attached by their secondary suspensors to a common suspensor, leading back to a single primary suspensor cell.

When the 4 primary embryos are sectioned in stages before they are completely separated, it is possible, in rare cases, that 2 embryos may be so cut as to appear to be at the tip of a single suspensor or embryonal tube. This might look as if the 2 embryos had arisen on the end of the same suspensor by the splitting of a single one, especially if some of the adjoining sections are lost. The writer had the opportunity of examining the original slide from which the drawing of a "twin embryo" had been published (8). Upon critical examination it proved to show traces of the wall of a second suspensor cell from which the stain had been washed out, and is more correctly shown in fig. 30. One of the adjoining sections, which happened to be a very thick one, is missing from the slide. It was possibly lost off during the staining, as the accidentally thick sections of a series often are, but the recognition of this second suspensor cell gives each of the 2 embryos in this figure its own suspensor and indicates that these 2 embryos were 2 of the primary group of 4, sectioned in a rather unusual position. The possibility that one of the 4 primary embryos could split to form 2 has been claimed by several investigators, but no other figures showing twin embryos of pines could be found in the literature on this subject.

Another type of twins is that found when 2 of the members of the embryo complex develop to fair size to form the mature seed embryos. Although polyembryony is such an extensive phenomenon in *Pinus*, the writer has never been able to find a mature seed with 2 fully and equally developed embryos; one was always considerably larger than the other, and these were not very common. When these 2 embryos are members of the same embryo system, the twin formation is due to a cleavage phenomenon, and is similar to that of duplicate twins in animals.

THE LATER EMBRYO

In embryos of *P. Banksiana*, the size of fig. 47, the apical cell may usually still be found, but by the time the stage shown in fig. 51 is reached it has disappeared. The cylindrical mass of cells

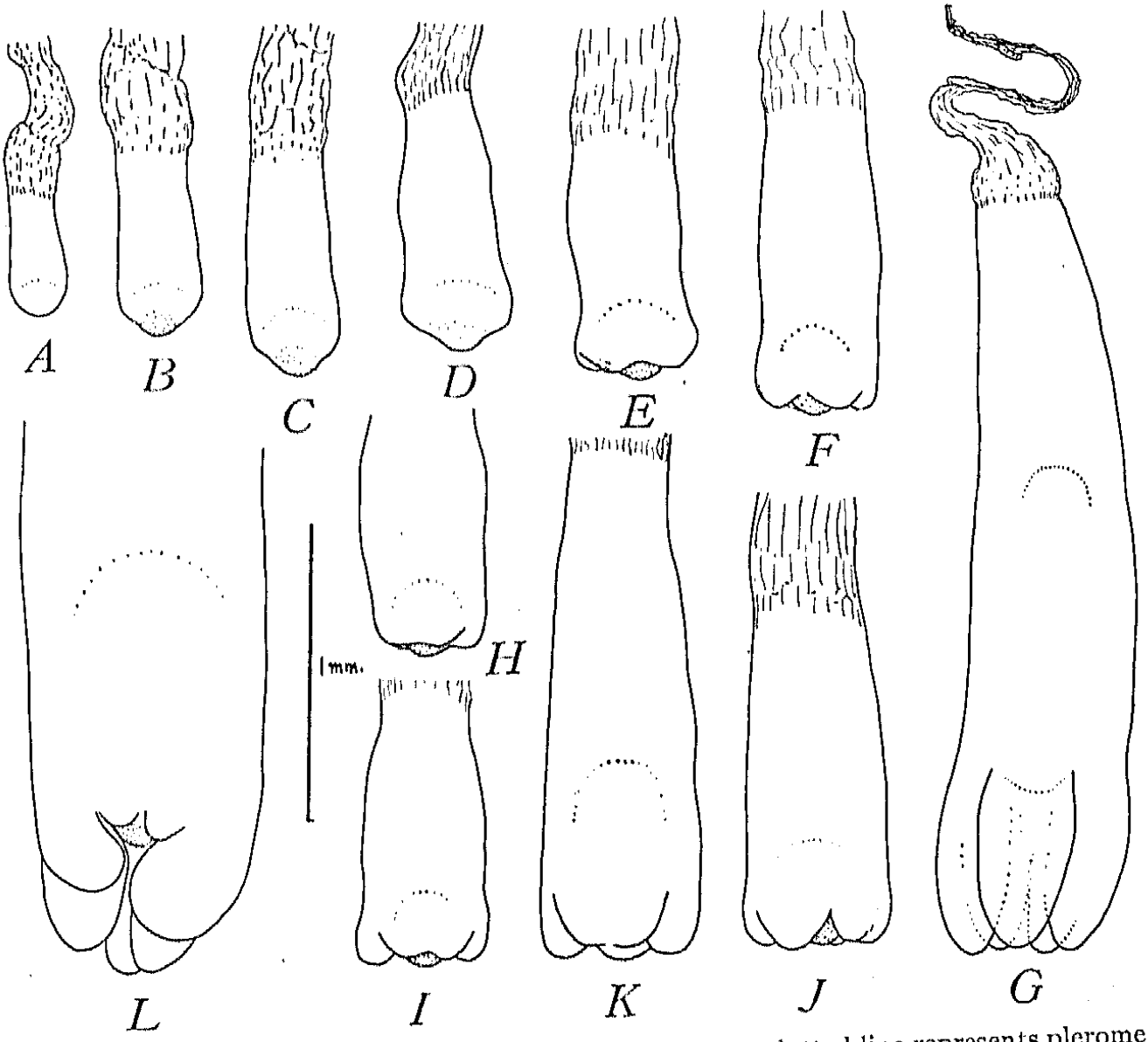

FIG. 3.—Development of stem tip and cotyledons; dotted line represents plerome of root tip; shaded area, meristem of stem tip; *H*, *I*, *J*, *K*, fusing cotyledons.

enlarges, and about the time the stage shown in fig. 52 is reached the cells near the tip begin to organize into an arch, shown by the dotted line of text fig. 34. Under this arch is the plerome of the root tip, the first body region to appear. The periblem organizes outside of this dome and is thickest above it on the side toward the suspensor, where it merges with the tissue of the massive root

cap. This curved cell arrangement may be recognized in the whole embryos mounted in Venetian turpentine or balsam, but sections show the details of this cell organization much better.

The stem tip may be recognized as a slight protuberance in the position formerly occupied by the apical cell, but long after this cell has disappeared. It may first be seen in embryos about 175 $\mu \times 400$ μ; and in living embryos dissected out under water a transparent area develops in the tissues near it, which is shown by the shaded area of *B*, text fig. 3. The embryo enlarges, and by the time it has reached the size of *D* the circle of cotyledonary primordia is recognizable. The number of these primordia, like the number of cotyledons, is not constant, and ranges from 3 to 7. Although the cotyledonary primordia are usually equally developed when they first appear, sometimes they are larger or appear sooner on one side than on the other. Figs. *J* and *K* show cases where 2 primordia formed only 1 cotyledon. Figs. *H* and *I* show the same thing in earlier stages, and since stages older than *K* do not reveal a double tip on the broad cotyledons it is doubtless rapidly outgrown. Many broad cotyledons may have a similar origin, but some of them seem to arise directly from 1 broad primordium. Although embryos like *H*, *I*, *J*, and *K* are not as common as *E* and *F*, those that do not show fusing primordia, there is no doubt a distinct tendency in *P. Banksiana* to reduce the number of cotyledons. The mature embryo frequently has only 3 cotyledons, and 4 or 5 are the usual numbers. In *P. Laricio* fusing primordia were not found, but here there are usually 10 or more cotyledons, and there seems to be no tendency to reduce their number.

The embryos of these 2 species show a tendency to grow slightly zygomorphically. In some cases this seems to date from the first appearance of the primordia. It is usually not very pronounced, but an embryo of *P. Laricio*, extremely abnormal in this respect, is shown in text fig. 3*L*. Here the suppression of the cotyledons on one side is nearly complete, a condition which, in the presence of a cotyledonary tube, would result in an embryo similar to the monocotyledonous embryo, as described in recent work (11). Although 2 primordia sometimes combine to form a single cotyledon, none of these pine embryos have a cotyledonary tube at any stage of their development.

ABNORMALITIES

Among the seeds of *P. Banksiana* one was found in a germinated lot which had developed in the reversed position. The cotyledons and hypocotyl were protruding about 15 mm. from the micropylar end of the seed, while the root tip was imbedded in the endosperm. It died without developing much beyond this stage. Some of the aborted embryos of the pine seed are frequently reversed, and LAND (20) described a young embryo of *Thuja* which was directed toward the micropyle. Embryos matured in this position are very rare; this case which was germinated was the one case of the kind found in connection with this investigation.

Among the many hundreds of ovules from which the testa was removed preparatory to dissection or imbedding, many cases (at least 15) were found with 2 gametophytes in the same ovule. They occurred in two ways, end to end and side by side. The end to end gametophytes often joined obliquely, and each gametophyte is necessarily formed by the functioning of 2 megapsores. Whether these gametophytes belonged to the same tetrad row or to different tetrads is a matter of conjecture, but one would think that the side by side and obliquely joined prothallia have more probably developed from megaspores of different tetrads. *P. Banksiana*, which was most largely dissected, yielded the most of these double gametophytes. Several were also found of *P. echinata* and two of *P. Laricio*. It is not surprising that a very primitive conifer like *Pinus* should occasionally show this feature.

A few ovules were found in which the terminal embryo aborted and the second one dominated over the others, which is very unusual. Two seeds were found which contained 2 embryos, but in each case the embryo pair was quite unequally developed.

One sectioned ovule of *P. Banksiana* was found in which the customary splitting of the embryo complex did not take place as completely as usual. By a careful study of the series it is clear that each of the 4 embryos is pursuing its own independent development and has its own apical cell. One of the 4 embryos is clearly the largest and will no doubt dominate over the others quite as well as if they were more completely separated.

Discussion

APICAL CELL.—STRASBURGER (38) was the first to cast doubt upon the existence of an apical cell in the embryo of the Abietineae. He felt doubtful of it because it did not appear to be a constant feature. The instances in *Pinus* described and figured by him in which he considered the apical cell absent are practically the same as some of the more unusual ones described in this paper. He regards embryos like figs. 13, 14, and 21*a* as having no apical cell; and while he recognized that in embryos like figs. 16 and 18 an apical cell seems to be present, he considered this apical cell growth not constant and that it has no phylogenetic significance.

COULTER (8) expresses the opinion that an apical cell is only simulated in *Pinus* and does not in reality exist. He is probably misled by the appearance of nearly vertical oblique walls in the terminal cell and by embryos like figs. 13 and 21*a*. COULTER and CHAMBERLAIN (9, 10) do not mention an apical cell, and thus imply that such a stage does not exist, but point out that the problem of the development of the pine embryo after the first few divisions is an open one.

SAXTON (33) overlooked STRASBURGER's work (38, 39), and although COULTER had expressed the opinion that an apical cell is only simulated, he is inclined to regard the terminal cell of the *P. pinaster* embryo as an apical cell. When he failed to find an oblique spindle he seemed not fully convinced about the existence of a true apical cell, which he figured only in young embryos up to 30 cells. For these reasons the writer considered it necessary to give considerable study and attention to the proof of the existence of an apical cell in the early embryo.

A series of embryos (figs. 6, 7, 8, 10, 13, and 14) may be selected showing the first oblique wall in all positions, from nearly transverse to vertical. This variation in the first oblique wall has made an occasional embryo hard to explain as having an apical cell. Fig. 15 shows how the next wall comes in, and after this stage the apical cell may easily be found, except that it is frequently very much to one side. The apical cell cuts the first oblique segment at no fixed stage, but probably at the time when the embryo is well separated from its neighbors in the embryo system. This same

cause may also account for the variation in the time of appearance of the vertical walls in the initial cells of the early embryonal tubes.

STRASBURGER (38) also cites the case of an embryo similar to fig. 21*a* as disproving the constant existence of an apical cell, but fig. 21*a* has an apical cell; it is one of the 4 cells of the apical tier, one of the adjacent cells is its last segment, while the two remaining cells constitute the next older segment.

An embryo like fig. 21*a*, but in which the c_2 tier of cells has elongated, looks so much as though it shows the original 4 embryonal cell rows going into a single embryo that doubtless this impression could be created from a study of serial sections only. When STRASBURGER found a stage very similar to fig. 21*a*, however, he was able to trace the suspensor back to a single tube and recognize that it is only one-fourth of the product of the egg. According to his explanation the embryo from this stage on develops like that of *Picea*, in which the whole of the fertilized egg unites to form 1 embryo, and has no apical cell. All of my investigations have failed to support this view, but, on the contrary, embryos slightly older than fig. 21*a*, such as figs. 16, 18, 22, and 28, always have an apical cell, and this cell may usually still be found in embryos of 500 or more cells.[1] Certainly the instances where the apical cell cannot be found in embryos having several hundred cells or less are rare, and the most exceptional cases found in this investigation have been figured and described. Every essential condition for an apical cell is satisfied. It has the proper position on the embryo, being at or near the apex of a body with polar differentiation; it has the same general shape as the apical cell at the stem tip of a fern; and it has recognizable segments which may be related in their regular turn to the 3 cutting faces, even in some embryos of 800 cells.

From a comparative study of the embryos of other conifers it is probable that this apical cell feature is retained more generally than one would suppose. According to STRASBURGER (38) the Cupressineae all have this feature. COKER'S (7) study of the embryo of *Taxodium* does not conflict with this view, for in many

[1] Estimated roughly by counting the average number of cells in diameter and length and applying the formula $l\pi r^2$.

cases he was able to determine the succession of walls in young embryos, which suggests the possibility that an apical cell may be found here. In *Podocarpus* COKER (6) figures a number of embryo stages, some of which may have an apical cell; in others it appears doubtful. ARNOLDI (1) and LAWSON (22) show figures for the early embryos of *Sequoia* and *Sciadopitys* in which the embryonal cell has formed 1 or more vertical walls, a condition which precludes the possibility of an apical cell, according to the views of some investigators. However, according to the later stages of *Sequoia*, figured by SHAW (37) and ARNOLDI (1), an apical cell arrangement exists, and it is possible that the vertical walls were only the first obliquely placed walls of the apical cell, a condition which occurs occasionally in *Pinus*, and is explained in connection with figs. 13, 14, 15, and 21*a*. SANTON (34) shows some of the stages in *Actinostrobus* which are suggestive of an apical cell, and doubtless it may be found in many of the conifers. It is just as certain to be absent, even in some of the Abietineae, if STRASBURGER's account of *Picea* (38) is correct, for if all 4 cells of the lower tier of the proembryo together produce an embryo, the apical cell loses its identity from the start.

It is evident that in *Pinus* a primitive condition is found, in which the apical cell is still functional for a considerable period, and that in some derived conifers this has been retained more or less, while in some evolutionary lines it has been suppressed or completely eliminated.

APICAL CELL IN RELATION TO PROEMBRYO. One of the great difficulties in accepting the apical cell as having phylogenetic significance has been the impression that if such a stage may be found it does not exist from the start. In looking over the literature it is apparent that many workers do not recognize an apical cell as such, unless it cuts off oblique segments from several cutting faces. Their apical cell would begin only with the first oblique wall. By studying the behavior of the segments in forming the suspensor the writer has shown that the embryonal cell is a hemispherical apical cell of a single cutting face, and that the primary suspensor cell is its first segment. We need not expect to go farther back in the proembryo than to when the embryo

initial is organized; the stage previous to this is a free nuclear division which organizes these several equivalent cells. Thus the proembryo stage in *Pinus* is the stage in which the divisions occur that bring about cleavage polyembryony.

PROEMBRYO.—The free nuclear division which occurs after the 4 nuclei descend to the bottom of the egg is followed by walls which are complete for the lower tier of cells, but leave the upper tier in open communication with the egg. Thus, when the upper tier divides to form the rosette and the open tier above it, the cleavage is still essentially a free nuclear division. According to FERGUSON (13) and KILDAHL (19), this upper tier of the 8-celled proembryo usually divides before the lower, and in the resulting 12-celled stage all of the cells with complete walls, namely, the 8 cells of the lower 2 tiers, are embryo initials, which henceforth grow by means of an apical cell. This fact suggests the possibility that the upper open tier may also represent a tier of similar initials which has become abortive. This seems probable when we consider that in *Pinus* the lowest tier produces embryos immediately; the rosette tier only after some delay, and then not always; while the upper open tier represents a group of initials that failed to organize. The presence of this upper abortive tier suggests a reduction from a more extensive form of polyembryony.

The lower tier of the 8-celled proembryo sometimes divides before the upper one, according to KILDAHL (19), who also confirms this order. No proof exists that the upper tier ever undergoes another division when the lower one divides first, and it is possible that the nuclei shown in her fig. 11 would have collapsed without undergoing further divisions. This would give us, in this case, only 8 embryo initials instead of 12 (counting the upper open tiers as potential embryo initials), of which only 4 function. It seems evident that this order of division is rather uncommon. According to the interpretation that the embryos are separately organized by means of initial cells in the proembryo, the latter stage has a new significance as a real preliminary stage in the embryogeny. However, the proembryo stage should be considered closed in *Pinus* when the 12-celled stage is reached, rather than the 16-celled stage, and in the instance shown by KILDAHL

the proembryo is complete in the 8-celled stage. Thus far "proembryo" has been recognized largely as a term of convenience to describe the stages preceding the elongation of the suspensor.

POLYEMBRYONY.—The writer has found it necessary to distinguish between the polyembryony caused by the simultaneous fertilization of several eggs and that brought about by the separation of the embryos of a single egg. The latter form of polyembryony, which is spoken of as "cleavage polyembryony," is no doubt a constant feature of *Pinus*, and may possibly be found in some of the other genera of this family. The statement that "all 4 cells of the lower tier may unite to form a single embryo, or they may separate to produce 4 embryos," may hold for the Abietineae as a group. The writer has found separated primary embryos in all of the species of *Pinus* examined, which includes *P. sylvestris*, for which STRASBURGER reports only 1 embryo per archegonium. Forms like *Thuja* (20) seem to show splitting of the embryos at times, while in other cases the archegonium produces only 1 embryo. COKER (7) found the embryos splitting apart in *Taxodium* and also in *Podocarpus* (6). Some of these more modern forms are therefore not constant like *Pinus* in this respect.

Polyembryony by cleavage from 1 egg is no doubt a primitive gymnosperm character, even though it has persisted to the *Ephedra* level, where it is on its way to elimination. No angiosperm has shown this form of polyembryony, which is a further proof that it is a primitive character. Aside from its phylogenetic significance, the feature of polyembryony is a wonderfully effective means for the possible elimination of unfit embryos, involving as it does in *Pinus* some 32 embryos when 4 archegonia are fertilized.

Although no matured twins have been found to arise by the cleavage of the egg in *Pinus*, this has been demonstrated for *Ginkgo* by LYON (26). Here we have a close parallel to the animal twins which are formed by cleavage, and LYON has shown that the twin embryos may originate from the same archegonium, remain organically connected, and develop equally to the maturity of the seed.

EARLY EMBRYO IN RELATION TO OTHER CONIFER EMBRYOS.—The known stages of the proembryos in *Picea* (28), *Abies* (29),

Pseudolarix (30), and *Tsuga* (31) are reported as similar to *Pinus;* the embryo is said to consist eventually of 4 tiers of 4 cells each.

In *Sciadopitys* LAWSON (25) found 8 free nuclei before organization into tiers takes place. This is very significant, for here we may have this extra free nuclear division result in more embryo initials, a thing which would bring about a greater display of cleavage polyembryony than in *Pinus*. Judging from the figures of ARNOLDI (1), this conclusion seems justified, for the central group of cells shown in several of his figures is doubtless made up of many embryo initials from which the embryos are elongating. The writer believes that cleavage polyembryony is a very primitive feature, and it is therefore possible that the embryo of *Sciadopitys* is more primitive than that of *Pinus*.

Little is known in regard to the rosette of other conifers. The work done on *Picea* (28), *Abies* (29), and *Tsuga* (31) does not include the stage showing the suspensor elongating. MIYAKE and YAZIN (30) have figured a stage in *Pseudolarix* with the suspensor elongated, which proves that a rosette group exists in this genus. It is not safe to conclude that a rosette exists in all forms in which the proembryo is organized in tiers like *Pinus*.

In *Pseudotsuga* LAWSON (24) reports a proembryo similar to *Pinus*, but does not show which tier of cells elongates, or whether a rosette exists. He applies the term "rosette" quite generally to the upper tier of free nuclei where no rosette cell group exists. Likewise, COKER, in his work on *Podocarpus* (6) and *Taxodium* (7), uses the term "rosette" to designate the group of free nuclei above the suspensor. While these investigators apply the term "rosette" here, it is evident from a comparison of the figures that a rosette homologous to that of *Pinus* does not exist in *Podocarpus*, *Taxodium*, or *Cryptomeria*. The term "rosette," as first used by MIRBEL and SPACH (27), applies to an unelongated tier of completely walled cells. LAND (20) showed that it is likewise the uppermost tier of completely walled cells that elongates in *Thuja*. The absence of a group of rosette cells and of rosette embryos is a more advanced character, found only in the more recent conifers.

SAXTON (34) has described the embryo of *Actinostrobus*, which repeats the proembryo of *Sequoia* in completely filling the egg

with walled cells. Four of the 6 cells in *Actinostrobus* organize as embryo initials and give rise to embryos. Neither ARNOLDI (1) nor LAWSON (22), in their work on *Sequoia*, followed the embryo development very far. They probably studied the development of only a single embryo from each egg. It seems probable that the other 3 cells which are cut off by walls in the first 2 divisions of the proembryo of *Sequoia* may represent embryo initials, and more careful study may perhaps reveal secondary embryos arising from 1 or more of these other 3 cells. Like the rosette embryos in *Pinus*, these possible secondary embryos in *Sequoia* may develop only after some delay, and thus easily be overlooked. *Actinostrobus*, and possibly *Sequoia*, represent forms in which cleavage polyembryony has been retained more or less.

The cleavage polyembryony of *Pinus* suggests an explanation of the proembryo of *Ephedra*, described by STRASBURGER (38) and LAND (21). Here the 8 free nuclei of the proembryo organize with walls as embryo initials, and from 4 to 5 of them produce embryos. The embryo initials organize only at the bottom of the egg in *Pinus*, while in *Ephedra* they organize with walls before reaching the bottom. *Ephedra* has thus retained, in a modified form, a very ancient character, that of cleavage polyembryony, a character which indicates that this plant has descended from the Coniferales. According to the testimony of their embryogeny, such forms as *Pinus* and *Actinostrobus* must be looked upon as the nearest conifer relatives of *Ephedra*.

A comparative study of conifer embryos suggests several possible evolutionary lines of advance. One of these is the one beginning with *Pinus* and culminating in *Ephedra*, in which cleavage polyembryony is retained in some modified form. The apical cell feature is retained among the more primitive embryos of this line, but apparently lost by the time the *Ephedra* level is reached.

The abietineous embryo of the type represented by *Picea* (38) would be produced when all the embryo initials together develop 1 embryo. Here the lower tier is an even one, and if the embryo develops uniformly a meristematic group of 4 cells replaces the apical cell from the first. Thus *Picea* may represent the culminating abietineous embryo type, while *Pinus* represents the primitive type.

In his work on *Cephalotaxus* STRASBURGER (38) shows in pl. 19, fig. 53, what is possibly a rosette embryo group, although he does not refer to it in the text. It is of interest in this connection to note also that the *Cephalotaxus* embryo has a cap which associates it with *Araucaria* (4, 38) and *Agathis* (12). All of this suggests another possible line of advance from a *Pinus*-like ancestor, through intermediate forms like *Cephalotaxus*, and a culmination in the embryo of the araucarian type. Thus it looks as though nearly all the embryos of Coniferales may be derived from an ancestor with cleavage polyembryony and an apical cell like *Pinus*, differentiating into the several more or less distinct lines of specialization. This is a strong argument in support of the theory that *Pinus* is a very primitive and ancient genus.

POLYCOTYLEDONY.—If polycotyledonous gymnosperms have been derived from dicotyledonous ancestors, one would expect that in the ontogeny of the cotyledons 2 primordial zones would first appear, and these 2 zones divide up and give rise to the primordia of the separate cotyledons. On the other hand, this investigation goes to prove the opposite; namely, that the polycotyledonous condition is the more primitive, and the tricotyledonous or dicotyledonous condition derived.

Most of the work which has been done on polycotyledony has been based upon the vascular anatomy of the seedling (16). The arguments that favor the derivation of polycotyledonous embryos by a splitting of cotyledons are based on anatomy and are well summarized by COULTER and CHAMBERLAIN (10), who state that "it must be remembered that these same facts may be used also as evidence that the dicotyledonous condition has arisen from the fusion of more numerous cotyledons."

SAXTON (33) also doubts the origin of polycotyledons from dicotyledons, and concludes from a study of cross-sections of *P. pinaster* that "the primordia are exactly equal and equivalent in origin." However, he produced no direct evidence to indicate that fusions of the many cotyledons may have occurred.

The study of the ontogeny of the cotyledons brings out facts not hitherto considered in connection with this problem. In speaking of cotyledonary fusions, it must be understood that full

grown cotyledons did not fuse, but 1 cotyledon is developed in the place formerly occupied by 2. The number of cotyledons is actually reduced by fusion of the primordia.

A zygomorphic tendency, which is usually only very slight, is evident in nearly all mature embryos of *P. Banksiana*, but only occasionally in the early stages of the embryo. This zygomorphy of the matured embryo is, no doubt, a secondary result due to the shape of the seed, for it is always oriented within the seed in the same manner, and the zygomorphy is less pronounced in the case of *P. Strobus*, which has a more regularly shaped seed. The zygomorphic tendency found in some early embryos cannot be related to the shape of the seed, and is no doubt due to certain hereditary tendencies. The most extreme case found (text fig. 3*L*) was that of *P. Laricio*. When zygomorphy is pronounced, as in this case, it furnishes an interesting parallel to the development of certain monocotyledonous embryos at the stage when primordia develop, as shown recently by COULTER and LAND (11). In *Pinus* the zygomorphy never goes to such an extreme as in the monocotyledonous embryo, and no cotyledonary tube is formed in any of the pines that were studied. That we have well developed cotyledonary tubes among the Abietineae is shown by the work of HILL and DEFRAINE (16) and by the recent work of HUTCHINSON (18) on *Keteleeria*. The cotyledonary tube would be formed as a natural accompaniment of a coalescence of the many cotyledons by fusion; its very existence among the historically recent gymnosperms is a further indication that cotyledonary fusion has taken place, rather than a splitting. It is interesting to note in this connection that the number of cotyledons in *Keteleeria* is 4, a rather reduced number.

Summary

1. A special technique for dissecting ovules, staining and mounting the embryos, and an improved method of staining embryos in serial sections have been described in detail.

2. The corrosion cavity results from an enzyme, which may be secreted by the unfertilized eggs as well as the embryo.

3. Two forms of polyembryony must be recognized in gymnosperms, namely, cleavage polyembryony and the polyembryony

due to plurality of archegonia. In *Pinus* one usually finds both types associated in the same ovule, and cleavage polyembryony always occurs in the several species of *Pinus* that were investigated. It is probably a constant feature of this genus.

4. The rosette consists of a group of embryo initials which usually produce embryos. Rosette embryos, like 3 of the 4 primary embryos, are always aborted.

5. Each embryo of a system may be traced back to an initial cell, one of the first completely walled cells of the proembryo. The 8 embryos formed by the cleavage of the egg are therefore definitely organized from the time of the last free nuclear division.

6. A further splitting of one of these 8 embryos into "twins" was not found to occur in *Pinus*. In rare cases 2 matured embryos were found in an ovule, but they were very unequal and due simply to the incomplete dominance of a single embryo.

7. The early embryo develops by means of an apical cell which exists from the time the first walls appear in the proembryo. This apical cell persists for a considerable period, being still recognizable in embryos of 500–700 cells.

8. The apical cell represents a primitive fern character, which is recapitulated in the embryogeny of *Pinus*.

9. Less than 4 primary embryos per archegonium may be produced in case one of the embryo initials, or the early apical cell, disorganizes.

10. The suspensor is formed by the elongation of cells in the basal portion of the embryo, a process that begins with the elongation of the first apical cell segment and continues until the maturity of the embryo.

11. Suspensor cells or embryonal tubes never divide after elongation, but rosette cells may elongate and later divide in forming the rosette embryos, showing their greater potentialities and their distinctness from the suspensor cells which they resemble.

12. Considerable variation occurs in the first secondary suspensor divisions, also in the time of appearance of the first oblique walls formed by the apical cell; both are doubtless related to the time of separation of the embryos.

13. Cleavage polyembryony is a primitive character which *Pinus*, *Sciadopitys*, *Actinostrobus*, and doubtless other genera have

retained. *Ephedra* has also retained it in a modified form, and this definitely associates Gnetales with the Coniferales rather than the cycads.

14. The other evolutionary lines suggested in the discussion likewise assign a primitive position to *Pinus*, so that this ancient type seems to be genetic to several conifer lines.

15. The body regions of the later embryo, so far as they have been determined, appear in the following order: plerome tip of root, periblem and root cap, stem tip, and cotyledons.

16. There is a distinct tendency in *P. Banksiana* toward a reduction in the number of cotyledons, attested by the fact that 2 primordia have been found to form 1 broad cotyledon. This suggests that the dicotyledonous condition has been derived from the polycotyledonous condition through cotyledonary fusions.

17. Cotyledonary tubes are the result of past cotyledonary fusions, and are found in embryos between the primitive polycotyledons and dicotyledons.

18. The zygomorphic feature of a monocotyledonous embryo is foreshadowed in the embryo of *Pinus*.

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DESCRIPTION OF PLATES VI–X

Figures of plate VI from dissected preparations, except figs. 1–5; figures of plate VII all from serial sections; figures of plates VI and VII ×300; figures of plates VIII–X drawn to same scale from dissected preparations and ×80; lettering in all figures as follows: *a*, apical cell; *e*, embryonal tubes; e_1, first embryonal tube (or its initial cell if it marks an unelongated cell); e_2, second

embryonal tubes; e_3, third embryonal tubes; *o*, portion of egg containing upper open tier of nuclei; *r*, rosette cells; *s*, suspensor (primary suspensor); *p*, basal plate (plate of thickening usually formed above rosette); all figures of *Pinus Banksiana* unless otherwise indicated.

FIG. 1.—Section through base of archegonium, showing suspensor cells (*s*) elongating before lower tier of tip cells (*a*) found in 16-celled proembryo have divided; early corrosion cavity forming; June 20, 1914.

FIGS. 2–5.—Sections through 4 separated embryos all coming from the same egg, still even with each other, but with their apical cell mitosis not simultaneous; mitosis shown results in second embryonal tube initial (e_2); June 20, 1914.

FIG. 6.—Vertical wall forming in second embryonal tube initial, which will result in a 2-celled suspensor division, as shown in fig. 8.

FIG. 7.—Later stage than fig. 6, in which an oblique wall has been formed by apical cell and no vertical wall has yet appeared in any embryonal tube initials.

FIG. 8.—Later stage than fig. 6, in which a 2-celled suspensor division has begun to elongate; first oblique wall cut off by apical cell has just been formed; June 30, 1916.

FIG. 9.—Later stage, in which first oblique wall of apical cell is only slightly tilted; 2-celled embryonal tube division has become well elongated; July 1, 1916.

FIG. 10.—Apical cell of *Pinus Laricio*, forming first oblique wall, in this case almost vertical; July 6, 1916.

FIGS. 11, 12.—Usual appearance of embryos with first oblique wall formed by apical cell; June 29, 1916.

FIGS. 13, 14.—Occasional appearance of embryo after vertical wall has been formed by division as shown in fig. 10; in fig. 14 embryonal tube initials (upper cells of group) have elongated, leaving only 4 cells below; fig. 13, June 22, 1914; fig. 14, July 1, 1916.

FIG. 15.—*Pinus Laricio*, showing how second oblique wall is formed by apical cell after first has appeared vertical; July 16, 1916.

FIG. 16.—Usual condition after first 2 oblique segments have been formed; July 1, 1916.

FIG. 17.—Later stage with distinct apical cell placed slightly to one side; apical cell has 3 cutting faces; June 30, 1916.

FIG. 18.—Usual condition of slightly older embryo; June 29, 1916.

FIGS. 19, 20.—Embryos with apical cell in rather unusual position; June 30, 1916.

FIG. 21.—Two views of same embryo; *a*, in a high plane of focus, showing shadows of lower nuclei; *b*, showing only nuclei of lower plane of focus and walls; apical cell difficult to distinguish with certainty; June 22, 1914.

FIGS. 22, 23.—Older stages than last, with distinct apical cells; July 5, 1916.

FIG. 24.—*Pinus Laricio:* unusual suspensor in which third embryonal tube initial (e_3) remained undivided, although suspensor section above it has 2 collateral tubes; very exceptional; July 6, 1916.

FIGS. 25–27.—Longitudinal sections showing successive stages in development of embryo by apical cell with well marked segments; June 30 and July 5, 1914.

FIG. 28.—Embryo about same stage as fig. 26, but with apical cell placed very much to one side; if section had been cut longitudinally and at right angles to this plane, apical cell would have been obscured; July 5, 1916.

FIG. 29.—Very late stage, in which apical cell is still active and segments very distinct; July 5, 1914.

FIG. 30.—Late stage of embryo with apical cell which has probably become inactive and is on verge of being eliminated; July 5, 1914.

FIG. 31.—Embryo smaller than fig. 29, which no longer possesses an apical cell.

FIG. 32.—Embryo past apical cell stage; July 5, 1914.

FIG. 33.—Two successive cross-sections through the tip of an embryo 100 $\mu \times 180\,\mu$, in which an apical cell may still be found; diagram 33*c* shows relations of segments; July 12, 1916.

FIG. 34.—Three successive cross-sections (*a*, *b*, *c*) through tip of an embryo 128 $\mu \times 280\,\mu$ (larger than any other embryo shown on this plate) in which apical cell may still be found, although doubtless it has become inactive; *d*, section through widest part of same embryo; *e*, segmentation as reconstructed from *a*, *b*, *c;* July 8, 1916.

FIG. 35.—Two successive sections through tip of embryo 108 $\mu \times 200\,\mu$, showing no trace of apical cell.

FIG. 36.—*Pinus Laricio:* drawing of an embryo described (8) as coming from splitting of a single embryo on end of a single suspensor cell, showing faint wall of another suspensor cell to right; it is no doubt a case where 2 primary embryos have not completely separated and are sectioned in an unusual position.

FIG. 37.—Two embryo groups of neighboring archegonia in early stage of suspensor formation; apical cell of embryo to left is in mitosis giving rise to first embryonal tube initials; all other embryos have already formed these cells; separation of embryos evident; June 29, 1916.

FIG. 38.—Embryos of same age showing early separation of the 4 primary embryos of each archegonium.

FIGS. 39, 40.—Successive stages in elongation of suspensors, first embryonal tubes, and separation of 4 primary embryos of archegonium; June 26–30, 1916.

FIG. 41.—More complete drawing of later stage with completely separated embryos and partly elongated embryonal tubes; one of the 4 primary embryos has broken loose from its attachment below and enlarged into a balloon at

lower end containing the nucleus; thick deposit of material, basal plate (*p*), is shown on upper wall of rosette; June 24, 1916.

FIG. 42.—Suspensor, of which lower end is beginning to enlarge into a balloon; June 24, 1916.

FIG. 43.—Embryo complex from 2 adjacent archegonia with 8 primary embryos present and 1 set of rosette embryos forming; all first embryonal tubes have elongated and some of second embryonal tubes are about to elongate; June 30, 1916.

FIG. 44.—Embryos from 1 archegonium of about the same stage as fig. 43; 1 embryo has been left far behind in the "struggle for supremacy"; June 22, 1914.

FIG. 45.—Embryo complex similar to fig. 43, but rather more advanced; a balloon-like enlargement may be seen at end of 1 primary suspensor; one of the 8 embryos has been aborted and one left far behind; July 1, 1916.

FIG. 46.—Embryo system from 1 archegonium in which primary suspensors and first embryonal tubes of secondary suspensors have fully elongated, while next divisions of suspensors are nearly half elongated; July 1, 1916.

FIG. 47.—Embryo in which third division of suspensor has completely elongated and succeeding portions of suspensor are beginning to form embryonal tubes of unequal lengths that break joints; suspensor slightly crushed below, thus separating embryonal tubes; July 5, 1916.

FIG. 48.—Embryo slightly older than in fig. 47, but with less developed suspensor becoming massive very suddenly; June 29, 1916.

FIG. 49.—Embryo with very typical suspensor forming fourth suspensor division (third secondary portion), with young embryonal tubes beginning at base; July 5, 1916.

FIG. 50.—Embryo of somewhat older stage than fig. 49.

FIG. 51.—Later massive embryo with characteristic secondary suspensor made up of dovetailed embryonal tubes (or tubes that break joints) in which suspensor divisions no longer appear; July 8, 1916.

FIG. 52.—Older embryo than fig. 51 shortly before differentiation of body regions; July 8, 1916.

FIG. 53.—Rosette in early stage of elongation (cases of elongating rosette cells are found in 5 per cent of embryos of *P. Banksiana*).

FIG. 54.—Rosette fully elongated, with a mitotic figure in lower end of one of its cells; June 30, 1916.

FIG. 55.—Detail of lower end of elongated rosette of fig. 54, showing division spindle.

FIG. 56.—Rosette elongated and divided into embryo of many cells, of which figs. 54 and 55 was a delayed beginning; June 30, 1916.

FIG. 57.—Rosette embryo similar to fig. 56, with suspensor tube broken off from below.

FIG. 58.—Embryo system with first division of rosette embryo showing in one of rosette cells, beginning of usual type of development of rosette embryos; June 27, 1916.

FIGS. 59–61.—Views of rosettes from above, showing stages in development of rosette embryos; June 29–30, 1916.

FIG. 62.—Rosette embryo of *Pinus echinata* in oblique view, showing apical cell; July 23, 1914.

FIG. 63.—Views of rosettes of 2 adjacent archegonia as seen from above, showing different stages in which various rosette embryos may be found at the same time; June 30, 1916.

FIG. 64.—Later rosette embryos well developed, but no tubes elongated to form a suspensor; July 8, 1916.

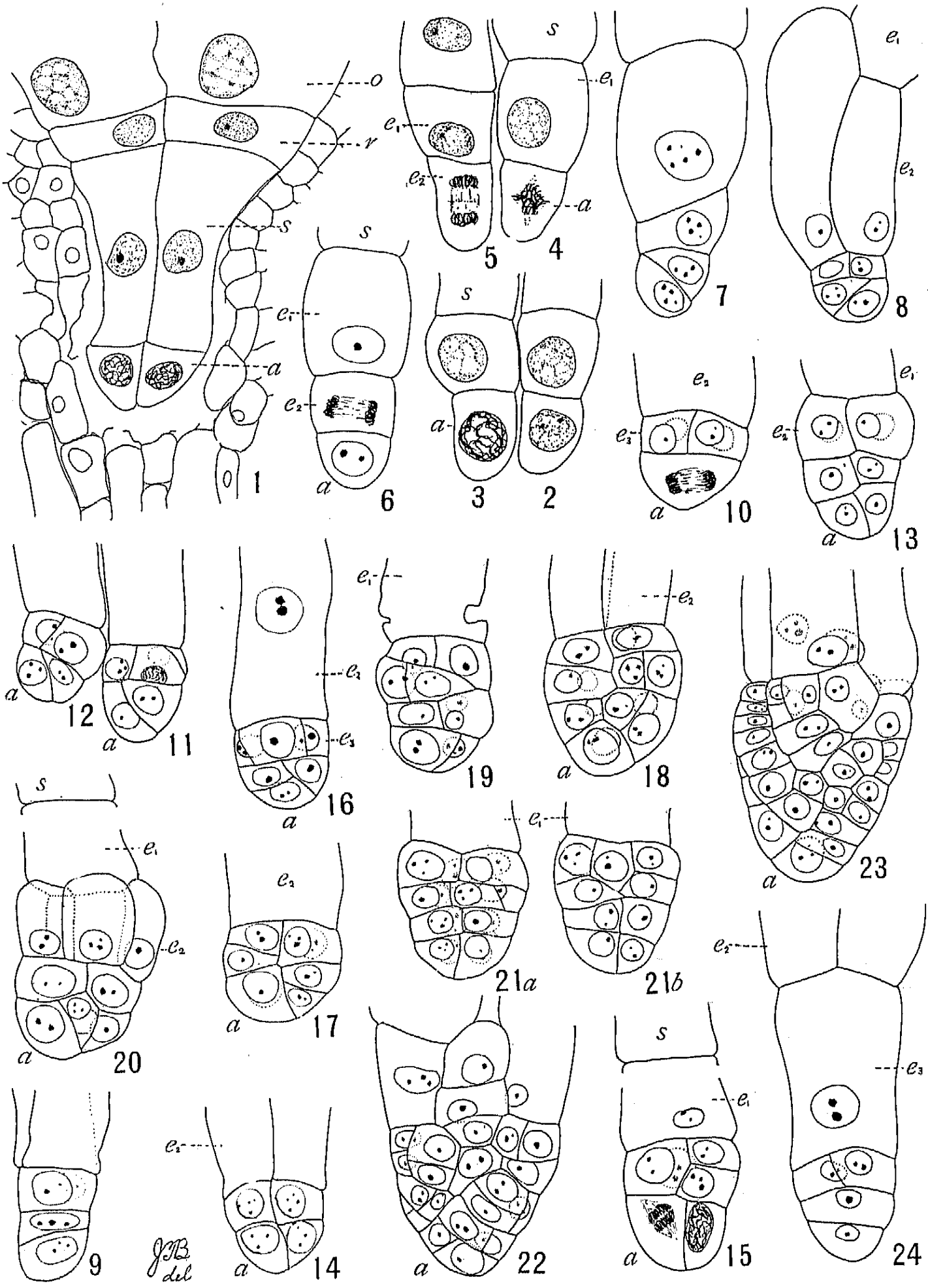
FIG. 65.—Side view of group of rosette embryos, one of which shows an apical cell and distinct segmentation; primary suspensor (*s*) of lower 4 embryos has entirely collapsed by the time this stage is reached; July 8, 1916.

FIG. 66.—Side view of rosette embryos from 2 adjacent archegonia, from some of which embryonal tubes have elongated to form a suspensor, showing that rosette-cell proliferations are real embryos.

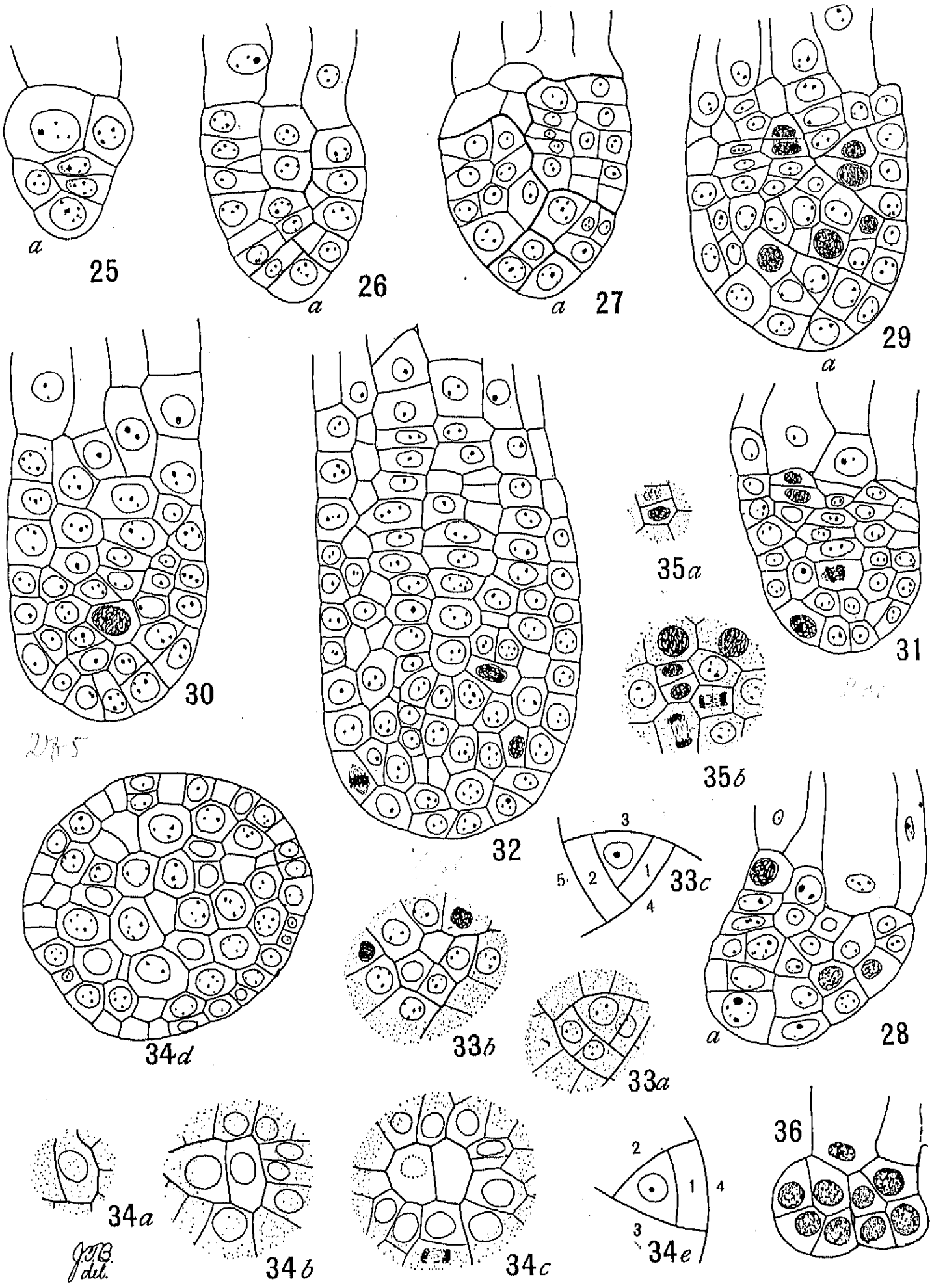
FIG. 67.—Group of rosette embryos which have suspensors elongated in various directions, although number of cells formed is less than in fig. 64, where no elongation has thus far occurred; July 5, 1916.

FIG. 68.—Rosette group showing 1 embryo with suspensor elongating under difficulty and distorted, on account of heavy wall found between rosette and suspensor cells.

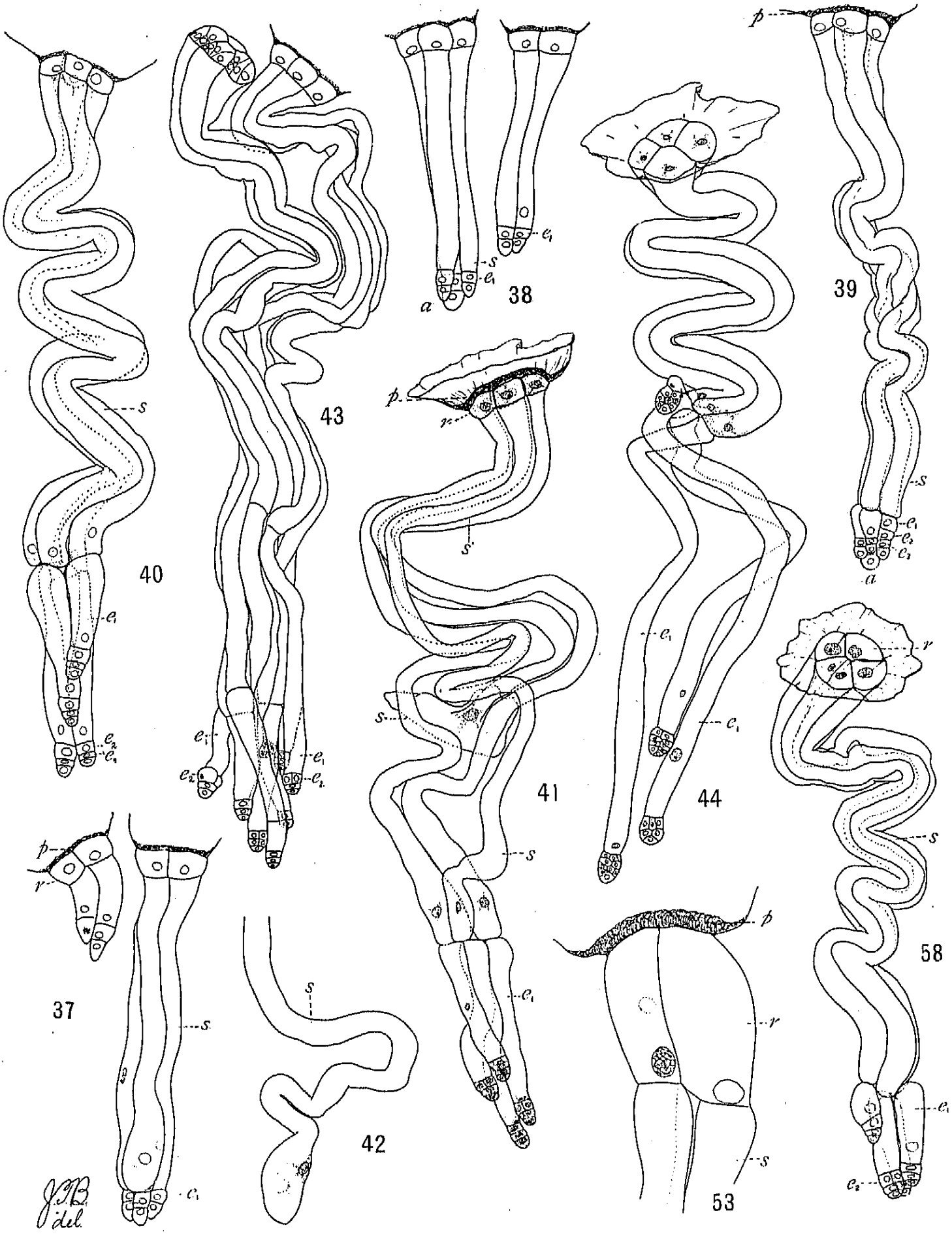
FIG. 69.—Embryo system which was badly stunted, due to delay in fertilization or development, while 2 or more adjacent embryo systems gained supremacy; June 29, 1916.



BUCHHOLZ on PINUS



BUCHHOLZ on PINUS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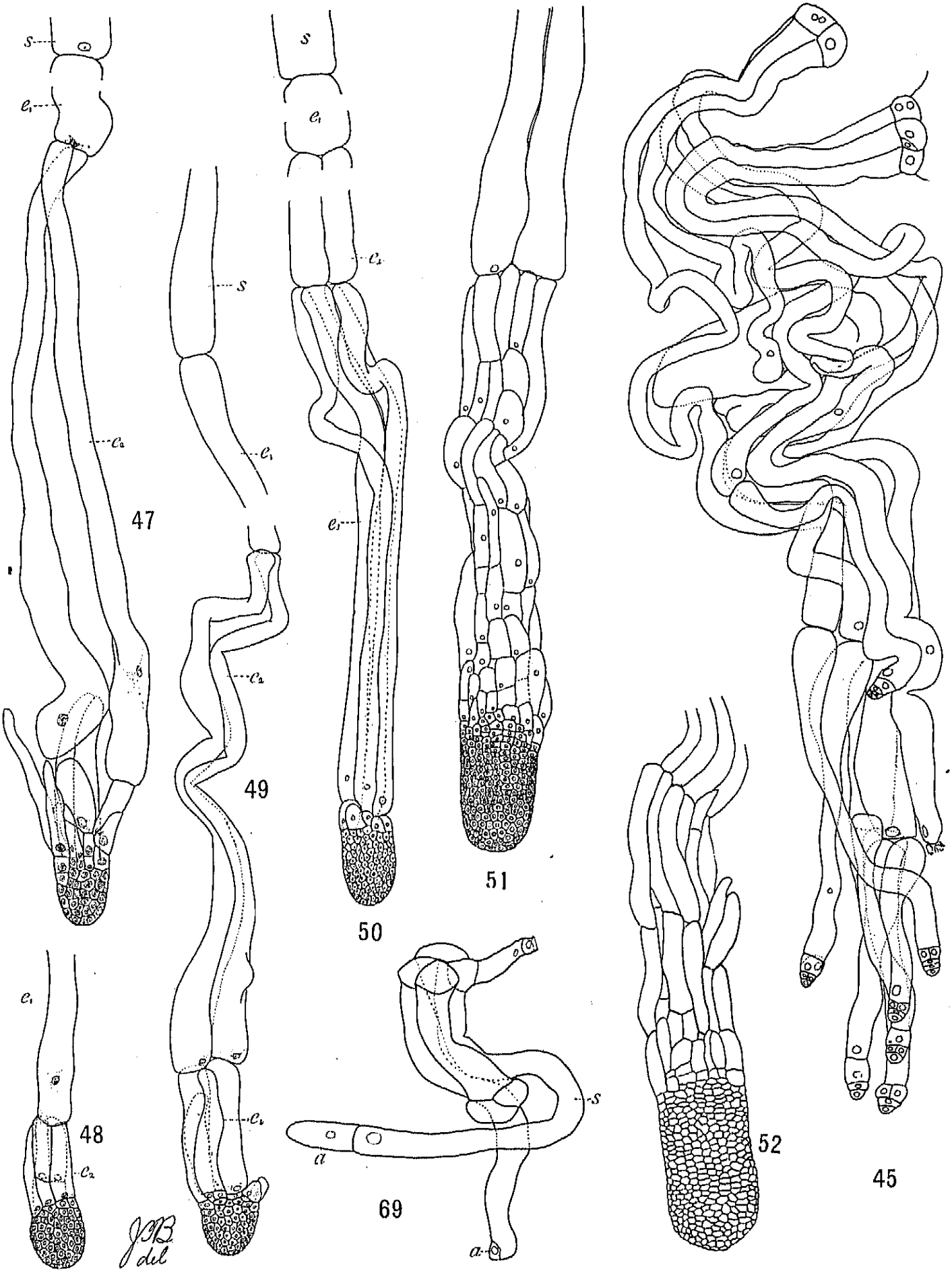
BUCHHOLZ on PINUS



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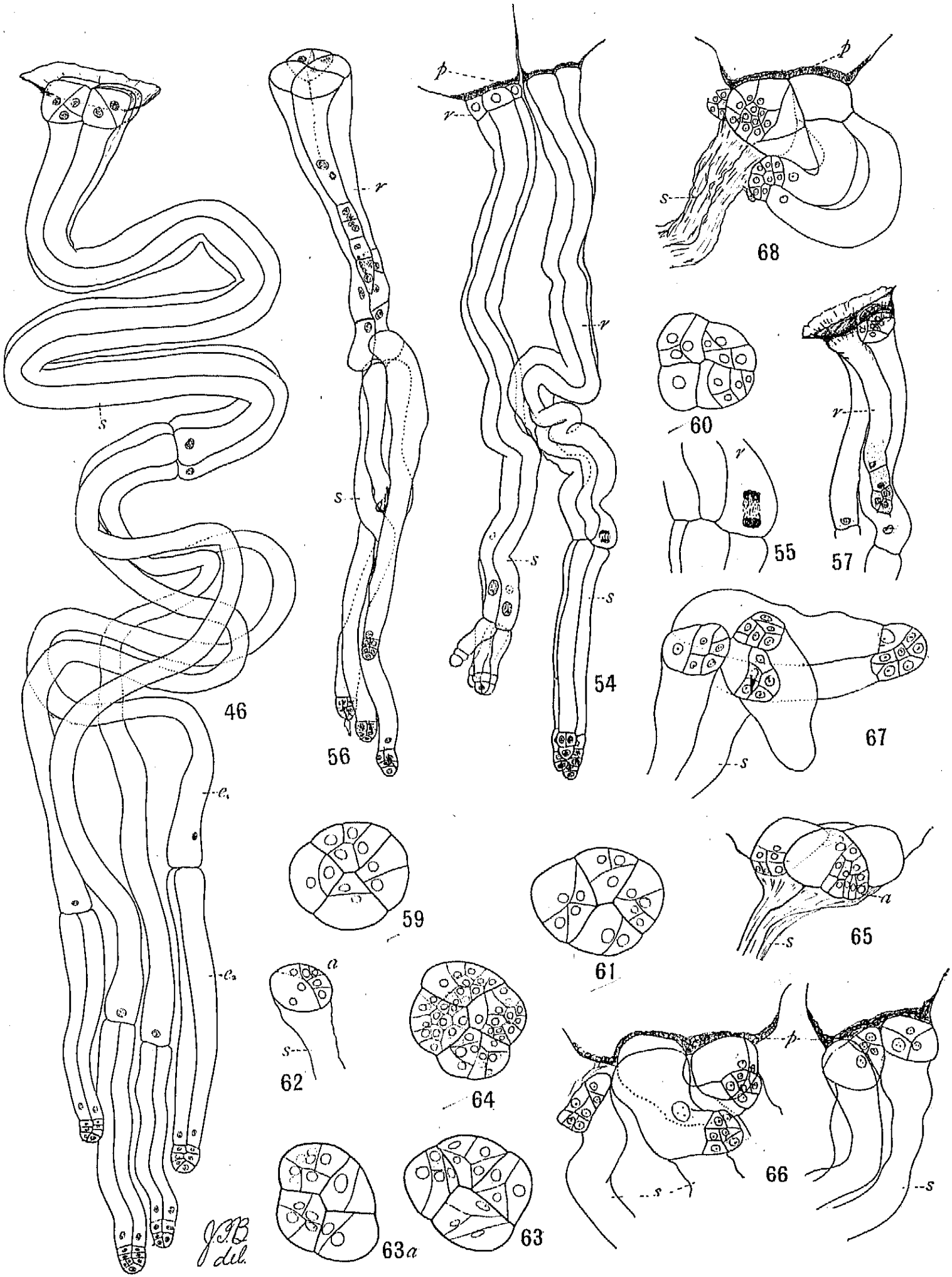

BUCHHOLZ on PINUS