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The initiation and early development of lateral vegetative, megasporangiate and microsporangiate buds is described. The subsequent development of the megasporangiate cone during its seventeen-month maturation cycle is described in detail. Initiation of lateral buds from cortical cells above the leaf axil occurs early in April and is similar in the three bud types. Apical zonation becomes apparent by mid-May but is less distinct in the microsporangiate primordium because of its smaller size. The microsporangiate bud remains distinctly smaller until microsporophyll initiation. Apical enlargement occurs in mid-July, at the end of the period of cataphyll initiation, and marks the onset of subsequent foliar initiation (leaves, bracts and microsporophylls). Like the cataphylls, these foliar organs are presaged by procambial differentiation from the peripheral zone outward to the base of the presumptive primordium. No distinct apical initials appear in any of the foliar organs. A group of subapical initials is active during early development but they soon differentiate and further elongation occurs by intercalary growth. Bract initiation ends early in October. The apices of the three bud types show a similar growth

periodicity and become greatly reduced in size and distinctness of zonation during foliar initiation. Scale initiation in the megasporangiate bud begins early in September and continues until the cones become dormant early in November. Scales are initiated from axillary cells at the base of the bracts. Apical zonation similar to other lateral shoots is present during early development but the organization changes to a type of submarginal growth. Growth of the megasporangiate cone is resumed in early March and the cone buds burst about one month later. The cone elongates rapidly by intercalary growth. Bracts enlarge rapidly but the shape of the bract established prior to dormancy is essentially maintained. The scale assumes a spoon-shaped appearance as a result of a form of marginal growth. Vascularization, and development of other tissues within the bract and scale, indicates that the bracts are homologous to leaves and basically similar in structure, but the scale is a modified fertile lateral shoot that is quite unlike other lateral shoots. Each bract is supplied by a single leaf trace and each scale by two separate branch traces. The seed wings differentiate from the outer adaxial layers of each scale. A large zone of macrosclereids differentiates in the basal abaxial portion of the scale. The cone reaches its maximum size early in July and maturation of tissues occurs in July and August, and is generally complete early in September. Cone opening is caused by drying and not by growth. Shrinkage of the large zone of macrosclereids in the abaxial basal portion of the scale is primarily responsible for cone opening.

ORIGIN AND DIFFERENTIATION OF THE SEED CONE OF *PSEUDOTSUGA MENZIESII* (MIRBEL) FRANCO

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ORIGIN AND DIFFERENTIATION OF THE SEED CONE OF PSEUDOTSUGA MENZIESII (MIRBEL) FRANCO

INTRODUCTION

The structure of cones has been the subject of careful study for over 200 years. Early studies were concerned primarily with the morphology and gross vascular anatomy of cones. This information, coupled with more recent paleobotanical data, provides a fairly detailed story of the evolution of the conifers and in particular, the cones. More recent studies have emphasized the ontogeny of the cones in order to better understand and interpret their structure at maturity.

The most recent work on cones deals primarily with the critical periods of initiation, pollination, fertilization and varied studies of the seed produced. These studies stem from the increased concern for reforestation and consequently an interest in all phases of seed production. Conifer seed has thus become an expensive commercial product. The field of forest genetics and the development of conifer seed orchards emphasize the need for a complete understanding of the development and structure of the cones. No previous studies have considered in detail the cone anatomy during the entire developmental cycle. The present study establishes the time and mode of lateral bud initiation in Douglas-fir and describes in detail the development of the megasporangiate cone throughout its seventeen-month developmental cycle. Although the times of the many developmental stages would vary from year to year and under different environmental conditions, the general time sequence described remains essentially the same.

LITERATURE REVIEW

The structure of the vegetative apex of gymnosperms has been the subject of rather intense investigation. A recent detailed review of the histogenesis of the embryo, root, shoot, leaf and axillary buds of many gymnosperms is given by Guttenberg (32). The concept of zonation is discussed by Foster (25) and reviewed by Clowes (13, p. 104-109). Lewis and Dowding (39) studied the anatomy of the vegetative buds of several conifers with special emphasis on the apex and crown region. Allen (5, 6) has described the embryogeny and development of the apical meristems of Douglas-fir. Changes within the vegetative apex of Douglas-fir throughout the year have been described by Sterling (49). Similar studies of growth periodicity have been made in other conifer species (18, p. 690-704; 26; 36, p. 505-516; 45).

Recently, the initiation of lateral buds has been studied in many conifers, with emphasis on the time of initiation. Mathews (42, p. 2-5), in a review of literature, reports that reproductive buds of many temperate conifers are initiated early in the growing season preceding the spring in which these cones appear. Species of Pinus have been studied most extensively and the results of these investigations are summarized by Gifford and Mirov (28). Additional studies of the species of Pinus have been made by Eggler (21) and Goo (3). The time of initiation in the pines varies from July to November. Allen (3), studying Douglas-fir, western hemlock and grand fir, noted that the different types of buds could be distinguished by August. Sterling (5) briefly described the initiation in the spring of lateral

vegetative buds of Douglas-fir. Comparisons of apical meristems of vegetative shoots and strobili in several conifers have been made by Gifford and Wetmore (29). They concluded that, in the limited number of conifers studied, there is no reserved portion of the apical meristem which is concerned primarily with the formation of reproductive shoots. The spatial relations of the zones may vary between the two categories of shoots but the basic organization present is invariable. Angiosperms have been studied more extensively concerning differences in vegetative and reproductive buds. Philipson (44) presents an extensive review of all aspects of this problem in dicots.

Coulter and Chamberlain (14, p. 245-300) and Haydon (33, p. 11-32) reported early studies of sporogenesis of several species of pines. Lawson (38) studied the gametophytes and embryos of Douglas-fir. The hypodermal origin of the microsporangium of Douglas-fir was studied by Allen (4, p. 548-550). The initiation of the microsporangiate bud was not studied but later stages of development were described. A recent study by Barner and Christiansen (9, p. 90-101) described the formation of pollen and the pollination mechanism, as well as the structure and function of the stigmatic tip of the ovule.

Few detailed studies have been made concerning the ontogeny of the cone, or the differentiation of tissues as the cone matures. Initiation and development of bracts and scales in several species of pine have been briefly described (52, p. 643-654; 28; 43; 18, p. 694-697). These descriptions were an incidental part of investigations designed to determine the time of cone initiation and gross changes in the cones during early development. Dupler (19, 20) described the

microsporangiate and megasporangiate structures of Taxus canadensis with respect to their development and vascular anatomy. Barner and Christiansen (9, p. 98-100) have briefly described the enlargement of bracts and scales of Douglas-fir but only with respect to the time and mechanism of pollination. Kemp (37) described, in detail, the general development and histogenesis in the megasporangiate shoot of Torreya californica prior to pollination. The origin of the bud, the apex and the histological resemblance to the vegetative shoot is stressed. No studies have been made which follow cone development of any species from initiation to maturity. Development of the megasporangiate cone of Douglas-fir from the time of pollination to maturity has been studied in relation to moisture content, stored food and respiration (12). Vascularization and sclereid formation have been described for the vegetative shoot of Douglas-fir (50, 51). Vascular anatomy of cones has been studied, but primarily in order to better understand cone morphology (1). The structure of the bract has been neglected except for descriptions of the arrangement of vascular tissue and its relation to the scale (1). Comparative studies of bracts and leaves of conifer species have not been made. Although the leaves of gymnosperms have received attention in numerous papers, the emphasis has been placed upon anatomical or taxonomic characteristics rather than histogenetic features. Cross (15, 16, p. 293-300), however, has described the origin and development of leaves in two conifers. Douglas-fir leaves have been studied in reference to sclereid formation (7), nature of the cuticle (11) and the structure and function of the endodermis (48, p. 283).

The early investigators during the 19th century, who studied and described the growth and anatomy of cones, were mainly concerned with the morphology and evolution of these structures. The literature on the interpretation of the microsporophylls and cone scales is very extensive and has been reviewed by Coulter and Chamberlain (14, p. 238-244) and others (17, p. 82-91; 53; 23). The most recent and widely accepted interpretation of the morphology and evolution of the megasporangiate cone is that by Florin (23). He concluded that the megasporangiate cone is a compound strobilus made up of a main axis with secondary fertile short shoots (scales) in the axils of bracts.

Willis (54, p. 995-1000) has shown that cone opening in Douglas-fir is a result of drying involving a complex interaction between temperature, humidity and air circulation. The tissues responsible for cone opening have not been described.

METHODS AND MATERIALS

The initiation of lateral vegetative, microsporangiate and megasporangiate buds of Douglas-fir occurs in the elongating vegetative shoot during spring (50). A study of the time and mode of initiation of the various kinds of buds is difficult because the site of initiation of a given bud type cannot be predicted with certainty. In addition, the buds cannot be easily distinguished until early August. A study was made in the fall of 1961 to determine the position on the twigs where each of the three types of buds was found most frequently. Information from this study, plus the careful selection of trees and sampling from specific areas of each tree, minimized the chances of confusing the different types of bud primordia.

Five trees were used in the study. Two trees approximately 15 feet high were selected for the study of initiation of vegetative lateral buds. These trees were growing in the open about five miles north of Corvallis, Oregon, at an elevation of approximately 300 feet. Two trees which had been observed to produce abundant microsporangiate buds on the lowest branches were chosen for the collection of microsproangiate bud primordia. Both trees were approximately 75 feet high and growing in the open in Corvallis. One tree was chosen which had produced very abundant megasporangiate cones in the past. This tree was about 100 feet high and growing on a steep south slope at an elevation of about 1000 feet in MacDonald Forest.

Collections were started in late March and made weekly during early stages of shoot elongation and biweekly during subsequent

stages. Elongating vegetative shoots, in which it was presumed that microsporangiate primordia were developing, were collected from the lower branches and the primordia studied were only those initiated near the base of the shoot. Elongating shoots in which lateral vegetative primordia were developing were collected from the upper half of the small trees in order to avoid confusion with possible microsporangiate primordia. Primordia studied were only those initiated in the terminal one-third of the shoot. Elongating shoots in which megasporangiate primordia were developing were collected from branches in the upper one-third of the large tree. Shoots were collected only from large lateral twigs which had produced megasporangiate cones in previous years, as evidenced by the many scars. Primordia studied were only those initiated in the terminal one-third of the shoot. Samples collected in an identical manner from this tree after the primordia could be readily distinguished, showed that 80 percent of these primordia were megasporangiate. The remaining 20 percent were about equally divided between lateral vegetative and microsporangiate primordia.

The elongating vegetative buds selected in this manner were marked with a notch at the base to identify upper and lower sides and the bud scales were removed. After the vegetative buds had burst, it was possible to remove the leaves and observe the developing lateral buds with a dissecting microscope. The section of the elongating shoot possessing the developing lateral buds was then dissected out. When the developing lateral buds had enlarged and formed bud scales, only the bud was collected and the bud scales removed. Collections

of all three types were continued until the buds became dormant in November. Subsequent collections during dormancy were made occasionally for the megasporangiate bud.

The buds collected were soaked for 15 minutes in a wetting agent consisting of a 0.013 percent solution of finely ground Ivory Flakes in distilled water to insure that the killing solution would penetrate between the bracts and scales of the megasporangiate buds. All buds were killed in Navashin's solution (34, p. 44) and left for one day.

During the second season of growth, megasporangiate cones were collected twice a week during early stages of elongation. Sections 1 cm long were dissected from the center of the small elongating cones and the bracts were trimmed off. Single scales and bracts were dissected from the larger cones. The lateral margins were removed from the scales which were to be sectioned longitudinally. The entire proximal half of the scale was retained for those to be sectioned transversely. Small central sections of the cone axis with all scales and bracts removed were collected during the latter part of cone elongation and maturation. Cones, scales and bracts were killed and fixed in either Navashin's solution or a formalin-acetic acid-alcohol mixture (FAA) prepared according to Sass (47, p. 18).

Air was evacuated from all samples while they were in the killing solution. Material killed in Navashin's solution was washed in running water for two hours and then dehydrated. Material killed in FAA was washed in 50 percent alcohol. All material was then passed through the tertiary butyl alcohol-paraffin oil series of Johansen

(34, p. 130-132) and embedded in 56°-58°C Tissuemat. The embedded samples were soaked in a solution of glycerin and a detergent (2) at approximately 37°C. Buds were soaked for one or two days, small cones for one week, and mature scales and cone axes for one to two months. Soaked material was sectioned immediately, or stored in water in a refrigerator until needed.

Buds were sectioned at 10μ and small cones at 15μ on the rotary microtome. Single scales, prior to maturation of all of the sclereids, were sectioned at 20μ on the rotary microtome. Mature scales were sectioned at 20 - 35μ on a sliding microtome and cellulose tape was used, as described by Bonga (10), to help prevent the hard and soft tissues from separating and tearing. Buds were stained with hematoxylin and fast green. Young cones and scales were stained progressively with safranin and hematoxylin. Where meristematic tissue was to be studied, an additional stain of fast green was often necessary.

Young cones were cleared by a modification of a technique described by Arnott (8). The cones were cleared in five percent sodium hydroxide at 37°C for about two weeks, or until they had only a brownish tinge, washed briefly in water and cleared for several days at 37°C in chloral hydrate at a concentration of 50 grams chloral hydrate to 30 cc of water. They were then washed overnight in water and stored in 95 percent alcohol. Dissected scales were treated similarly but required one month in five percent sodium hydroxide and about one week in chloral hydrate. Scales never became as clear as the young cones because of the slightly lignified epidermis and

hypodermal layers. Fully elongated whole cones were soaked in five percent sodium hydroxide for about two months. All the tissues except the vascular tissue were carefully picked loose and washed away by a stream of water under high pressure. The vascular tissue was then stained in a dilute solution of safranin in 70 percent alcohol.

OBSERVATIONS

Zonation in the Apical Meristem

Sterling (49) and Allen (6) have described the zonal pattern of the fully differentiated vegetative shoot apex of Douglas-fir. It is characterized by a small group of apical initial cells at the summit of the apex which divide anticlinally, giving rise to the protoderm, and periclinally, giving rise to a central mother cell zone below (fig. 19). Central mother cells and their nuclei enlarge, become lighter staining, and the cell walls become slightly thickened. Cell division is infrequent in the interior of this zone but is active on its periphery. Products of divisions on the flanks of the central mother cell zone form a mantle-like peripheral zone of small, densely staining and less differentiated cells. Some authors (22, p. 100-102) include the protoderm in the peripheral zone. The peripheral zone gives rise to cortex, procambium, and foliar and bud primordia. Divisions at the base of the central mother cell zone form a central rib meristem. Pith mother cells arise basally from the rib meristem. The rib meristem and the uppermost cells of the peripheral zone are sometimes grouped into one zone called the eumeristem (22, p. 100-102; 49). Since both rib meristem and peripheral zone are derived from the central mother cells, there is necessarily a gradation in cell size at this level. This gradation becomes more abrupt as the apex approaches dormancy, and the distinction between rib meristem and peripheral zone is more certain in apices where there is little cell division and differentation occurs

nearer the summit of the apex.

Ontogeny of Axillary Buds

Three types of axillary buds develop in Douglas-fir: (1) vegetative, (2) megasporangiate and (3) microsporangiate. Minor variation exists in the site of initiation of the three types along the axis of the expanding vegetative bud. Axillary vegetative and megasporangiate buds are usually restricted to the terminal half of the developing lateral shoot while microsporangiate buds are found along the entire length of the shoot but predominantly in the proximal half. Also, most buds of all types are located along the sides of the lateral shoot, a few on the lower surface and still fewer along the upper surface.

The time of initiation does not vary among the three types, and begins shortly after the onset of elongation of the buds in which they develop. This, of course, varies with the environment but in the trees from which the collections were made, microscopically visible axillary bud initiation began during the second week of April. This is about one month before the vegetative buds burst, and coincides with bursting of the current season's megasporangiate buds. Earliest stages of axillary bud primordia are most easily detected in transections of the expanding shoot while subsequent stages are best followed in median longisections.

The three types of axillary buds show the same general sequence in the early stages of development; i.e., initiation of an axillary meristem, apical enlargement, and the differentiation of an apical

zonation. Megasporangiate and vegetative buds are not distinguishable from each other until nearly all cataphylls are initiated. Microsporangiate buds are similar in their initial stages but fewer cells are involved so the developing bud apex is smaller. The general pattern of development, however, is essentially the same in all buds, and a description of development up to the differentiation of a zoned apex will suffice for all three types of buds. Once zonation is established, variations become distinct enough to warrant a separate discussion of each bud type.

Axillary buds of Douglas-fir are not truly axillary in origin but arise from cortical and epidermal cells just above the axil of the leaf (fig. 4). The epidermis of an axillary area prior to bud initiation consists of uniformly rectangularly shaped cells which contain ergastic materials. Cortical cells in the axillary areas appear as a wedge-shaped zone of meristematic cells with the vertex of the wedge extending toward the vascular cylinder of the shoot. Cortical cells adjacent to the axillary area are more vacuolate and tend to be arranged in rows several cells deep. The adaxial surface of the leaf is pressed against the epidermis of the cortex in a deep concavity formed by emerging leaves on either side (fig. 1).

The epidermal cells at the site of bud initiation become larger, less vacuolate, lose the ergastic materials, develop much larger nuclei, and some of them differentiate as apical initials of the developing bud primordium. The wedge-shaped zone in the cortex becomes more pronounced than a comparable area without a bud as the cells within tend to become slightly larger, stain darker and show

no definite orientation. This less oriented arrangement of cells is due to frequent cell divisions in all planes, as well as to cell enlargement. This activity soon causes a slight swelling in the concavity and forces the subtending leaf outward (fig. 2). Apical initials divide, at first primarily anticlinally, and both cells and nuclei enlarge to twice the size of other cells in the primordium, and soon form a group of ten to fifteen cells at the apex. The apical initials then divide periclinally and give rise to cells beneath which, at this time, do not differ from other subepidermal cells of the axillary meristem but later assume the characteristics of central mother cells.

In the epidermis at either side of the primordium, as seen in transection, occur a few enlarged cells with large nuclei similar to the apical initials. These divide periclinally while the subepidermal cells divide in all planes, but predominantly periclinally and give rise to the first two cataphylls (fig. 3). These cataphylls elongate rapidly and divisions become restricted to radially elongated subepidermal cells, while the outer two or three cell layers accumulate ergastic materials and become highly vacuolated. The cataphylls become flattened on the end by contact with the subtending leaf but due to pronounced abaxial growth by cell division, elongation and vacuolation, they extend between the leaf and apex of the bud, over-arching the latter.

When the bud primordium is only about three cells high, as viewed in longisection, the third and then the fourth cataphylls arise at its upper and lower limits, respectively. The epidermal cells at the upper side of the developing apex enlarge and produce a slight swelling at

this point. Subepidermal cells divide anticlinally and periclinally and give rise to the third cataphyll (fig. 4). The enlarged epidermal cells and subepidermal cells of the cataphyll become vacuolate and rapidly accumulate ergastic materials but are still capable of division.

Shortly following the initiation of the third cataphyll, the fourth cataphyll primordium arises similarly at the lower side of the bud. The delay between the initiation of third and fourth cataphylls is the first indication of a spiral arrangement of cataphylls, for the first two were seemingly initiated simultaneously. Procambial strands differentiate basipetally from the bases of these first cataphylls and form connections with the vascular cylinder of the shoot. Procambial differentiation occurs by elongation of cells at the base of the cataphyll primordium, and longitudinal divisions within these cells produce a strand many cells wide. As in later formed cataphylls, the procambium does not extend into the cataphyll but only to the base.

The apex, which is that portion of the bud primordium extending above the cataphylls, is, at the time of initiation of the first four cataphylls, a group of meristematic cells apparently dividing in all planes and lacking any pattern of zonation (fig. 4).

Zonation of the apex is achieved by a number of simultaneously occurring processes. Central cells of the apex enlarge and divide primarily transversely. As a result, the apex rapidly assumes a hemispherical appearance, the cell divisions become oriented and zonation becomes evident (fig. 5). At first zonation is very indefinite but becomes more distinct as the apex enlarges. Transverse divisions become most frequent in the central portion of the apex at the level

of the cataphyll primordia. These divisions give rise to vertical rows of cells forming the rib meristem zone. This zone is at first only a few cells high but rapidly becomes higher by transverse divisions of cells above and below (fig. 6). At the same time, along the sides of the apex just below the protoderm, cells divide primarily longitudinally and form a cone of elongated cells enclosing the rib meristem zone and joining with the differentiating procambium at the base. Near the summit of the apex, this cone-shaped zone, termed the peripheral zone, is continuous with a group of undifferentiated central mother cells from which it is derived. At its uppermost point, the peripheral zone is only one or two cells wide but it broadens basipetally by longitudinal divisions, and becomes many cells wide at the site of cataphyll initiation (fig. 6). The peripheral zone, although arising from the central mother cells, differentiates acropetally from the area of cataphyll initiation and differentiates the procambium for future cataphylls and leaves, and basipetally to form the connection with the vascular cylinder of the shoot. The differentiation of distinct rib meristem and peripheral zones occurs before differentiation of central mother cells. Actually both of these zones arise from this indistinct subterminal group of cells by frequent divisions; that is, many of the lower cells of this zone divide transversely and add to the rib meristem, while cells at the flanks divide transversely to form the peripheral zone. These subapical cells, forming the central mother cell zone, assume the characteristics of central mother cells only after further apical growth.

At this stage, the three apices show essentially the same pattern

of cell arrangement but due to the smaller size of the microsporangiate apex its zonation is much less distinct. Compare figures 7-9 which are essentially the same age (approximately 35 days after visible initiation) and have an equal number of cataphylls differentiated.

Vegetative Lateral Bud

When zonation first becomes recognizable, the apex is about 120μ tall and 200μ wide and has the appearance of a rather broad cone with a dome-like summit (fig. 9). This general shape is retained throughout cataphyll initiation by the maintainance of a balance between elongation and broadening, while zonation within the apex becomes more distinct. This general enlargement is due primarily to rib meristem activity which increases the number of vertical rows of cells by oblique and occasional longitudinal division of rib meristem and pith mother cells, and enlargement of the latter. Axis elongation occurs by transverse divisions in the rib meristem and pith mother cells as well as cell elongation. Differentiation of pith mother cells is not rapid at first; therefore, the rib meristem remains many cells deep. Activity of the rib meristem and pith mother cells continues throughout cataphyll initiation. During this time, the amount of ergastic material in these cells increases compared to that present in the bud before zonation was established. This material is found most abundantly in cells in the future crown region and in the outer rows of the rib meristem and pith mother cells.

The central mother cell region becomes delimited more gradually than other zones. Cells of the region do not assume their

characteristic form until nearly all of the cataphylls are formed and the apex has enlarged to about 160μ tall and 230μ wide. At this time they form a small group of conspicuously enlarged cells with less dense cytoplasm, large nuclei and slightly thickened cell walls (fig. 11). These remain distinct throughout the remaining period of cataphyll and leaf initiation.

The peripheral zone broadens as the apex elongates and becomes three to four cells wide at its base during active cataphyll formation and increases to twice this width by beginning of leaf initiation. This increase occurs basipetally by longitudinal or oblique divisions of one or two rows of cells derived from the central mother cell zone. Procambium differentiates acropetally in the peripheral zone from the level of the uppermost cataphyll or leaf in older apices. Differentiation of procambium, preceding the initiation of the foliar organ it will supply, has been shown previously in the terminal shoot of Douglas-fir (50).

The initiation of a cataphyll primordium is first evidenced by differentiation of a procambial strand in the peripheral zone to supply the presumptive primordium. This occurs by oblique divisions of peripheral cells, followed by tangential elongation, which extends the procambial strand to the base of radially elongated protodermal cells. These protoderm cells divide periclinally, and the derivatives divide in all planes to produce a slight swelling at the base of the apex immediately above a lower, more differentiated cataphyll. When the primordium consists of only a few cells, vertical growth of its apex is evidenced by an increase in abaxial cell division and elongation.

Transverse division and elongation of hypodermal cells, similar to that in the shoot apex, produce vertical rows of cells within the developing cataphyll and give it a pointed aspect. Longitudinal and oblique divisions in these vertical rows increase the number of rows and broaden the base of the cataphyll. Procambium differentiates acropetally into the base of the cataphyll but extends no further, even in the fully differentiated cataphyll.

By the time the cataphyll has attained a length of 70μ the abaxial epidermal cells have accumulated ergastic materials but still divide anticlinally and elongate. A small group of apical initials lacking abundant ergastic materials is present at this stage but later becomes difficult to distinguish because of marked marginal growth of the cataphyll. Subsequent cataphyll elongation results from intercalary growth by transverse division, vacuolation and elongation of cells throughout the subapical ground meristem. Marginal and intercalary growth results in the production of very long and broad bud scales, which are about ten cells thick at the base and become thinner acropetally. Outer bud scales develop abundant ergastic materials, especially in the greatly elongated abaxial epidermal cells. Subepidermal cells enlarge and large intercellular spaces form.

In the dormant vegetative bud there are about ten layers of bud scales at a given level, or a total of approximately twenty bud scales enclosing the bud. As a result of marked marginal growth, the largest bud scales enclose about one-half the circumference of the bud, with the smaller bud scales enclosing proportionately less. Outermost, or first formed, bud scales are very short, broad and thin, while the

median layers of bud scales are longer, attaining a length of about 7 mm, are thicker and more coriaceous in texture. The innermost, or last formed, bud scales are scarious in texture and may only slightly exceed the length of the bud which they enclose.

Following initiation of the first few cataphylls, a region of growth activity arises in the cortex of the bud axis between the base of the apex and the juncture with the shoot on which the bud is borne. Cortical cells along this axis divide periclinally and the derivatives elongate tangentially and increase the diameter of the short axis. During subsequent cataphyll initiation, this "receptacular meristem" (49) differentiates acropetally as new cataphylls are initiated so that the base of the axis is always broader than that just below the apex. In addition to this broadening of the cortex, transverse expansion of the pith occurs throughout the bud axis below the apex. Both of these processes tend to raise the cataphylls until they appear borne on a flattened receptacular structure which gives the bud a very broad base by which it attaches to the shoot. As in the terminal bud described by Sterling (49), abundant sclereids differentiate in this region and large intercellular spaces form.

Leaf primordia are initiated acropetally along the flanks of the conical apex to within about 100μ of the summit. Details in leaf initiation are the same as those for cataphyll initiation except that the base of the cataphyll primordium is much broader, enclosing more of the apex. Once elongation of the leaf primordium begins, differences become more apparent. Leaf primordia elongate perpendicular to the bud axis rather than assuming an early vertical growth.

Elongation is by transverse division and elongation of hypodermal cells to form long rows of cells. Longitudinal and oblique divisions of the hypodermal cells are more frequent in a leaf primordium than in a cataphyll primordium. Also, there is no marginal growth in the leaf primordium. The leaf, therefore, assumes a rounded rather than flattened aspect as seen in transection. Procambium differentiates acropetally in the elongating leaf, forming a core of elongated cells which extends nearly to the leaf tip. The apex of the differentiating leaf never assumes the pointed aspect present in the cataphyll.

Leaf primordia in the lower two-thirds of the dormant bud are essentially the same size while those near the summit decrease in size acropetally. Most leaves of the dormant bud, therefore, are about 400μ long and 150μ wide. There is no vascular differentiation in the dormant leaf. A central core of procambium, consisting of greatly elongated cells, extends to within 70μ of the apex (fig. 12). A group of isodiametric, meristematic, subapical initials extends from the tip of the procambium to the epidermis. A layer one cell thick, consisting of shorter, meristematic and more vacuolate cells, surrounds the procambium and joins with the subapical initials near the apex. Surrounding this layer is another layer three or four cells deep in which the cells are less meristematic, more vacuolate and contain abundant ergastic materials. This layer tapers to a thickness of one or two cells where it joins the subapical initials. The epidermis encloses this layer and consists of elongated cells which contain less ergastic materials than the subjacent layer (fig. 12).

Abaxial elongation exceeds adaxial elongation in the terminal

portion of the leaf, which causes a slight upturning of the leaf apex. In transection, the leaf is round at the base and becomes elliptical in distal portions as a result of marginal growth.

Changes occur in size and shape of the bud and in zonation of the apex throughout bud development. During the latter part of cataphyll initiation, the vegetative lateral bud (fig. 11) takes on the appearance described by Sterling (49) for the terminal bud (49, fig. 5, 14). Until this time the lateral apex shows less distinct zonation than the terminal apex (fig. 10). The lateral bud is generally smaller throughout development than the terminal bud at corresponding stages, and these stages may occur up to two weeks later than in the terminal bud. This is probably due to the fact that the lateral bud, though arising at the time of terminal shoot expansion, must pass through the additional stages of apical enlargement and the formation of zones which already exist in the terminal apex.

Changes in the apex during leaf initiation were not followed closely in the lateral buds but appear to duplicate stages of development in the terminal bud as described by Sterling (49). At the onset of leaf initiation, the apex is approximately 200μ high and 320μ wide, or about double the size it was when zonation became apparent. Elongation of the apex continues with leaves being initiated in close order up the apical cone. A characteristic mamillary apex is formed. This arises by abundant transverse and longitudinal divisions in the rib meristem which increases greatly in width as well as depth. Shoulders of this tissue are thus formed below the apex and give it a mamillary aspect. Pith mother cells extend closer to the summit and the central

mother cell zone diminishes somewhat while its cells decrease in size.

During the latter part of leaf initiation, the walls of cells just below the pith mother cells of the new bud begin to thicken and differentiate the crown region characteristic of the dormant conifer bud. In the dormant vegetative bud, this zone is about 1000μ wide and 200μ to 300μ high, completely spanning the pith region. Walls of the adjacent cortical cells also thicken, extending the crown region outward from the vascular cylinder to the epidermis of the bud axis just above the last formed cataphylls. This portion of the crown region is only half as high as that portion present in the pith. A similar thickening of cell walls is not present in the vascular tissue at the level of the crown region.

Microsporangiate Bud

When the apices of both lateral vegetative and megasporangiate bud show distinct zonation, comparable stages of the microsporangiate apex show only a very slight indication of zonation (fig. 7). This is primarily because of the smaller size of the microsporangiate apex, which at this time is only half the size of the other two types, and consists of only about half as many cells; yet, the same general sequence of enlargement and early cataphyll formation has occurred. Darkly staining ergastic material is also more prominant in the pith mother cells of the microsporangiate apex.

During subsequent cataphyll formation, the apex enlarges but more gradually than in the other types. The greatest change at first is due to lateral enlargement of the pith mother cells which cause a

broadening of the base of the apex (fig. 13). During this expansion, rib meristem cells become vacuolate as do the peripheral cells. The only cells in which dense cytoplasm persists are those of the protoderm and a small group of about ten central mother cells.

Rapid transverse divisions occur in the rib meristem toward the end of cataphyll initiation causing the apex to elongate and forming the more conical structure (fig. 14), characteristic of the other apices at a much earlier stage (fig. 10). Oblique and occasional longitudinal divisions also occur, which increases the number of vertical rows of cells. The rib meristem and pith mother cells continue to accumulate ergastic material throughout cataphyll initiation. Just prior to microsporophyll initiation, in some apices, nearly all cells of the rib meristem and pith mother cell region are filled with darkly staining ergastic material. The amount of ergastic material is, therefore, much greater in the microsporangiate apices than in the other types. Below the future crown region, in the area of transverse expansion, much less ergastic material accumulates. During microsporophyll initiation much of the ergastic material in the bud disappears.

Central mother cells become more easily distinguished as they gradually increase in number. They enlarge slightly over other apical cells, stain about the same as peripheral cells and develop slightly thickened walls (fig. 14). The central mother cell zone, however, never attains the size and distinctness seen in the other apices.

The peripheral zone broadens basipetally while procambium differentiates acropetally to the base of presumptive cataphylls and

microsporophylls. A "receptacular meristem" develops but is not as active as in other bud types. Transverse expansion of the pith in the bud axis below the crown region is also less extensive. The cataphylls, therefore, are borne on a smaller, less flattened receptacular structure and the bud is attached to the shoot by a much smaller area of tissue (fig. 15). For this reason the microsporangiate buds are attached to the shoot much less firmly than either vegetative or megasporangiate buds.

The apex at the onset of microsporophyll initiation (about three and one-half months after initiation of the bud), is about 200μ tall and 200μ wide at the base. This is slightly shorter and more narrow than other apices at a corresponding stage. The entire bud enlarges during subsequent microsporophyll initiation. The most conspicuous enlargement is the broadening of the bud as microsporophylls differentiate along the cone-like apex. A portion of this broadening results from increased cell size and number of vertical rows of pith mother cells which cause a general thickening of this zone from the future crown region to the base of the central mother cells (fig. 16). Additional broadening is due to an increase in width of the peripheral zone which becomes more pronounced as microsporophylls differentiate. The increase in width of the bud and differentiation of microsporophylls to within 70μ of the summit causes the apex to assume the aspect of a flattened dome (fig. 17). Rapid differentiation of pith mother cells restricts the rib meristem to a height of only one or two cells. As a result of these processes, the central mother cell zone also becomes very flattened and wide, and is restricted to only a few cells (fig. 17).

The apex retains this shape throughout the subsequent microsporophyll initiation to dormancy.

The initiation of microsporophylls is the same as that for the other foliar organs (cataphylls, leaves and bracts) and is described in some detail by Allen (4) in his discussion of the origin of the microsporangium. The microsporophyll primordium arises by periclinal divisions of radially elongated protodermal cells at the base of the apex. Continued periclinal and anticlinal divisions of the surface cells and their derivatives produce a symmetrical, hemispherical swelling (fig. 17). Enlargement continues by a form of apical growth until the microsporophyll reaches a length of about 75μ. Cells of the adaxial surface then begin to vacuolate, elongate and divide infrequently, while abaxial cells divide actively in all directions. Divisions in the abaxial surface layers soon become primarily periclinal and result in an abaxial swelling. The microsporophyll thus assumes the aspect of a blunt rather than a pointed appendage, and stands nearly perpendicular to the cone axis with only a slight upturning of the tip (fig. 18).

The dormant bud has microsporophylls differentiated all along the flanks to within 60μ of the summit. The apex becomes very small (60μ tall and 200μ wide), retains the flattened dome aspect, but zonation within becomes obscure. Cells in the apex become vacuolated, develop slightly thickened walls and many accumulate ergastic substances. The crown region in the dormant bud is similar in size and appearance to that in the vegetative lateral bud.

Megasporangiate Bud

Few differences exist between the megasporangiate and the vegetative lateral buds from the time of bud initiation to initiation of bracts and leaves. At the time zonation becomes established, the megasporangiate apex may be slightly larger than the vegetative lateral apex (fig. 8). This difference is maintained throughout the period of cataphyll initiation and apical enlargement.

As in the vegetative lateral apex, zonation becomes more marked as the apex enlarges, and is most distinct at the onset of bract initiation (fig. 19). The apices are similar in the extent of comparable zones throughout the enlargement period. Although both are initiated at about the same time, development of the megasporangiate bud lags behind that of the vegetative lateral bud. The megasporangiate apex begins to initiate bracts about two weeks after vegetative lateral apices on the same shoot have begun to initiate leaves. This time lag may be related to the fact that the megasporangiate bud passes through a longer period of enlargement.

Cataphyll initiation and differentiation are essentially the same as for the vegetative bud except that slightly fewer cataphylls develop in the megasporangiate bud. In the latter, nine layers of cataphylls are most common with the total number of cataphylls being approximately 16. Cataphylls are borne on a receptacular structure which develops in the same manner but to a lesser extent than in the vegetative bud. The dormant megasporangiate bud, therefore, rests on a more narrow receptacular structure and is attached to the shoot less firmly than

the vegetative buds.

Near the end of cataphyll initiation, the bud reaches a length of about 600μ and the apex, that portion rising above the cataphylls, attains a height of approximately 250μ and a basal width of 300μ (fig. 19). Apical enlargement continues but the apex broadens more rapidly than it elongates. This occurs by cell enlargement and increased number of vertical rows in the rib meristem and pith mother cells. The peripheral zone almost doubles in width throughout its length due to longitudinal cell divisions. This broadening is similar to that occurring in the vegetative buds prior to leaf initiation except that the megasporangiate apex does not assume the mamillary appearance of the vegetative buds. Therefore, at the onset of bract initiation, the apex is a very broad cone about 300μ high and 350μ wide (fig. 20).

Bract initiation begins about mid-July, or three and one-half months after initiation of the megasporangiate bud. The initiation of a bract is similar to that of a cataphyll, leaf or microsporangium; i.e., procambium differentiates acropetally in the peripheral zone to the base of the presumptive bract primordium, enlarged protodermal cells divide periclinally and the derivatives divide in all planes; this produces a swelling approximately 400μ broad at the base of the apex. Four to six adjacent protodermal cells take part in the initiation. Subsequent anticlinal and periclinal divisions of subepidermal cells produce a very broad, flattened primordium (fig. 23). This shape is maintained until the primordium is about 200μ high. Cells in the abaxial portion of the primordium then divide and elongate more rapidly than those in the adaxial portion and result in upward growth of the

tip of the bract (fig. 24). This becomes more pronounced by increased division and elongation of the central subepidermal cells, which gives the primordium a pointed aspect (fig. 25). Ergastic materials accumulate first in the abaxial epidermal cells and later in the adaxial epidermis.

No distinct apical initials differentiate, but a group of four to eight subapical initials become evident when the bract begins vertical growth (fig. 24, 25). These cells become larger, more vacuolate and divide less frequently than cells deep within the bract. The subapical initials give rise at the flanks of the bract to a peripheral zone (fig. 26, 28). Cells of this region differentiate rapidly by elongation, vacuolation and accumulation of ergastic materials. This zone is continuous beneath the epidermis from the subapical initials to the base of the bract. Just below the subapical initials, this zone is one or two cells wide but it broadens basipetally by oblique and longitudinal divisions. The adaxial peripheral zone becomes two to three cells thick while the abaxial peripheral zone becomes four to five cells thick. Cells throughout the length of the peripheral zone rapidly accumulate ergastic materials, first in the epidermis and then in subjacent layers (fig. 26). The appearance of the ergastic materials gradually changes as the cells which contain them continue to elongate and become more vacuolate. This change occurs first in the abaxial epidermis and then in the peripheral zone just below the subapical initials, and finally in all the abaxial and adaxial peripheral cells. Within a given cell, the ergastic materials first appear as yellow droplets and later darkly staining granular material which finally

completely fills the cell. When the bract has elongated to 400μ, most of the subapical initials have passed through this sequence of differentiation and only a few remain meristematic.

The central subapical initials give rise basally to a central column of cells which elongate but retain darkly staining cytoplasm and show little sign of vacuolation. Cells within this column divide transversely, which increases the length of the column, and longitudinally and obliquely, which increases the diameter of the column basipetally. This central column is made more conspicuous by the accumulation of ergastic materials in the outer cell layers of the bract (fig. 26).

The subapical initials cease division and differentiate early in the development of the bract. Subsequent elongation of the bract, therefore, is a result of intercalary cell division and elongation. Intercalary growth occurs at first throughout the length of the bract, but later predominates in the basal half. This pattern of development closely follows that of the leaf, except that the bract exhibits very early vertical growth and a more pointed apex.

From the time of its initiation until the bract reaches a height of 250μ, the primordium is generally elliptical in transection although it may be slightly flattened on the adaxial surface (fig. 27). The first indication of marginal growth appears at the base of the primordium just above the axillary region (fig. 28). One to three marginal epidermal cells and about ten submarginal cells on either side, as seen in transection, show a slight enlargement and lack ergastic materials. Certain of these cells become marginal and submarginal initials which

are responsible for growth of the laminar portions of the bract. These initials differentiate acropetally from the base of the primordium until they appear as rows of cells on either side of the bract primordium.

Marginal initials are never very distinct in the bracts of Douglas-fir, and the classical interpretation of a single row of marginal initials is difficult to apply. The marginal initials, at the onset of marginal growth, consist of two or three rows of protodermal cells. These are slightly larger than other protodermal cells and lack ergastic materials (fig. 28). As the lamina broadens, the margins become less rounded and the marginal initials are reduced to one or sometimes two rows of cells (fig. 30). The adjacent cells, previously functioning as marginal initials, become smaller, accumulate ergastic materials and become indistinguishable from other epidermal cells. Marginal initials divide periclinally, giving rise to submarginal cells, and anticlinally, contributing cells to the epidermal layer. Anticlinal divisions are not restricted to marginal initials but occur frequently in cells throughout the epidermis.

A single row of submarginal initials does not occur in bract development of Douglas-fir. At the onset of marginal growth, the submarginal meristem appears in transection as a group of about ten subepidermal cells at the margins of the elliptical primordium. These cells are characterized by a lack of ergastic materials, slightly enlarged cells and nuclei, and show frequent cell divisions (fig. 28) which occur in all planes but are most frequently periclinal. Many of of the innermost cells of the submarginal meristem and their derivatives accumulate ergastic materials, and the number of meristematic cells

decreases. During subsequent laminar growth, the rapidity with which derivatives differentiate varies, and consequently the number of cells in the submarginal meristem also varies.

The early activity of the marginal and submarginal initials produces only a rudimentary lamina (fig. 28, 29). Derivatives continue to divide periclinally, enlarge and elongate perpendicular to the marginal initials. As a result, the lamina rapidly becomes broad and flattened in most bracts (fig. 30). The lamina, in the dormant bud, extends about one-half the length of the bract, and the midrib of the bract distal to the lamina remains elliptical in transection (fig. 33). Lower bracts have wide obcordate lamina, median bracts have wide obdeltoid lamina, and bracts near the apex have narrow rhombic lamina. Bracts just below the summit of the apex are very short and undergo no marginal growth.

Transverse sections of bracts that are about 600μ long show the origin of resin canals (fig. 31). Resin canals are initiated near the distal end of the lamina and differentiate both acropetally and basipetally. In the dormant bud, therefore, resin canals extend from about 200μ below the bract apex to nearly the base of the bract. A single canal is initiated below the abaxial epidermis on either side of the provascular strand. A group of about five large cells containing ergastic materials divide, and the daughter cells become oriented into a strand, circular in transection. These daughter cells, like the cells from which they were derived, are elongated as seen in longisection of the bract. Daughter cells continue to divide, lose their ergastic contents and a small intercellular space forms in the center

of the strand (fig. 31). The intercellular space enlarges as a result of further divisions and the derivatives form an inner core of about ten cells surrounded by an outer, but less distinct, cylinder of cells having a similar appearance. The inner core becomes the epithelial tissue of the resin canal and may undergo further divisions. In the dormant bud, the resin canal attains a maximum diameter of about 40μ and the elongated epithelial cells become flattened (fig. 32). This type of schizogenous development is like that described for leaves of other conifers (15, p. 477).

There is no vascular differentiation in the bract prior to dormancy. Cells within the provascular strand continue to elongate and longitudinal divisions are frequent. Subsequent divisions become oriented so that the central cells in the procambial strand become arranged in radial rows (fig. 34). A layer two cells thick of meristematic cells surrounding the provascular strand are shorter and more vacuolate than the provascular cells. These undergo some additional divisions and, following dormancy, differentiate into transfusion tissue and endodermis in the terminal half of the bract. A region of enlarged parenchyma cells with abundant ergastic materials encloses the procambial strand and surrounding layer. This region may be four or more cells thick on the abaxial and adaxial surface and vary to a greater or lesser extent at the margins of the bract, depending upon the extent of marginal growth. Following dormancy, the inner cells differentiate into mesophyll tissue and the outer one or two layers into hypodermis.

Bracts are initiated over a period of three and one-half months,

but the rate of bract initiation is not constant during this time. Half the final number of bracts are initiated during the first month, while subsequent bracts are initiated at a slower rate. The apex, at the onset of bract initiation, is a broad cone about 300μ high and 350μ wide (fig. 20). The rapid initiation of the first bracts is accompanied by a comparable rapid apical enlargement resulting from divisions in the rib meristem and peripheral zones so that the apex assumes the aspect of a large, flattened dome 500μ broad and 250μ high (fig. 21, 22). During subsequent bract initiation, apical enlargement does not keep pace and the apex becomes a very low, broad dome 50μ high and 180μ wide (fig. 44).

Changes in the extent and distinctness of the apical zones occur throughout bract initiation. All zones can be readily delimited at the onset of bract initiation. The central mother cell zone is distinct and consists of about 20 cells (fig. 20). This zone becomes smaller during early bract initiation as more rapid division of cells on the flanks causes the peripheral zone to broaden at the top and extend closer to the summit of the apex. Divisions at the base of the central mother cell zone also increase, which extends the rib meristem closer to the summit of the apex. Central mother cells gradually decrease in size. Thus, the number of central mother cells decreases, and they become more like the cells of the peripheral and rib meristem zones (fig. 44).

The length of the bud increases during bract formation from 700μ to 2700μ because of rib meristem activity and intercalary growth (fig. 20, 45). Pith mother cells divide transversely many times and form

long vertical files of genetically related cells (fig. 45). Longitudinal and oblique divisions of pith mother cells increase the number of vertical rows and the bud broadens throughout its length. Intercalary growth causes a separation of bract primordia which, at their initiation, were spirally arranged in close order along the axis of the bud.

Scale initiation begins about the first of September, about five months after the megasporangiate bud is initiated. This is one and one-half months after the onset of bract initiation, or when approximately half the final number of bracts have been initiated. Scale initiation is delayed in the first few bracts and begins first in bracts just above the base of the cone. Initiation then proceeds acropetally and basipetally; however, the lowest bracts never develop scales and the lowest scales formed never become large and seldom develop functional ovules. All functional scales are initiated by the time the buds become dormant in the fall.

Although scales, morphologically, are modified lateral shoots, their initiation and early development are quite different from all other types of lateral shoots. The scale is more truly axillary in origin instead of arising from cortical cells above the axillary region. A scale is initiated in the axil of a bract when the bract has attained a length of approximately 600μ and has just begun marginal growth. The first indication of initiation is the enlargement of epidermal and subepidermal cells in the axil of the bract (fig. 35, 36). These then undergo rapid periclinal divisions which result in a bulge in the median axillary region. This activity continues and extends along the proximal adaxial surface cells of the bract to produce a low and broad

swelling of enlarged meristematic cells (fig. 37).

When the primordium has become three or four cells high, divisions predominate in the lower layers of cells, where it joins with the bract, and thus increase the height of the primordium (fig. 38). The primordium rapidly broadens until it nearly equals the width of the bract, and also extends acropetally along the adaxial bract surface. When the primordium is viewed from above the adaxial bract surface, it has the appearance of an arc of slightly raised tissue extending the width of the bract. This extension of the primordium laterally and acropetally results from activities of the epidermal cells of the bract, which lose their ergastic contents, enlarge and divide periclinally (fig. 36-39). The derivatives enlarge, develop large nuclei, and continue to divide which increases the height of the primordium as it advances along the bract surface. Transverse divisions in cells at its base also increase the height of the primordium. This layer of cells, therefore, assumes the aspect of a eumeristem which differentiates first in the axil of the bract and extends acropetally along the bract beneath the advancing scale primordium (fig. 38). The cells above the eumeristem remain larger and divide less frequently. The eumeristem of the scale extends into the cortex of the bud axis while the remainder of the eumeristem forms a flattened, asymmetrical, cup-shaped zone between the bract tissue and the scale primordium. Continued transverse divisions in the eumeristem produce files of cells perpendicular to the adaxial bract surface (fig. 39). The scale primordium increases in size until it becomes hemispherical or conical, and about 130μ high and 200μ wide. For a short time, a rudimentary pattern of zonation is present in the

scale primordium. A few enlarged apical initials are differentiated at the summit of the primordium, and divide anticlinally to form the protoderm, and periclinally to form a small, indistinct group of central mother cells. Periclinal divisions, however, are not restricted to the apical initials but may occur in all cells of the protoderm. The central mother cells are slightly enlarged with large nuclei, have lightly staining cytoplasm, do not have thickened walls and divide less frequently than cells of the eumeristem (fig. 39). The central mother cell zone gives rise along the flanks and below to the eumeristem, which includes cells of both the rib meristem and peripheral zone of other apices. There is no central cylinder of pith formed in the scale as in other shoots, and the peripheral zone is most evident at the axillary side of the scale primordium. For these reasons, usage of the term "eumeristem" is more accurate than the terms rib meristem and peripheral zone. The eumeristem gives rise to vertical rows of cells by repeated transverse divisions, and is most active at first in the axillary region of the bract. The later formed vertical rows become progressively shorter toward the advancing margin of the scale primordium. The longest vertical rows of cells in the axil of the bract bend abruptly, from a direction almost perpendicular to the bract surface to a direction parallel to the bract surface. Cells within these curved rows continue to divide transversely and elongate to form a procambial strand which differentiates basipetally, connecting the scale primordium to the vascular cylinder of the cone axis (fig. 40). The young scale primordium at this time, therefore, consists primarily of eumeristem with apical initials and central mother cells present only

in the median portion. The broad, lateral portions of the expanding primordium consist only of meristematic eumeristem cells (fig. 40, 41).

When the scale primordium has expanded acropetally along the adaxial bract surface for a distance of about 200μ, a different type of growth begins within the primordium. Periclinal divisions of bract epidermal cells at the margin of the scale cease, and divisions within the scale primordium increase all along the rounded margin. In the median portion of the scale primordium, cells in the eumeristem closest to the cone axis divide and rapidly elongate, while eumeristem cells at the margin of the primordium divide but do not elongate. This causes the apex of the primordium to be tipped outward from its earlier vertical position (fig. 42). The central mother cells then undergo rapid periclinal divisions, and the zonation pattern is lost; thus apical growth of the primordium is changed from perpendicular to the bract surface to almost parallel with it (fig. 39, 42). The apical initials, which were never very distinct, also divide periclinally as do other cells in the protoderm. In the non-median portion of the primordium, which consists at first entirely of eumeristem, divisions are also primarily periclinal (fig. 41). The result is an overgrowth of the margin of the scale primordium over that portion which joins with the adaxial bract surface. This overgrowth becomes very extensive so that the lateral margins of the primordium are completely free from the bract (fig. 43). This growth, along the curved margin of the scale primordium, continues by periclinal division of protodermal and subprotodermal cells. Rows of genetically related cells are thus produced that extend nearly parallel with the bract surface (fig. 42).

Transverse and oblique divisions occur within the rows, and increase the number of cell rows. Enlargement of the free scale surface continues for only a short time before the bud becomes dormant.

In the latter part of September, megaspore mother cells begin to differentiate, and most have differentiated by mid-October, from the eumeristem tissue in the lateral portion of the scale (fig. 43). The megaspore mother cell in the dormant bud is enclosed by many concentric layers of eumeristem cells. The mother cell is many times larger than surrounding cells and has a very large nucleus which stains lightly except for a darkly staining, large nucleolus. The site of the megaspore mother cell, therefore, foreshadows ovule differentiation but it is not known whether it predetermines ovule differentiation.

In the dormant megasporangiate cone bud (fig. 45), the scale is about 300μ long and 600μ wide at the widest portion. The portion of the scale that is free from the bract surface varies. Along the sides of the scale, the free portion extends about 100μ beyond the scale. This increases somewhat midway between the base and the rounded summit of the scale and again decreases at the summit of the curved margin. In face view, the shape of the scale primordium is similar to that of the mature scale except that the ratio of width to length is much greater in the scale primordium. There are no vascular tissues or resin ducts differentiated in the scales of the dormant bud.

The megasporangiate cone bud shows mitotic activity in both scales and bracts until early November, when they become dormant. The dormant bud is about 10 mm long and the cone within is about 2.7 mm long (fig. 45, 46). Megasporangiate cone buds are larger and readily

distinguishable from vegetative lateral buds and microsporangiate buds during dormancy (fig. 46).

Bract Enlargement

The shapes of the mature bracts are primarily determined by the extent of laminar growth prior to dormancy. Bracts elongate rapidly following dormancy, increasing their length from 1.5 mm to about 2 cm in two months, and vary in appearance in different parts of the cone at maturity (fig. 47). The lowermost bracts without axillary scales are very needle-like, pointed and have no lamina. Bracts subtending the sterile basal scales have a very rudimentary lamina restricted to the basal one-third of the bract. Midway in the cone the bracts are longer and the lamina extends three-fourths the length of the bract and has prominant, pointed lobes. Bracts in the distal half of the cone become somewhat shorter, more narrow and the lamina extends nearly to the tip of the bract. The basal one-third to one-half of each bract is completely covered by scales and is colorless, while the exposed portions of the bract are green. The covered portion of each bract is generally wider than the exposed portion, and also varies in the differentiation of tissues.

Elongation is primarily a result of intercalary cell division and elongation since the subapical initials differentiate early and never divide extensively. Derivatives of the submarginal meristem of the lamina, and the layer of cells surrounding the provascular strand, divide frequently by insertion of walls perpendicular to the longitudinal axis of the bract, thereby producing vertical rows of

genetically related cells which increase the length of the bract more rapidly than the width (fig. 49). Cells in the procambial strand divide transversely and obliquely and become more elongated than other cells of the bract.

All cells outside the differentiating vascular bundle and below the subapical initials divide frequently during early development. Maturation of these cells, however, begins in the distal portion and gradually extends basipetally so that finally cell divisions become restricted to an intercalary meristem at the base of the bract. By the time the bract attains a length of about 1 cm, very few transverse divisions occur, even at the base of the scale, and subsequent bract elongation results primarily from cell elongation.

Marginal growth of the bract proceeds until the largest bracts become about 7 mm wide (fig. 47). At first, this is primarily because of the resumption of mitotic activity of the submarginal meristem. Reduction in the frequency of divisions both oblique and parallel to the surface of the lamina cause it to gradually become thinner nearer the margin. Divisions in the submarginal meristem cease before the bract has attained its final width and subsequent broadening results from enlargement of the differentiating mesophyll cells.

Both epidermal and mesophyll cells in the distal part of the lamina elongate obliquely to the lamina. This causes the distal portion of the bract to become slightly wider than the proximal half. Frequent divisions of these cells early in bract development, followed by elongation, cause the lobes of the lamina to extend distally and become rather pointed. This gives the mature bract the characteristic

three-pointed appearance (fig. 47).

Epidermal cells divide anticlinally and most elongate parallel to the midrib as the bract elongates. Small groups of cells on the adaxial and, to a lesser extent, on the abaxial side of the midrib, remain small and divide frequently (fig. 49). These actively dividing strips of cells ultimately produce rows of guard cell initials. The stomata are thus restricted to three or four discontinuous rows on either side of the midvein separated by elongated epidermal cells. In the mature bract, they extend from slightly beyond the forked portion basally into the distal part of the covered portion. The mature guard cells are slightly elongated parallel to the midrib, sunken and are overtopped by subsidiary cells (fig. 48). They are quite similar to those reported for leaves of other conifers (41, p. 360; 16, p. 297; 22, p. 145-153).

The epidermal cells become elongated and slightly flattened or cylindrical in cross-section (fig. 48, 50). The cell walls are uniformly thickened with a thick overlying cuticle and the cells become partially filled with granular ergastic materials. Unicellular epidermal hairs differentiate on the covered portion of the abaxial bract surface and also occur infrequently on the adaxial surface along the marginal portion of the lamina.

The outermost subepidermal cells differentiate into hypodermal tissue. Following dormancy, these cells occasionally divide transversely or obliquely and elongate (fig. 49). The hypodermal layer in the mature bract becomes one to three cells thick on the abaxial side of the midrib and one or sometimes two cells thick in the rest of the

bract (fig. 53, 56). It may be absent or represented only by occasional cells on the adaxial side of the midrib. Near the thin margin of the lamina only hypodermis and epidermis are present. Hypodermal cells are elongated, generally cylindrical cells often having tapering, overlapping ends and thick, lignified cell walls (fig. 50). They generally contain darkly staining, granular ergastic materials.

Differentiation of the mesophyll tissue begins at the tip of the bract, when the bract is about 8 mm long, and proceeds basipetally. The cells elongate parallel to the midrib and become lobed, thus assuming the shape characteristic of mesophyll cells of most conifer leaves. The mature bract has lobed mesophyll cells with large intercellular spaces throughout the distal portion where the broad lamina is absent (fig. 50, 51). In the uncovered portion where the lamina is present, the lobed mesophyll cells are most conspicuous in the midrib region and extend half the width of the lamina. In the covered portion, mesophyll cells are less lobed and more uniformly cylindrical throughout the width of the bract (fig. 55). Mesophyll in the marginal portion of the lamina is only one or two cells thick, and consists of unlobed, elongated, cylindrical cells. Intercellular spaces are large in the midrib, especially in the exposed portion of the bract, but they become progressively smaller near the margin of the lamina.

Astrosclereids, and occasionally macrosclereids, differentiate from parenchyma cells in the mesophyll of the midrib. None were found in the narrow lamina of the bract, except in the hypodermal layer. They are similar in general appearance and size to sclereids found among the parenchyma cells of the scale.

Resin canals differentiate acropetally nearly to the tip of the mature bract, and basipetally to just below the distal end of the covered portion. They do not anastomose with the canals of the scale or the cone axis.

The structure of the vascular strand, including the surrounding transfusion tissue and endodermis, varies in different parts of the bract. The strand in the exposed part of the bract is similar to that in many conifer leaves in that both endodermis and transfusion tissue are present (fig. 50, 51). In the covered part of the bract, however, neither endodermis nor transfusion tissue differentiate (fig. 55). The transition from a strand without endodermis and transfusion tissue to one where these tissues are present occurs approximately where the bracts emerge from between the scales.

Differentiation in the provascular strand begins immediately following dormancy. The central cells become arranged into distinct radial rows because of increased orientation of cell divisions in a tangential plane. A cambial region develops in the center of the radial rows of cells and produces phloem to the abaxial side and xylem to the adaxial side. Differentiation of vascular tissue begins at the nodes of the cone axis, extends into the base of the bract, and then proceeds acropetally in the bract (fig. 57). Primary phloem differentiates before primary xylem. Phloem differentiation is marked by elongation of precursor cells followed by an apparent disintegration of the nucleus and thickening of the cell walls. The primary phloem becomes crushed as secondary phloem is produced. Only a few strands of primary xylem differentiate and these become stretched but most are

not crushed during elongation.

Vascular tissues in the mature bract form a single strand in the center of the midrib. The size of the strand and the number of elements within decreases gradually acropetally. Secondary xylem elements consist of tracheids with bordered pits and tertiary spirals. Xylem and phloem parenchyma are arranged as a uniseriate, vertical, ray-like plate separating the bundle into two equal parts (fig. 56). This is similar to the vascular bundles of some species of spruce described by Marco (41, p. 264).

A distinct endodermis differentiates in the midrib of the bract beyond the lobes. The endodermis completely encloses the transfusion tissue and consists of large elongated cylindrical cells with slightly lignified radial walls (fib. 50-52). They contain a thin parietal layer of cytoplasm and occasionally granular ergastic materials. The endodermis becomes less distinct below the forked part of the bract (fig. 53, 54) and is absent in the covered portion of the bract (fig. 56).

Transfusion tissue is present throughout the exposed portion of the bract. The transfusion tissue consists of elongated living parenchyma cells with non-lignified walls, and thin-walled but lignified tracheids with bordered pits (fig. 50, 52, 54). Laterally and adjacent to the vascular tissue are albuminous cells (22, p. 437-440) which are slightly elongated, with dense cytoplasm and prominent nuclei (fig. 54, 56). The layer of transfusion tissue is thicker on the abaxial side of the vascular bundle than on the adaxial side (fig. 51). At the proximal end of the exposed portion of the bract, the transfusion

tissue becomes less extensive and becomes confluent with the surrounding mesophyll. Basipetally it becomes restricted more to the lateral margins of the vascular tissue adjacent to the albuminous cells, and still lower is represented by only scattered transfusion tracheids. In covered portions of the bract, transfusion tissue is absent and mesophyll cells adjoin the vascular tissue except at the lateral margins where a few albuminous cells remain (fig. 56).

Scale Development Following Dormancy

Growth of the scale is resumed following dormancy and the median scales elongate to about 2.5 cm in about three months. Scales of the mature cone are approximately spoon-shaped but vary in size and shape in different parts of the cone (fig. 67). The basal five to ten scales are small, sterile and slightly broader than long. Scales from the center of the cone are about 3 cm wide and 2.5 cm long and enclose about one-third of the circumference of the cone. Distal scales decrease in size acropetally, becoming very tiny at the tip of the cone. They curve more than basal scales and thus enclose more of the cone. The exposed portion of the scale is green and that portion covered by adjacent scales is white during enlargement of the cone. In the mature cone, the scales turn brown prior to cone opening.

Early growth determines the ultimate shape of the scale and initiates certain zones which later differentiate into distinct tissues during enlargement and maturation. The first activity, following dormancy, is frequent cell divisions all along the abaxial half of the scale. Most divisions are periclinal but occasional anticlinal and

oblique divisions also occur. Cells in the adaxial half of the scale divide less frequently, enlarge, become vacuolate and some accumulate ergastic materials (fig. 57). The increased activity of abaxial cells marks the beginning of marginal growth which is responsible for much of the further enlargement of the scale (fig. 58). There is no real distinction between apical and marginal meristems at this time. Hence, the terms marginal meristem and submarginal meristem will be used to describe both increase in length and in width.

A small group of subepidermal meristematic cells forms a submarginal meristem all along the margin of the scale (fig. 58). Divisions of submarginal cells are in all planes at first, and the margin becomes rounded. Divisions later become predominantly transverse and the number of rows of meristematic cells decreases as a result of more rapid differentiation of derivatives. The margin of the scale thus becomes very pointed in section (fig. 59). By the time the scale is about 2 mm long, outer cells of the submarginal meristem become vacuolated, enlarged and cease to divide (fig. 60). They accumulate ergastic materials during subsequent development and are borne passively on the margin of the scale while deeper cells of the submarginal meristem continue to divide.

Cells of the submarginal meristem, in the first stage of marginal growth, give rise below to three zones as seen in median longisection, i.e., zones along the abaxial and adaxial sides and a central procambial zone. The origin of these zones becomes obscure in the more mature scale when the outer cells of submarginal meristem become differentiated. The submarginal meristem gives rise along the abaxial

scale surface to a zone consisting of two or three layers of subepidermal cells (fig. 58). This zone broadens basipetally by longitudinal and oblique divisions and elongates by transverse cell division and elongation (fig. 59). Cell layers just beneath the epidermis elongate, remain very narrow, and constitute a distinct hypodermis while cells of deeper layers enlarge, remain isodiametric and elongate at a much later stage. Cells of the hypodermis accumulate ergastic materials as do cells in the overlying epidermis. The abaxial zone becomes thicker in the median portion of the scale than in the marginal portions. The basal cells of this zone later differentiate into sclereids.

The submarginal meristem gives rise directly below to a central zone of meristematic cells which differentiates into procambium (fig. 58). Cells of this zone remain meristematic longer than cells in the abaxial zone. The zone is about four cells wide just below the submarginal meristem and broadens basipetally by longitudinal and oblique divisions. Elongation occurs by transverse and oblique divisions and cell elongation. The zone forms a continuous band of meristematic cells in the very young scale but differentiates acropetally into many procambial strands alternating with interfascicular areas as the scale enlarges (fig. 61, 63). When cells making up the original submarginal meristem differentiate, deeper cells at the distal end of the procambial zone continue to divide, serving as a less conspicuous submarginal meristem.

The submarginal meristem gives rise along its adaxial flank to a zone of cells which enlarge greatly but remain isodiametric, divide

occasionally in all planes and many accumulate abundant ergastic materials (fig. 58, 59). This cell enlargement causes the adaxial side of the young scale to be more swollen than the abaxial surface. During early enlargement of the scale, cells in this zone may divide to form short vertical files of genetically related cells. Ergastic materials accumulate first in cells scattered throughout this zone but soon become concentrated in two or three layers of subepidermal cells (fig. 58, 59). A hypodermis differentiates later which is less distinct than the one on the abaxial side. The adaxial zone becomes much thicker in the median portion of the scale than either the abaxial or procambial zones (fig. 59). Lateral portions of the adaxial zone are much different for it is here that the ovules differentiate.

Early activity of the submarginal meristem thus establishes the shape of the scale and causes it to enlarge uniformly both vertically and laterally. The general shape of the scale is maintained throughout subsequent enlargement. Outer cells of the submarginal meristem differentiate when the scale is about 2 mm long. Subsequent enlargement of the scale occurs by less conspicuous meristematic cells at the tip of the procambial zone, which form a rudimentary submarginal meristem, by an intercalary meristem at the base of the scale, and by intercalary growth throughout the scale.

Cells of this rudimentary submarginal meristem divide transversely and obliquely. Derivatives of oblique divisions may form part of either the abaxial or adaxial zones or broaden the central procambial zone. As the procambial zone differentiates into procambial strands and interfascicular areas, only the procambial strands remain very

meristematic. Cells at the tips of the procambial strands continue to divide throughout growth of the scale and differentiate in the fully enlarged scale.

Growth by a basal intercalary meristem occurs during early stages of scale enlargement. This appears to be a resumption of activity in the rib meristem region which was responsible for much of the enlargement of the scale during its initiation. Frequent transverse divisions occur for a brief period following dormancy, but scale elongation by this means appears to be of little importance. These divisions do enlarge the base of the scale where it joins the cone axis and are, therefore, more conspicuous in lateral portions of the scale than in median portions. Subsequent scale enlargement is a result of cell division and enlargement throughout the scale.

Vascular Differentiation

The procambial tissue differentiated at the base of the scale during scale initiation is continuous above with the central band of meristematic tissue derived from the submarginal meristem of the scale. Following dormancy, branching strands of provascular tissue differentiate acropetally throughout the band of meristematic tissue. Groups of cells within this meristematic band divide frequently longitudinally and the derivatives become arranged into numerous concentrically arranged strands of cells (fig. 61). Cells between the strands divide less frequently, enlarge and accumulate ergastic materials. This results in the delimitation of distinct provascular strands and interfascicular areas (fig. 63). Provascular strands enlarge by continued

longitudinal divisions which soon become oriented primarily in a tangential plane and form radial rows of cells in each strand (fig. 62). Cells in the cambial region divide rapidly during scale enlargement (fig. 62, 64) but the numerous xylem and phloem mother cells thus produced do not begin to differentiate until scale elongation is essentially complete (fig. 66). There is an indistinct sheath of undifferentiated cells around the differentiating vascular tissue (fig. 64).

Primary phloem differentiates on the adaxial side of the bundle followed by primary xylem on the abaxial side. The first primary phloem cells formed develop slightly thickened walls and are stretched and crushed during scale elongation (fig. 62, 66). Additional primary phloem, which is arranged in radial rows, differentiates during elongation but is not crushed in the distal half of the scale. The sieve cells of this later phloem have thicker cell walls. Primary phloem consists only of sieve cells and a few parenchyma cells. The parenchyma cells elongate slightly and often greatly enlarge. Primary sieve cells are elongated with overlapping end walls and have faint small sieve areas present on the radial walls.

Only two or three tangential rows of secondary phloem cells differentiate in the distal half of the scale once elongation is complete. Here also the phloem consists only of sieve cells and phloem parenchyma (fig. 66). Near the base of the scale, where more secondary growth occurs, fusiform parenchyma is also present. Both primary and secondary phloem parenchyma cells enlarge tangentially and continue to accumulate ergastic materials during subsequent maturation of the

scale (fig. 66). Only secondary phloem persists at the base of the scale and all primary phloem is crushed.

The xylem consists of two or three rows of primary tracheids, secondary fiber-tracheids and parenchyma. The primary tracheids have large bordered pits and distinct tertiary spirals. The cells are short at first with distinct end walls, but become very long and narrow or often crushed as the scale elongates. Most of the cells derived from the cambial region during elongation differentiate into fiber-tracheids once elongation is complete (fig. 65, 66). The secondary xylem mother cells remain nucleate and elongate throughout scale elongation. At maturity they are longer than primary tracheids, thicker walled, have faint tertiary spirals and tapering, overlapping ends. Fiber-tracheids are roughly oval in transection with the widest cell surface being the radial wall in which most of the bordered pits are found. Xylem parenchyma is not abundant in the small bundles in the distal half of the scale but is common in the very large bundles at the base of the scale (fig. 65). The cells are generally arranged in uniseriate, ray-like sheets which extend through the width of the bundle. The cells are elongated vertically and have thick, lignified walls with numerous simple pits in the mature scale. The vascular bundles at the base of the scale become very large and form an almost continuous band of vascular tissue the width of the scale (fig. 65, 70, 71). As a result of this enlargement, the interfascicular parenchyma cells become compressed between the vascular bundles (fig. 65). These parenchyma cells become crushed, or some differentiate into thick walled sclereids similar to those in the adjacent abaxial base of the scale.

The indistinct sheath of cells around the vascular tissue is usually only one or two cells thick next to the primary xylem but becomes several cells thick next to the primary phloem (fig. 64). The cells of this sheath elongate slightly and many remain undifferentiated throughout the period of vascular differentiation. This tissue, at maturity, resembles transfusion tissue in that it encloses the vascular tissue and consists of tracheids similar to transfusion tracheids, parenchyma cells, albuminous cells and macrosclereids which occur in the proximal half of the scale. It resembles transfusion tissue of Douglas-fir leaves and bracts further in that it is thickest adjacent to the phloem. It differs, however, in that no surrounding endodermis is present in the scale. The tissue is most conspicuous in the distal half of the scale where the vascular bundles are small. It is less abundant at the base of the scale where there is abundant secondary vascular tissue. Here, the vascular bundles undergo considerable enlargement and the surrounding tissue does not keep pace and often becomes stretched and crushed (fig. 65).

Differentiation in this tissue begins with the onset of vascular differentiation in adjacent tissues. The tracheids develop thin, lignified, secondary walls with numerous faint bordered pits. Thick lignified tertiary spirals develop in the mature tracheids which are slightly elongated longitudinally and tend to be rather irregular in shape. The tracheids develop primarily adjacent to the xylem, however, a few differentiate adjacent to the phloem. The parenchyma cells also differentiate most frequently adjacent to the xylem with only a few differentiating among the meristematic cells adjacent to the phloem.

The albuminous cells of the scale are abundant adjacent to the phloem but are only scattered adjacent to the xylem. In the proximal half of the scale, numerous macrosclereids differentiate adjacent to the xylem (fig. 65) and are contiguous with the larger macrosclereids near the base of the scale (fig. 70).

Sclereids

Sclereids are found abundantly throughout the mature scale as groups forming distinct tissues, or as isolated cells dispersed among the parenchyma tissue. Three of the four principle types of sclereids distinguished by Foster (24, p. 67-70) are found in the mature scale: (1) macrosclereids which are elongate, rod-like cells, form an extensive and distinct tissue on the abaxial side of the scale and less distinct hypodermal layers on most of the adaxial scale surface (fig. 77); (2) brachysclereids, or stone cells, which are short, roughly isodiametric sclereids resembling parenchyma cells in shape, are found as a subepidermal layer over nearly all of the adaxial scale surface (fig. 78) and in the seed coat; and (3) astrosclereids, which are branched sclereids found scattered throughout the parenchyma tissue of the scale.

Macrosclereids in the mature scale are found most extensively in the median, basal, abaxial portion of the scale extending from the abaxial side of the vascular bundles to the epidermis (fig. 69, 70). They form a wedge-shaped zone, as seen from the abaxial surface, which extends from the base of the scale along one-third the length of the scale (fig. 99, 100). Two morphologically distinguishable forms of

macrosclereids are present in this zone which form outer and inner layers (fig. 75, 76). Cells which will later differentiate into one or the other type are distinguishable when the scale is only 2 mm long (fig. 73). Both zones increase in thickness during the three and one-half months following the breaking of dormancy, each becoming about fifteen cells thick at the base of the scale.

When the scale is 2 mm long, the cells of the outer zone are only about 35μ long and 10μ wide (fig. 73). These cells elongate more rapidly than they increase in width and, when secondary cell walls begin to form, are about 350μ long and 30μ wide (fig. 74). When elongation is complete, abundant ergastic materials accumulate and are retained during secondary cell wall formation. These macrosclereids thus form a thick layer at the base of the scale and extend as a hypodermal layer one or two cells thick over most of the remaining abaxial surface (fig. 69). The layer is represented by only scattered macrosclereids near the margin of the scale.

The cells of the inner zone remain isodiametric during early development (fig. 73) and are indistinguishable from cells which later differentiate into parenchyma tissue. Cells of this zone, in the young scale, divide in all planes to increase both breadth and length of the zone. The inner zone becomes thicker than the hypodermal zone primarily because the cells increase more in diameter (fig. 74). The cells are about 25μ in diameter when the zone can first be delimited (fig. 73). Cell enlargement is equal in all directions at first until the cells become about 40μ in diameter, after which they elongate rapidly to about 300μ long and 60μ wide by the time secondary cell

walls begin to form. Cell divisions occur throughout this time, but become very infrequent in later stages of elongation.

Both types of cells in these zones differentiate similarly into macrosclereids by the even deposition of thick secondary walls which become heavily lignified (fig. 75-77). The lumens of the narrow outer macrosclereids become almost completely filled as the secondary wall becomes about 11μ thick. The secondary walls of the inner macrosclereids become about 23μ thick, but because the cells have a larger diameter, a distinct lumen remains. The transition between these two zones is not abrupt. The secondary walls of these cells are laminated and penetrated by simple pits which frequently are branched (fig. 76, 77). The secondary wall of the mature sclereid often appears to overarch the small pit chamber. There is a slight amount of apical intrusive growth during the final period of cell elongation so that the ends of the elongated mature macrosclereids are often pointed and overlapping (fig. 77). The lumen of the mature macrosclereid is usually completely filled with uniformly darkly staining ergastic materials, as are the canal-like cavities of the pits.

A hypodermis consisting of macrosclereids differentiates beneath the adaxial epidermis and under the differentiating seed wings. They are absent only directly below the seed and along the margins of the mature scale.

Brachysclereids in the mature scale form a layer one to many cells thick in a continuous zone below the hypodermal macrosclereids except along the margin of the scale where they occur just below the epidermis (fig. 78). The layers of brachysclereids are thickest in the

central adaxial portion of the scale.

Brachysclereids in the scale differentiate by secondary sclerosis (22, p. 216) of ordinary parenchyma cells and generally remain isodiametric. Brachysclereids differentiate first form the outer subhypodermal parenchyma cells distal to the developing seed wing on the adaxial side of the scale. Mature brachysclereids have uniformly thickened, lignified, secondary cell walls which may be 15μ to 35μ thick, and numerous ramiform pits (fig. 78). They differ from macrosclereids in size and shape only and, where brachysclereids and macrosclereids occur in adjacent layers, the distinction becomes rather arbitrary.

Astrosclereids are scattered throughout the parenchyma tissue of the scale. They differentiate from parenchyma cells by apical intrusive growth into intercellular spaces and become large irregularly branched cells. They undergo secondary sclerosis and develop lignified secondary cell walls 20μ to 30μ thick which have many simple and often ramiform pits. A small lumen remains that is filled with darkly staining ergastic materials when the sclereid is mature.

Parenchyma cells which do not differentiate into sclereids remain essentially isodiametric or become slightly elongated longitudinally during growth of the scale. They become vacuolated and gradually accumulate darkly staining granular ergastic materials which often completely fill the cells in the mature scale. Cell walls remain thin and have abundant simple pits. Small intercellular spaces form which are most conspicuous in transection of the scale. Parenchyma tissue makes up most of the central part of the scale between the abaxial

and adaxial layers of sclereids (fig. 69-72).

Resin Canals

Resin canals in the parenchyma tissue of the scale are continuous with those in the cortex of the cone axis. They begin to differentiate in the base of the scale when the scale is about 2 mm long. A cortical resin canal on either side of the vascular bundle supplying the scale branches dichotomously and the branch differentiates acropetally into the base of the scale above the vascular bundle. The two resin canals branch many times in the base of the scale to form numerous smaller canals in the adaxial half of the scale (fig. 70). Dichotomous branching continues during subsequent enlargement and the number of resin canals may reach about 60 in the central and distal part of a mature scale. In the distal half of the scale, resin canals differentiate between the vascular bundles, generally alternating with them. Resin canals extend, in the same general pattern as the vascular bundles, to all regions of the scale.

Ovule and Seed Wing Development

Divisions occur throughout the concentric layers of cells forming the nucellus which encloses the elongating megaspore mother cell. This increases the number of layers of cells in the nucellus and increases the size of the bulge caused by the developing ovule on the adaxial scale surface. As the concentric layers of the nucellus increase, the outer three or four layers of cells divide transversely and elongate in that portion of the nucellus nearest the axil of the

scale. A slight overgrowth of cells is produced which extends over the megaspore mother cell and its surrounding nucellus. This overgrowth is continuous around the nucellus and marks the initiation of the integument. Elongation of the integument continues by rapid cell division in the proximal portion of the integument and cell enlargement and vacuolation in the distal portion. The integument thus forms a long hollow cylinder with many unicellular hairs differentiated at the tip. The lower portion of the integument elongates more than the upper portion to produce a one-sided stigmatic tip which terminates at the axil of the scale. The longer lower tip is oriented upward during the period of pollination. Details of the nature and function of the stigmatic tip preceding and following pollination are discussed by Barner and Christiansen (9, p. 97-101) and Lawson (38, p. 168-175).

The scale becomes thicker by cell division around the developing ovule, which then appears to be sunken deeper into the adaxial scale surface (fig. 79). Before the time of pollination, the micropylar ends of the developing ovules are directed toward the basal lateral portion of the scale. As the scale broadens, the area between the two ovules becomes wider and there is a shift in their placement on the scale. The chalazal end of the developing ovule is shifted toward the lateral margin of the scale and the developing ovules become nearly parallel with the longitudinal axis of the scale following the period of pollination.

The integument differentiates into the three layers characteristic of most conifer seeds (14, p. 254). An outer layer, which is most extensive on the upper surface of the seed, is continuous with the

adaxial surface of the scale and its cells become vacuolated and many accumulate ergastic materials (fig. 79). This layer changes little during ovule and seed development and forms the thin outer covering of the seed which attaches to the seed wing (fig. 79). Cells of the middle layer remain smaller, isodiametric, retain dense cytoplasm and divide frequently, forming a distinct layer up to six cells thick (fig. 79). This layer differentiates into a stony layer in which the cells are small, isodiametric or slightly elongated, and have thick, lignified, secondary cell walls with numerous simple pits (fig. 80). The inner layer of cells adjacent to the nucellus is most distinctly developed in the free portion of the integument and consists of small undifferentiated cells (fig. 79). Cells of this layer become slightly elongated and vacuolated during ovule development. In the mature seed, all three layers are quite thin because of the increase in the size of the seed. Cells of both the inner and outer layers become much elongated and flattened against the stony layer. Some cells of the inner and outer layers may also become slightly lignified.

Seed wings begin to differentiate about the time of pollination when the scales are only about 2 mm long. Initiation of an adaxial hypodermis occurs by divisions in the subepidermal layer which gives rise to a layer, usually three cells thick, continuous with the outer layer of the integument (fig. 81). Unlike other adaxial subepidermal cells, hypodermal cells here become slightly elongated and accumulate no ergastic materials during their early stages of development. As the hypodermis thickens, it causes a slight lifting of the overlying epidermis (fig. 81, 82). Cells of the outer hypodermal layer divide

transversely and become greatly elongated, while in the underlying layer the cells become only slightly elongated (fig. 82). The latter layer is present between the seed wing and the scale and also extends between the ovule and the scale. Cells of this layer in the mature scale become irregularly elongated, remain undifferentiated and do not accumulate ergastic materials (fig. 78, 83).

The outer layer of hypodermal cells accumulates ergastic materials and the cells elongate more rapidly than the overlying epidermal cells so that the latter becomes stretched and flattened. The fully developed seed wing, therefore, consists of two cell layers. The outer hypodermal layer is most conspicuous, consisting of a single layer of narrow elongated cells usually filled with darkly staining ergastic materials and with slightly thickened, lignified secondary cell walls (fig. 78). The epidermal layer consists of very flat cells containing less ergastic materials and having slightly thickened, lignified secondary cell walls. No epidermal hairs are present on the seed wings.

The seed wing is fully formed during the first part of July, about two months before the cones open and seeds are released. Separation of the seed wing from the scale generally occurs during the latter part of August. Separation of both seed and wing from the scale is apparently a result of dissolution of the middle lamella between the two layers of undifferentiated cells beneath the outer hypodermal layer. The cells separate along the middle lamella with little breakage of cell walls. The separated wing thus has a layer of undifferentiated cells attached.

Epidermis and Epidermal Hairs

The epidermis of the mature scale consists of elongated, flattened rectangular cells and many unbranched hairs which are generally unicellular but a few consist of two cells. The walls of both epidermal cells and hairs may become slightly thickened and lignified and are covered by a thin cuticle (fig. 84). Epidermal hairs vary in length from short erect hairs less than 60μ long to hairs 120μ long which may be arched, or bent parallel to the scale surface.

Epidermal hairs begin to differentiate first on the adaxial surface when the scale is about 1 mm long, and on the abaxial surface when the scale is about 2 mm long. A hair is initiated as a protuberance from an isodiametric or slightly elongated epidermal cell or as a protuberance from the outer derivative resulting from the oblique division of an epidermal cell. During the elongation of the protuberance, a cell wall may form at its base separating the cell into an outer cell forming the hair and the subjacent epidermal cell (fig. 84).

Hairs differentiate on all epidermal surfaces of the scale except that portion covering the seed wing. The adaxial surface of the seed coat has a limited number of epidermal hairs. Hairs become less abundant and often shorter along the margin of the scale. Hairs on the covered portions of the scale have thin slightly lignified secondary cell walls and are seldom erect.

Elongation of the Cone Following Dormancy

Dormant megasporangiate cones are about 2.7 mm long (fig. 45, 46). Growth resumes in early March and the cone elongates to about 7 or 8 cm by the first of July (fig. 68). During this three and one-half months, vascular tissues are initiated and partially differentiated. From July to September, subsequent differentiation and maturation of tissues occurs throughout the cone.

Elongation of the cone is a result of both cell division and cell elongation throughout the cone axis. Transverse divisions within the rib meristem of the apex are frequent at the onset of elongation but gradually become less frequent and cease by the time of pollination (fig. 85). Transverse cell divisions of pith mother cells throughout the cone axis are frequent during this same period of time. About the time of pollination (fig. 98), divisions of pith mother cells become restricted to the terminal portion of the cone and then cease. Cell elongation is responsible for most of the cone elongation following pollination.

Some bract primordia are initiated following dormancy; however, these remain small and form a compact cluster at the tip of the mature cone. Rudimentary sterile scales may be initiated in the axil of many of these bracts.

Cells in the pith of the mature cone are elongated and have thick, lignified cell walls with simple pits (fig. 96). Cell wall thickening occurs first adjacent to the metaxylem when cone elongation is nearly complete. Ergastic materials increase gradually in the pith cells

during elongation and maturation.

Vascularization

Vascularization of the megasporangiate bud is similar to that in the vegetative shoot described by Sterling (50).

Procambial initiation is like that of cataphylls and leaves in that procambium differentiates acropetally in the peripheral zone of the apex to the base of the presumptive bract primordium. The procambium appears, in transection, as a group of concentrically arranged cells set apart from surrounding cells by their orientation, more darkly staining nuclei and higher nucleocytoplasmic ratio (fig. 86). Frequent longitudinal cell divisions occur which increase the diameter of the strand basipetally. These divisions soon become oriented in a tangential plane in the center of the strand and produce radial rows of cells. Bundles having this appearance are present at the base of the megasporangiate bud prior to dormancy.

The bundles enlarge rapidly in the elongating bud following dormancy by the activity of a definite cambial region (fig. 87). The number of radial cell rows also increases by radial or oblique division of cells within the cambium, and especially by tangential division of cells in the surrounding residual meristem. Bundle size increases throughout elongation until, in the fully elongated cone the vascular tissue forms a complete cylinder dissected only by fused leaf and branch gaps (fig. 94).

The first primary phloem cells differentiate about when radial seriation appears in the bundles (fig. 87, 89). The outermost

concentrically arranged cells differentiate first (fig. 89), followed immediately by radially arranged cells. Phloem differentiation occurs rapidly in the radial rows and so that three or four layers of protophloem are present before the first protoxylem differentiates. Walls of the primary sieve cells are slightly thickened and stain lightly with safranin. When the tiers of primary sieve cells are four or five cells deep, the oldest cells enlarge. These enlarged cells possess very little cytoplasm and the nucleus becomes small and irregular (fig. 88, 89). Continued cone elongation causes these cells to be passively stretched and eventually crushed against the bordering cortical cells. No sieve areas are visible in the first primary sieve cells and phloem parenchyma and crystals are also absent. Succeeding sieve cells are larger and show more conspicuous sieve areas. Secretory cells, which form outside the bundle in the vegetative shoot (50), do not develop in the cone.

Protoxylem is differentiated shortly after primary phloem, first at the nodes near the base of the cone followed by rapid basipetal and acropetal differentiation (fig. 87, 89). Protoxylem elements have thin, lignified, secondary walls with faint pits and thick spiral thickenings (fig. 88, 89). Metaxylem differentiates in the radial rows of cells adjacent to the protoxylem (fig. 90, 91). Metaxylem elements are larger than protoxylem elements with thicker, more heavily lignified secondary walls. As a result of rapid cone elongation, the protoxylem is greatly stretched and the spiral thickenings become widely spaced and irregular. Many of these cells are eventually completely crushed. Metaxylem elements elongate more and have more

closely spaced spiral thickenings and bordered pits.

The only sound criteria for determining the beginning of secondary growth is that the first secondary trachery elements are significantly shorter than the last primary elements (22, p. 381-383). The distinction is less clear in plants such as Douglas-fir where radial arrangement of cells in the vascular bundle occurs long before any secondary tracheids are formed.

Secondary tissues are initiated in the cone axis before elongation is complete. Differentiation of the secondary xylem, however, is delayed until that portion of the cone is nearly or fully elongated (fig. 92). The primary xylem becomes about seven layers thick while the vascular cambium produces 20 or more layers of undifferentiated cells before the first differentiation of secondary tracheids begins (fig. 92). When differentiation is initiated, it proceeds from the oldest of these cells to the youngest in about three weeks (fig. 93). During this time, the cambium is still very active so that when the cone is fully elongated, the secondary xylem region may be over 50 cells wide and the undifferentiated cambium region less than 10 cells in width (fig. 94). This type of differentiation is not unusual in vegetative stems; however, it has not been described for the vegetative shoot of Douglas-fir.

The secondary xylem, in transverse section (fig. 96) appears quite different in many respects from that of the vegetative shoot. Tracheid walls are somewhat thicker than walls in the spring wood of the vegetative shoot, and they are generally more elongated in the

radial direction. The cells are quite variable in size in transection and tend to be either oval or rhomboid rather than rectangular. This irregular shape makes the radial rows of cells less distinct than in the vegetative shoot, and causes numerous intercellular spaces. Distinct bordered pits are present about equally in all walls. Two rows of pits are not uncommon in the radial walls of large tracheids but only one row is found in the more narrow tangential walls.

Vertical resin ducts with thick walled epithelial cells are scattered throughout the secondary xylem of the mature cone, and are similar in size to those of the vegetative shoot. Radial ducts were not observed. Vascular rays are numerous, uniseriate and often difficult to distinguish from interfascicular parenchyma. Xylem parenchyma, which occurs terminally in the summer wood in the vegetative shoot, was not observed.

The distinction between primary and secondary phloem in the cone axis is uncertain. The amount of secondary phloem increases much more gradual than the secondary xylem and attains a maximum width of about 20 cells (fig. 95). Three cell types are present in the secondary phloem. The last formed sieve cells have thicker cell walls and less conspicuous sieve areas than those in the early secondary phloem. Thin walled phloem parenchyma cells are scattered throughout the phloem and usually contain ergastic materials. Some of the parenchyma cells contain very dark uniformly staining material while others contain more lightly staining droplets. Phloem parenchyma is not arranged into tangential bands as in the vegetative shoot (31). In the older phloem, parenchyma cells enlarge to about twice their original size. Ray

cells, two to three times the diameter of the sieve cells, are present and appear either to have no ergastic materials or the same type of cell contents as the phloem parenchyma. Both phloem parenchyma and ray cells proliferate in older phloem and disrupt the radial arrangement of the sieve cells. Fusiform parenchyma, vertical albuminous cells and sclereids were not observed.

Morphology

The primary vascular system of the megasporangiate cone consists of an anastomosing system of vascular strands similar to that of the vegetative shoot (22, p. 661). Differences are that the cone maintains much shorter internodes and all but the most basal and distal foliar organs (bracts) each have a modified axillary shoot which is the scale. Cleared cones, collected at the time of pollination, show that the primary vascular system consists of leaf (bract) traces in sympodial linkages and of branch traces supplying the scales (fig. 97). Each bract is supplied by a single unbranched leaf trace which branches from an axial strand and passes upward nearly parallel with the strand for a considerable distance before bending abruptly and passing almost horizontally through the cortex to the base of the bract. A single collateral vascular bundle passes unbranched to the tip of the bract. The phloem occurs on the abaxial side of the bundle and the xylem on the adaxial side within the bract.

Two branch traces separate from adjacent axial strands just above the point where the leaf trace bends outward (fig. 97). One branch comes from the same axial strand that gave rise to the leaf trace.

Branch traces immediately bend outward horizontally, rather than passing parallel to the cone axis. The close approximation of the two branch traces to the single leaf trace forms a common leaf and branch gap after secondary tissue has formed. The bract and scale traces do not fuse in Douglas-fir cones as they do in some of the pines (1). Fusion in these species generally occurs at the base of the cone where there are very short internodes. The length of the fused gap does vary in the mature cone of Douglas-fir. The length of the gap increases, as a result of increased elongation, in the distal portion of the cone. The bract and scale bundles are, therefore, nearly fused at the base of the cone (fig. 103). In the center of the cone they are separated somewhat more (fig. 102), and still more in the distal portions (fig. 101).

The primary phloem of the two strands leading into the scale fuse as the procambial strands enlarge, and form an arch above the primary xylem. In a lateral vegetative shoot this arch enlarges and fuses to form a cylinder with phloem outside the xylem. The scale, however, is a flattened rather than a cylindrical structure, and vascular tissue forms a broad band of bundles the width of the scale (fig. 70, 101). The provascular arch becomes flattened in the base of the scale and is reflexed opposite to that in the vegetative shoot as it differentiates acropetally. The xylem thus differentiates on the abaxial side of the collateral bundle, with the phloem on the adaxial side. The fused bundles branch dichotomously many times in the base and the margin of the scale and less frequently in the central portion of the scale (fig. 99-101).

DISCUSSION

The time of initiation of lateral vegetative, microsporangiate and megasporangiate primordia of Douglas-fir in the Corvallis, Oregon area has been established as the second week of April for the 1962 growing season. The time is dependent upon environmental factors as reviewed by Mathews (41, p. 2-3) and, therefore, varies from year to year. The time of initiation, however, can be accurately determined by using the stages in growth periodicity of the vegetative shoot as points of reference. On this basis, lateral bud primordia are initiated shortly after the onset of mitotic activity in the vegetative shoot in which they develop, or about one month before the vegetative buds burst. Initiation coincides with bud bursting of the current season's megasporangiate cones.

The three bud types are not truly axillary in origin. Primordia arise similarly from cortical cells just above the axil of the leaf, while cells at the juncture of the leaf and cortex do not appear to take an active part in bud initiation. This distinction becomes apparent when the origin of the scale is carefully studied because the cells in the axil of the bract are primarily responsible for scale initiation, while those in the cortex above are not involved. The origin of lateral buds, however, has been termed axillary in studies made in several conifer species (42; 16, p. 31; 48).

The characteristic zonal pattern in the apex of conifers is recognizable in all lateral bud primordia after an early period of apical enlargement. The lateral vegetative and megasporangiate apices

are essentially indistinguishable until all bud scales have been initiated; however, the megasporangiate apex may be slightly larger during this phase of growth. The smaller size of the microsporangiate shoot during cataphyll initiation is a result of fewer cells being involved in the initiation as well as the reduced activity of the cells during apical enlargement. As a result, zonation is apparent within the lateral vegetative and megasporangiate buds within two or three weeks of the time of initiation, which is one or two weeks earlier than in the microsporangiate apex.

Comparison of lateral reproductive and vegetative apical meristems shows that there is no marked difference in the zonal pattern. There is no reserved portion of the apical meristem which is concerned primarily with the formation of reproductive shoots. This is contrary to the interpretation in many dicots (43, p. 38-44) and in agreement with observations for the few conifer species that have been studied (28, 26). The spatial relations of the zones may vary between the two categories of shoots but the basic organization remains. In apices of comparable size, zonation is no more apparent in the vegetative than in the reproductive shoot. The distinctness in size shown by the microsporangiate bud from the time of its initiation suggests that the bud types are distinct throughout development and that reproductive buds do not arise by the stimulation of a previously latent portion of a single common bud type (43, p. 38-44). Slight variation in zonation and size of the different apices loses much of its significance when the apices are studied throughout the year. In plants having a growth periodicity, such as Douglas-fir, the vegetative apex alone

shows as much variation during different times of the year (47) as there is between the vegetative and reproductive shoots during early stages of development.

Cataphylls are initiated over a three and one-half month period from early April to mid-July. Cataphyll initiation is the same in the three bud types. The simultaneous initiation of two oppositely placed cataphylls as the first two bud scales on the axillary shoot is consistent in the three bud types and corresponds to the situation reported for vegetative and reproductive lateral buds in many seed plants (36, p. 258). These two cataphylls are initiated when the bud primordium is only a few cells high. Subsequent cataphylls are not initiated in pairs, which marks the beginning of the spiral arrangement of foliar organs. Cataphylls, although initiated like other foliar organs, undergo more extensive laminar growth similar to that described for Pinus lambertiana (45). The lack of vascular tissue beyond the base of any of the cataphylls agrees with the observations of the terminal vegetative bud of Douglas-fir (47).

The apices of lateral buds undergo a marked enlargement at the end of cataphyll initiation. This is similar to that described for the apex of the terminal shoot (47) except that no mamillary apex was observed in either the lateral vegetative or reproductive apices. The subsequent rapid initiation of foliar organs (leaves, bracts and microsporangia) begins about mid-July and is similar to cataphyll initiation. In all cases, procambium differentiates acropetally to the base of the presumptive foliar primordium. A varying number of protodermal cells opposite the procambial strand then divide periclinally, followed by

both periclinal and anticlinal divisions of the derivatives, to originate the foliar primordium. Similar observations in Douglas-fir have been reported by Allen (4, p. 548-550) for microsporophyll initiation. Distinct apical initials have been described in the leaf primordia of some conifers (14, 15, p. 293-295) but they are not present in any of the foliar organs of Douglas-fir. A group of subapical initials appears in the cataphyll, leaf and bract primordia which assume an early vertical growth. These, however, function for a relatively short time, then differentiate and subsequent elongation results from intercalary growth. Subapical initials are not evident in microsporophyll primordia and these primordia do not develop the pointed tip present in other foliar primordia.

Bract initiation in the megasporangiate primordium is continuous from mid-July to early October. The apex, which showed the most conspicuous zonation at the onset of bract initiation, becomes restricted to a broad flat zone with indistinct zonation by the end of bract initiation. The decrease in distinct zonation is more rapid and complete than in the vegetative shoot (47). This appearance is maintained in the dormant megasporangiate shoot. Distinct zonation does not appear again in the spring, although there is some apical activity and a few rudimentary bracts are initiated. Proliferation that results in the formation of a complete leaf-bearing vegetative bud which elongates the following spring occurs but very infrequently (39). A similar pattern of apical reduction is evident in the dormant microsporangiate apex, except that here the apex is more differentiated. The present study agrees with observations on the relationship between apical

zonation and growth periodicity for the vegetative and megasporangiate shoot of Torreya(35, p. 505-510; 36, p. 258-259), the vegetative shoot of Douglas-fir (47), Pinus lambertiana and P. ponderosa and species of Abies and Picea (44). In all these cases, the zonal pattern is most distinct during foliar initiation but is reduced to a minimum degree of clarity during periods of inactivity. This relationship apparently does not exist in Pinus montana (44).

Scale initiation begins early in September and continues until the cones become dormant early in November. Although the morphology of the scale has been studied extensively (16, p. 82-92; 22, p. 367-384), scale initiation in the Abieteneae has not been studied in detail. The present study shows that scales are initiated from truly axillary cells rather than from cortical cells. It is, therefore, distinct from any other type of lateral shoot initiation reported in the conifers. Scale initiation is similar to other lateral shoots of Douglas-fir and certain pteriodiophytes (48) in that they are not presaged by the development of procambial strands. Bundles develop later which link the primordium with the vascular system of the shoot. Scales differ from other lateral shoots in that a recognizable apical zonation similar to vegetative shoots is present for only a brief time in the fall. The onset of marginal growth following dormancy changes this organization to a type of submarginal meristem. The submarginal meristem is similar to that of many conifer leaves but soon differentiates and is replaced by a rudimentary submarginal meristem at the tips of the procambial strands. The dorso-ventrally flattened nature of the scale, and the related band of vascular tissue rather than a

vascular cylinder, further distinguishes the scale from other lateral shoots.

Megasporangiate buds become dormant early in November and resume growth again in early March. Growth in the spring is marked by rapid elongation and enlargement of bracts and scales. Within one month, the buds elongate from 2.7 mm to nearly 2 cm when they burst through the bud scales. The bracts enlarge more rapidly than the scales and are first to reach their maximum size. The shape of the bract established prior to dormancy is essentially retained during subsequent enlargement. The scales assume a spoon-shaped appearance as a result of a form of marginal growth soon after dormancy is broken. This appearance is maintained throughout scale enlargement. The cone reaches its maximum size in early July and maturation of tissues occurs in July and August. Maturation is generally complete early in September when the cones become dry and open.

Seventeen months elapse between initiation and maturity of the megasporangiate cone. Table 1 summarizes the time sequence during the early development of all bud types and the complete development of the megasporangiate cone.

TABLE 1
TIME OF ORIGIN AND PERIODS REQUIRED
FOR DEVELOPMENT OF BUDS AND CONES

Observation	Date	Time Elapsed From Bud Initiation
All Buds		
Lateral bud primordia initiated	Early April	(megasporangiate buds burst)
Zonation becomes apparent	Mid-May	1½ months
Cataphyll initiation complete, apical enlargement occurs, leaf, bract or microsporophyll initiation begins	Mid-July	3½ months
Megasporangiate Cone		
Beginning of scale initiation	Early September	5 months
All bracts initiated	Early October	6 months
All scales initiated and megasporangiate buds become dormant	Early November	7 months
Megasporangiate buds resume growth	Early March	11 months
Megasporangiate buds burst and pollination occurs	Early April	12 months
Fertilization	Early June	14 months
Elongation of cone is complete	Early July	15 months
Maturation is complete, cones open and seeds are released	Early September	17 months

The work of Florin (22, p. 367-384) and his synthesis of the work of others has contributed significantly to the understanding of the structure and evolution of conifers and, in particular, the megasporangiate cone. Since the studies began over 200 years ago, the problem has been whether to consider the megasporangiate cone a simple flower or a compound strobilus, i.e., an inflorescence. This problem rests on the morphological nature of the scale. The ovuliferous scale is interpreted as a highly modified, lateral, fertile shoot and not a sporophyll. It has evolved from an ordinarily leafy, ovule bearing dwarf shoot, and its present appearance is a result of the fusion and specialization of both the sterile and fertile components found in the ancestral structure. The present study supports this interpretation of the megasporangiate cone.

The bract of Douglas-fir is homologous to a leaf. The exposed portion of the bract is similar in structure to the leaf except for the broad lamina. A characteristic endodermis and transfusion tissue is present in both, as well as a single vascular strand and two resin canals. The transition in structure in the basal portion of the bract may be a response to being covered by the overlapping scales. Two branch traces supplying each scale support the interpretation of the scale as being a modified, fertile lateral shoot. The separate origin and path of bract and scale vascular bundles agrees with the findings for most members of the Podocarpineae and Abietineae, while fusion of these bundles is most extensive in the Araucarineae (1, p. 295-297). In Douglas-fir, as well as some other members of the Abietineae (1, p. 278-282), no vascular tissue supplies the ovules.

Cone opening is caused by drying and not by growth. An early study on the amount of moisture which must be lost before cones will open was done by Willis (52, p. 995-1000). He showed that the degree of cone opening increased with a decrease in moisture content. No opening was observed until 19 to 34 percent of the wet weight of the cone was lost. Further drying increased the degree of opening until, when 51 percent of the wet weight had been lost, all cones were fully opened. Drying beyond this point does not result in increased separation of the scales. Drying and degree of opening was shown to be a complex result of temperature, humidity and air circulation.

The large zone of macrosclereids in the abaxial basal portion of the scale has not been described for any of the conifers. The position of these cells and their very hygroscopic nature make this tissue responsible for opening of the scales in the mature cone. Thin, dehydrated sections of this tissue have been observed under the microscope to swell with the addition of water and to shrink again when dehydrated. Hand cut longisections about 1 mm thick, which were left attached to a 1 cm section of the cone, have been observed under a dissecting microscope. The basal abaxial zone of sclereids swells when placed in water and the scale closes. Subsequent drying causes this tissue to shrink and the scale opens again. The highly lignified secondary walls of the macrosclereids are evidently responsible for their very hygroscopic nature (33, p. 174-175).

SUMMARY

Lateral vegetative, megasporangiate and microsporangiate buds of Douglas-fir are initiated in early April. Subsequent development and maturation of the megasporangiate cone occurs during the following seventeen months. All lateral buds arise similarly from cortical cells just above the axil of the leaf. A period of apical enlargement and cataphyll initiation follows and apical zonation becomes apparent. The pattern of zonation is similar in the three bud types, but is less distinct in the young microsporangiate apices because of their smaller size. The three bud types are evidently distinct throughout development and reproductive buds normally do not arise by transition from a vegetative type.

Cataphylls are initiated over a three and one-half month period, from early April to mid-July. Apical enlargement occurs at the end of cataphyll initiation and marks the onset of subsequent foliar initiation (leaves, microsporophylls and bracts). Like the cataphylls, these have no distinct apical initials but a group of subapical initials appears for a short period of time and then differentiates. Subsequent foliar enlargement occurs by intercalary growth.

Bract initiation in the megasporangiate primordium is continuous from mid-July to early October. Apical zonation becomes less distinct during the latter part of bract initiation. The decrease in distinct zonation is more rapid and complete than in the vegetative shoot. Distinct zonation does not appear again in the spring, although there is some apical activity and a few rudimentary bracts are initiated.

The relationship between apical zonation and growth periodicity agrees with that of the vegetative shoot of Douglas-fir and several other conifers.

Scale initiation begins early in September and continues until the cones become dormant early in November. Scales are initiated from truly axillary cells unlike other lateral shoots. Apical zonation similar to other lateral shoots is present for a brief time in the fall.

Growth of the megasporangiate cone is resumed early in March and the cone buds burst about one month later. Growth in the spring is marked by rapid elongation of the cone and enlargement of bracts and scales. The shape of the bract established prior to dormancy is essentially maintained during subsequent enlargement. The scales assume a spoon-shaped appearance after dormancy is broken as a result of a form of marginal growth. The cone reaches its maximum size in early July and maturation of tissues occurs in July and August. Maturation is generally complete early in September when the cones become dry and open.

The present study confirms the interpretation that the megasporangiate cone is a compound strobilus and the scale is a highly modified lateral fertile shoot. The bract is homologous to a leaf and basically similar in structure.

Cone opening is caused by drying and not by growth. Drying and the degree of opening is a complex result of temperature, humidity and air circulation. Shrinkage of the large zone of macrosclereids in the abaxial basal portion of the scale causes the scales to open in the mature cone.

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APPENDIX

Figures 1-6

Figures 1-3. Cross sections of elongating vegetative shoots showing stages in lateral bud initiation. X200.

Figure 1. Axillary area of leaf having no lateral bud primordium.

Figure 2. Axillary area of leaf with lateral bud primordium arising from cortical cells of the shoot.

Figure 3. Axillary area of leaf with a cushion-like lateral bud primordium showing the initiation of the first two cataphylls.

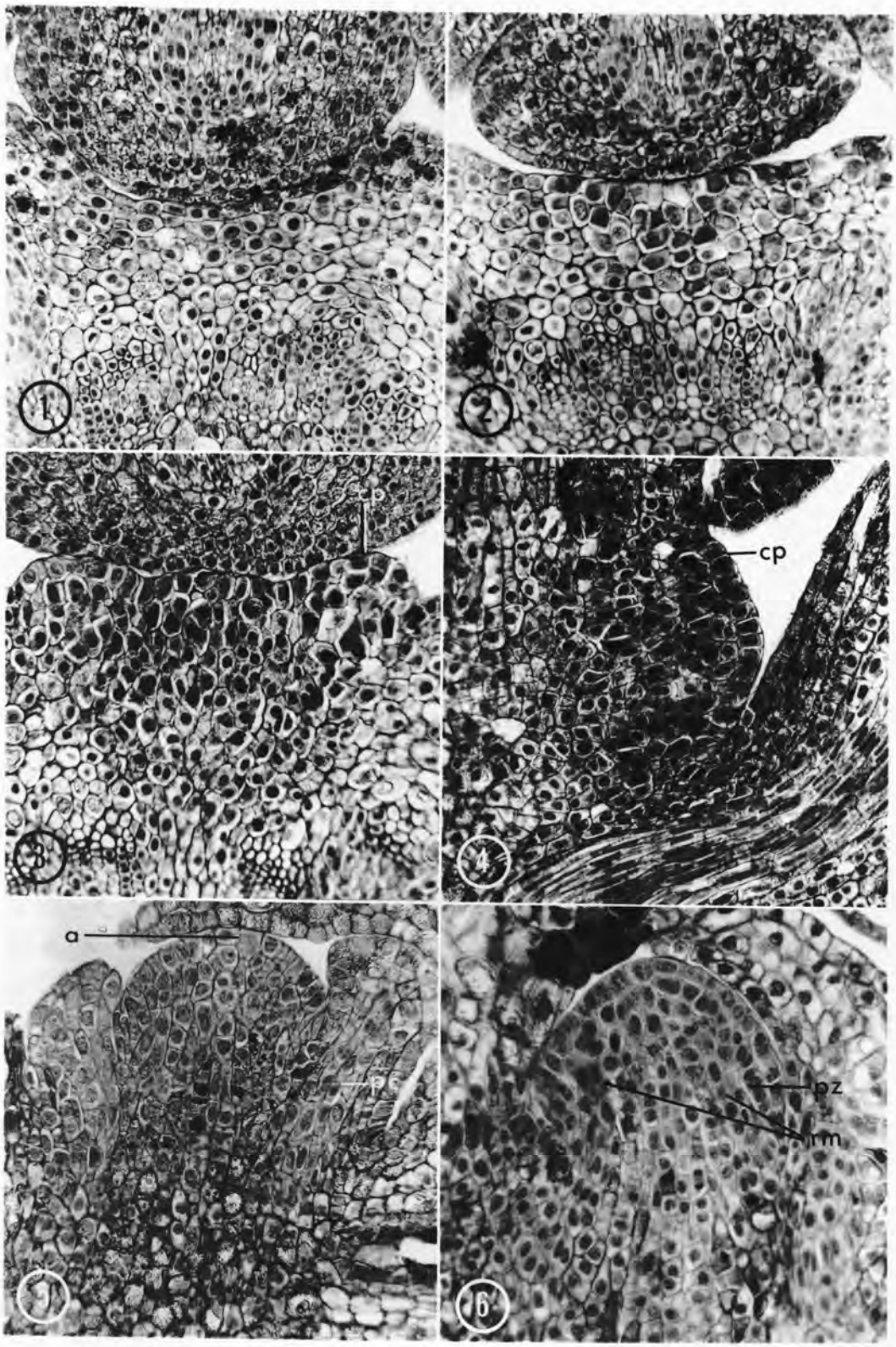
Figures 4-6. Longitudinal sections of stages of lateral bud initiation and enlargement. X200.

Figure 4. Axillary area of a leaf with a lateral bud primordium showing the region of initiation of a cataphyll.

Figure 5. Enlarged lateral bud primordium before zonation has been established. Apical initials of bud and procambial strands supplying cataphylls are evident.

Figure 6. Lateral bud when zonation is first apparent. Rib meristem and peripheral zones are present.

cp - cataphyll primordium
a - apical initials
pc - procambial strand
rm - rib meristem
pz - peripheral zone


1
2
3
cp
4
cp
5
a
p
6
pz
rm

Figures 7-12

Figures 7-11. Median longitudinal sections of lateral buds. X200.

Figure 7. Microsporangiate bud approximately 35 days after bud initiation. Note smaller size and fewer cells in apex than in figures 8 and 9.

Figure 8. Megasporangiate bud approximately 35 days after bud initiation.

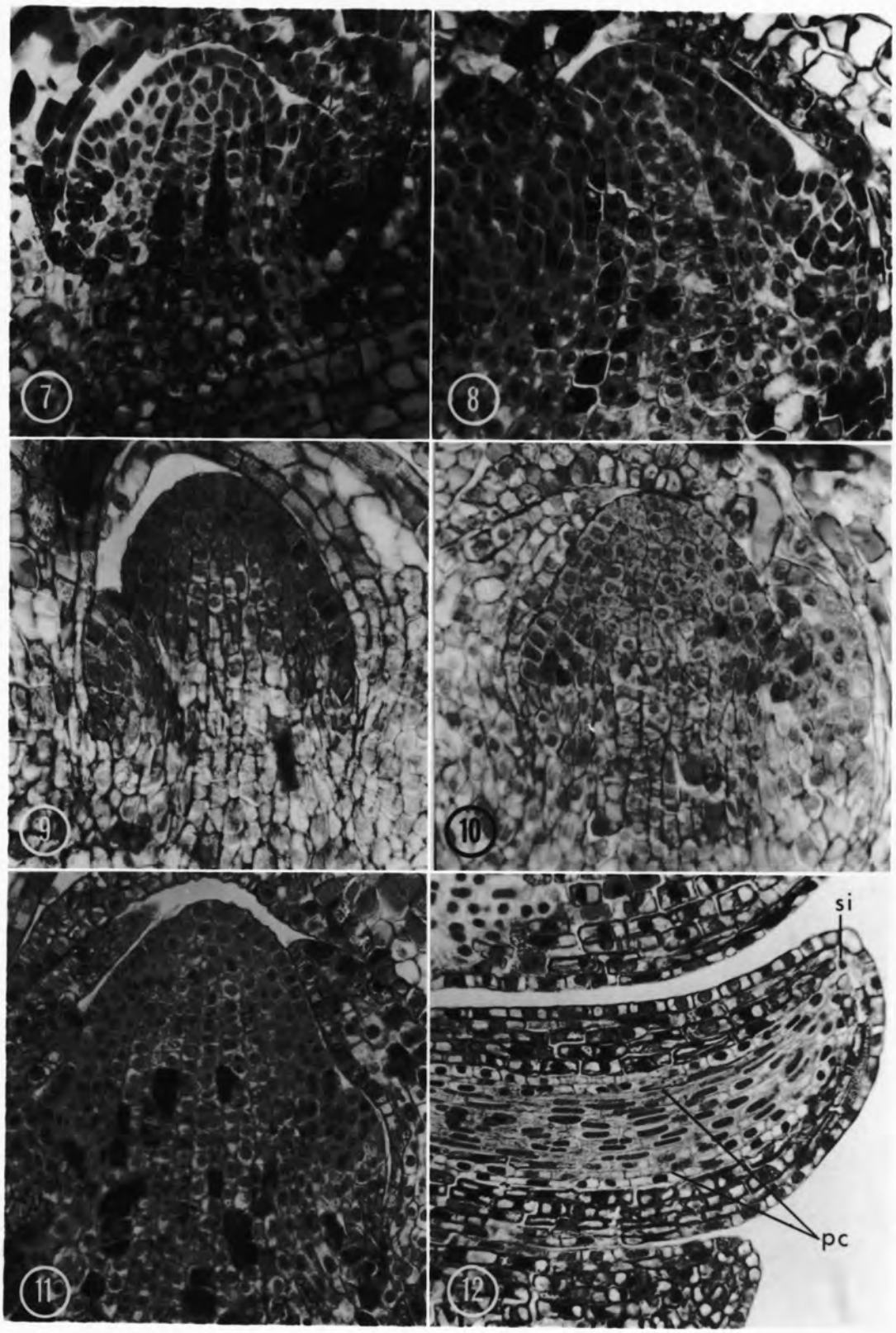
Figure 9. Lateral vegetative bud approximately 35 days after bud initiation.

Figure 10. Lateral vegetative bud during cataphyll initiation.

Figure 11. Lateral vegetative bud during the latter part of cataphyll initiation.

Figure 12. Longitudinal section of a leaf primordium in the dormant vegetative bud. X200.

pc - procambium
si - subapical initials


7
8
9
10
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12
si
pc

Figures 13-18

Figures 13-18. Longitudinal sections of microsporangiate buds.

Figure 13. Apex during cataphyll initiation approximately two and one-half months after bud initiation. Note rudimentary zonation in the small, broad apex. X200.

Figure 14. Enlarged conical apex in early July near the end of the period of cataphyll initiation. X200.

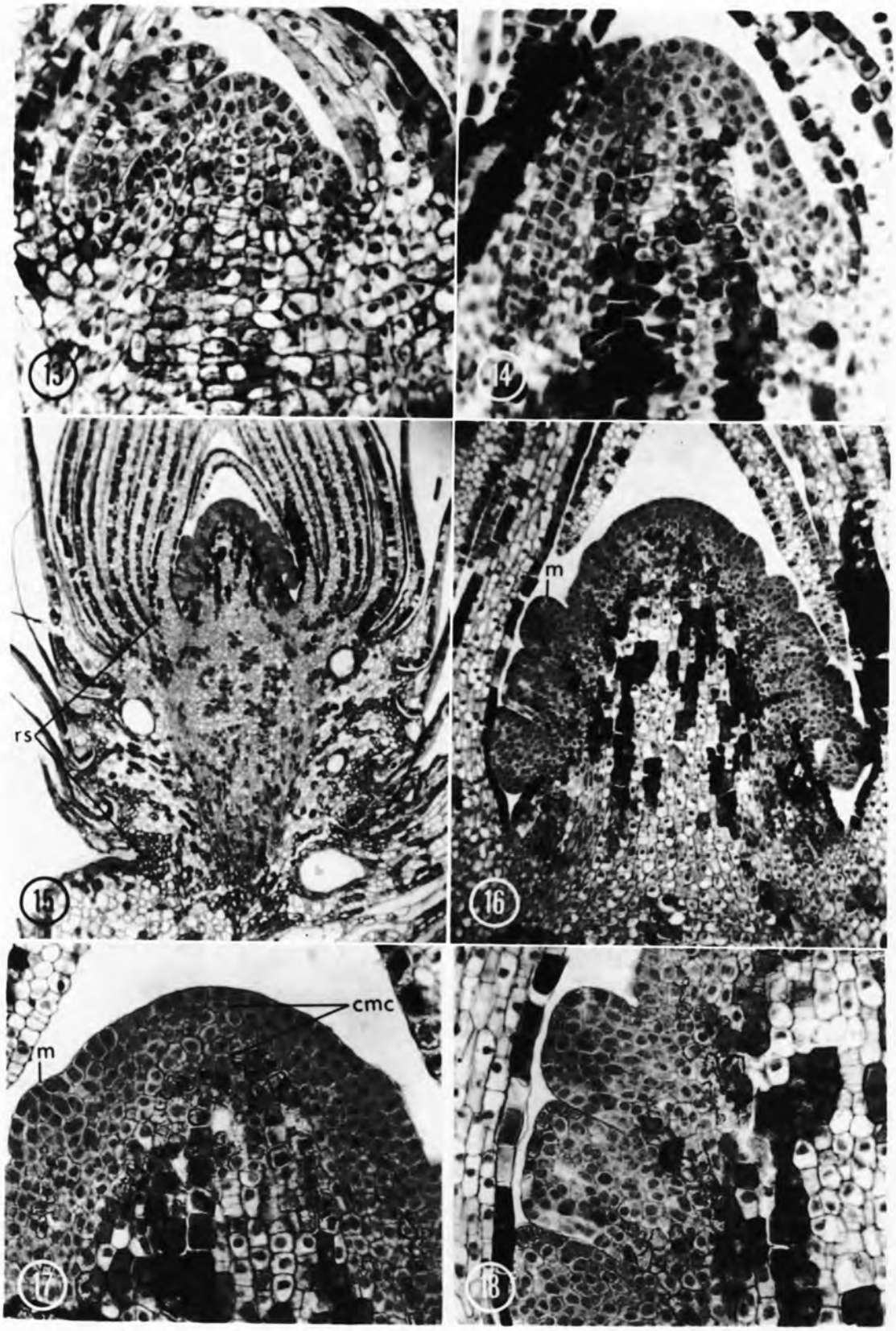
Figure 15. Bud with about half the microsporophylls initiated. Note the receptacular structure on which the cataphylls are borne. X31.

Figure 16. Bud about four and one-half months after bud initiation showing microsporophyll initiation and broadening of the central column of pith mother cells. X100.

Figure 17. Broad bud apex during microsporophyll initiation showing very restricted central mother cell zone, wide peripheral zone and initiation of microsporophyll. X200.

Figure 18. Blunt microsporophyll primordia. X200.

m - microsporophyll
rs - receptacular structure
cmc - central mother cells


13
14
rs
15
m
16
cmc
m
17
18

Figures 19-24. Longitudinal sections of megasporangiate buds. X200 (Fig. 21, X31).

Figure 19. Apex with distinct zonation during cataphyll initiation.

Figure 20. Apex at the end of cataphyll initiation and the onset of bract initiation, about mid-July.

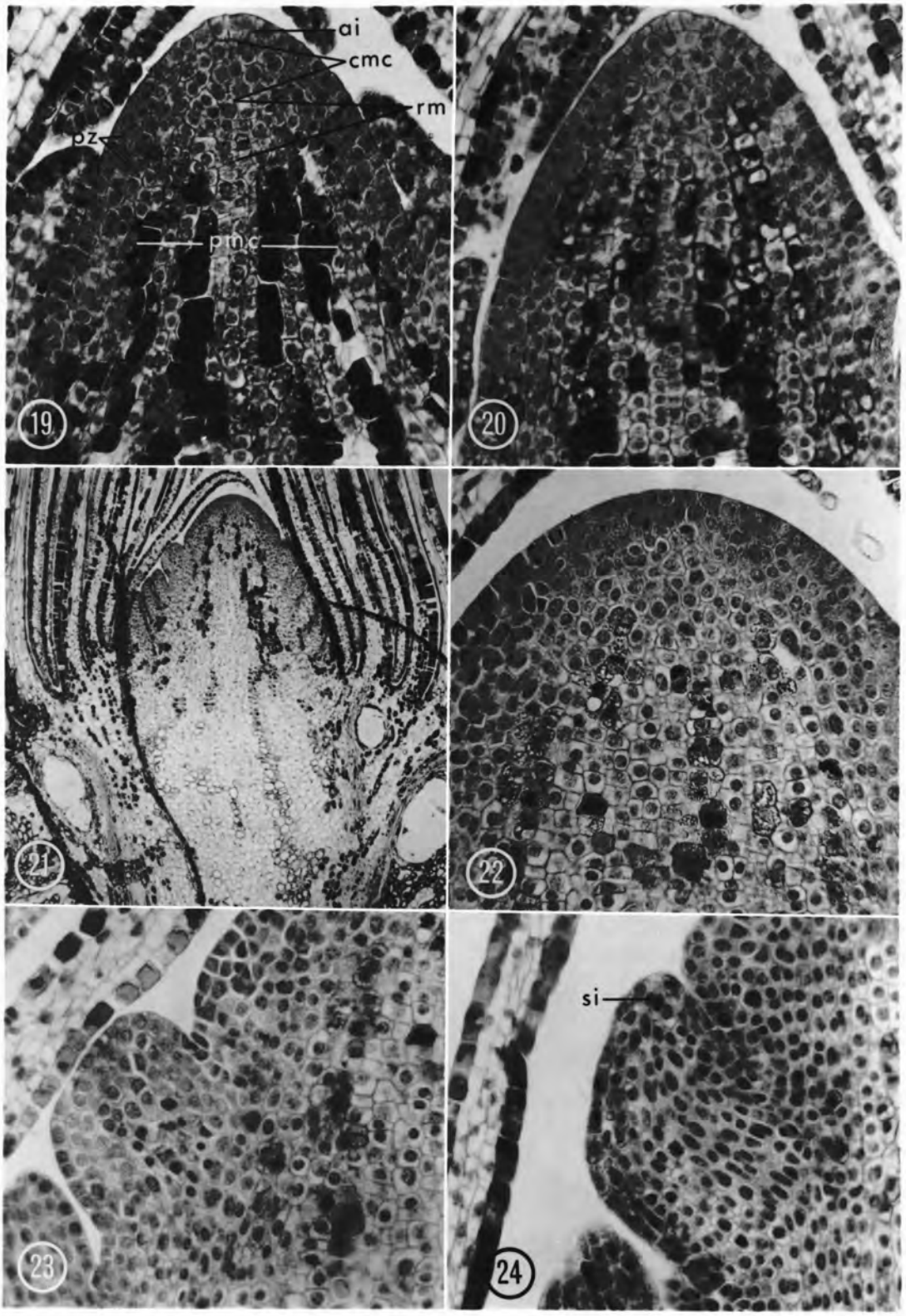
Figure 21. Megasporangiate bud when half the final number of bracts have been initiated, about mid-August. Note broad pith mother cell zone.

Figure 22. Flattened dome-shaped apex with indistinct zonation, about mid-August. Note both anticlinal and periclinal divisions in protoderm and broad pith mother cell region.

Figure 23. Bract primordia.

Figure 24. Beginning of upward growth of the bract primordium showing subapical initials.

ai - apical initials
cmc - central mother cells
rm - rib meristem
pz - peripheral zone
pmc - pith mother cells
si - subapical initials


ai
cmc
rm
pz
pmc
19
20
21
22
23
si
24

Figures 25-30

Figures 25-26. Longitudinal sections of elongating bracts.

Figure 25. Elongating bract with meristematic central column of cells. X200.

Figure 26. Elongating bract showing subapical initials which give rise to the darkly stained peripheral zone beneath the epidermis and the central column of cells. X100.

Figures 27-30. Cross sections of developing bracts. X200.

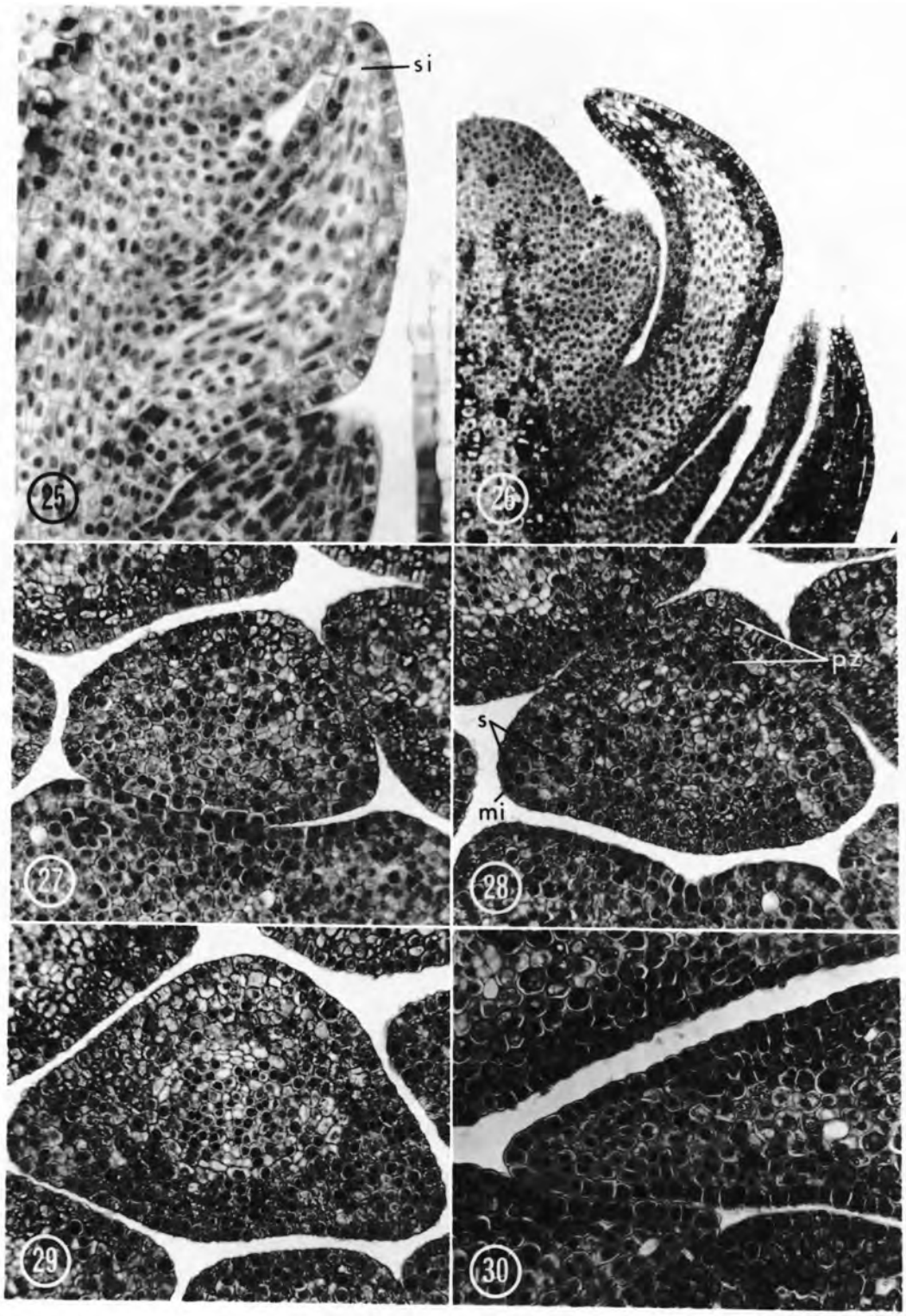
Figure 27. Elliptical bract primordium prior to initiation of marginal growth.

Figure 28. Bract at the onset of marginal growth. Note peripheral zone, marginal initials and submarginal initials.

Figure 29. Bract with a rudimentary lamina.

Figure 30. Lamina of bract showing a distinct marginal initial and a group of submarginal initials.

si - subapical initials
pz - peripheral zone of bract
s - submarginal initials
mi - marginal initial


si
25
26
27
pz
s
mi
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29
30

Figures 31-34

Figures 31-34. Cross sections of developing bracts. X200.

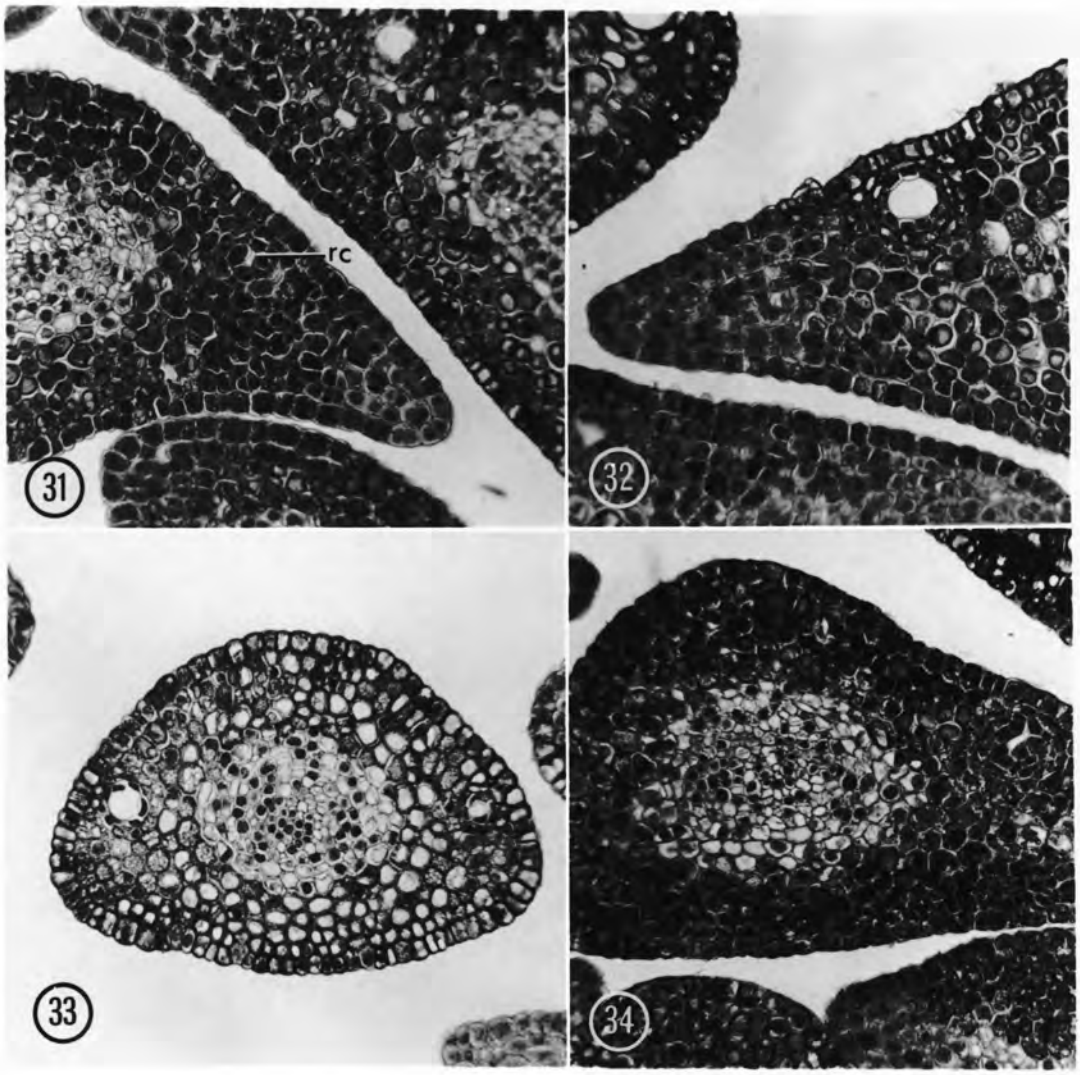
Figure 31. Initiation of resin canal below the abaxial epidermis.

Figure 32. Resin canal in a bract in the dormant bud.

Figure 33. Distal portion of bract where no lamina differentiates.

Figure 34. Bract in dormant bud showing central procambial strand of somewhat radially arranged cells, surrounding light meristematic cells and outer darkly stained peripheral zone of parenchyma cells.

rc - resin canal


rc
31
32
33
34

Figures 35-40

Figures 35-38. Median longitudinal sections of a megasporangiate cone showing stages of scale initiation in the axillary region of the bract. X400.

Figure 35. Axillary region of bract before scale initiation. Cone axis is to the left.

Figure 36. Initiation of scale from axillary cells. Cone axis is to the left.

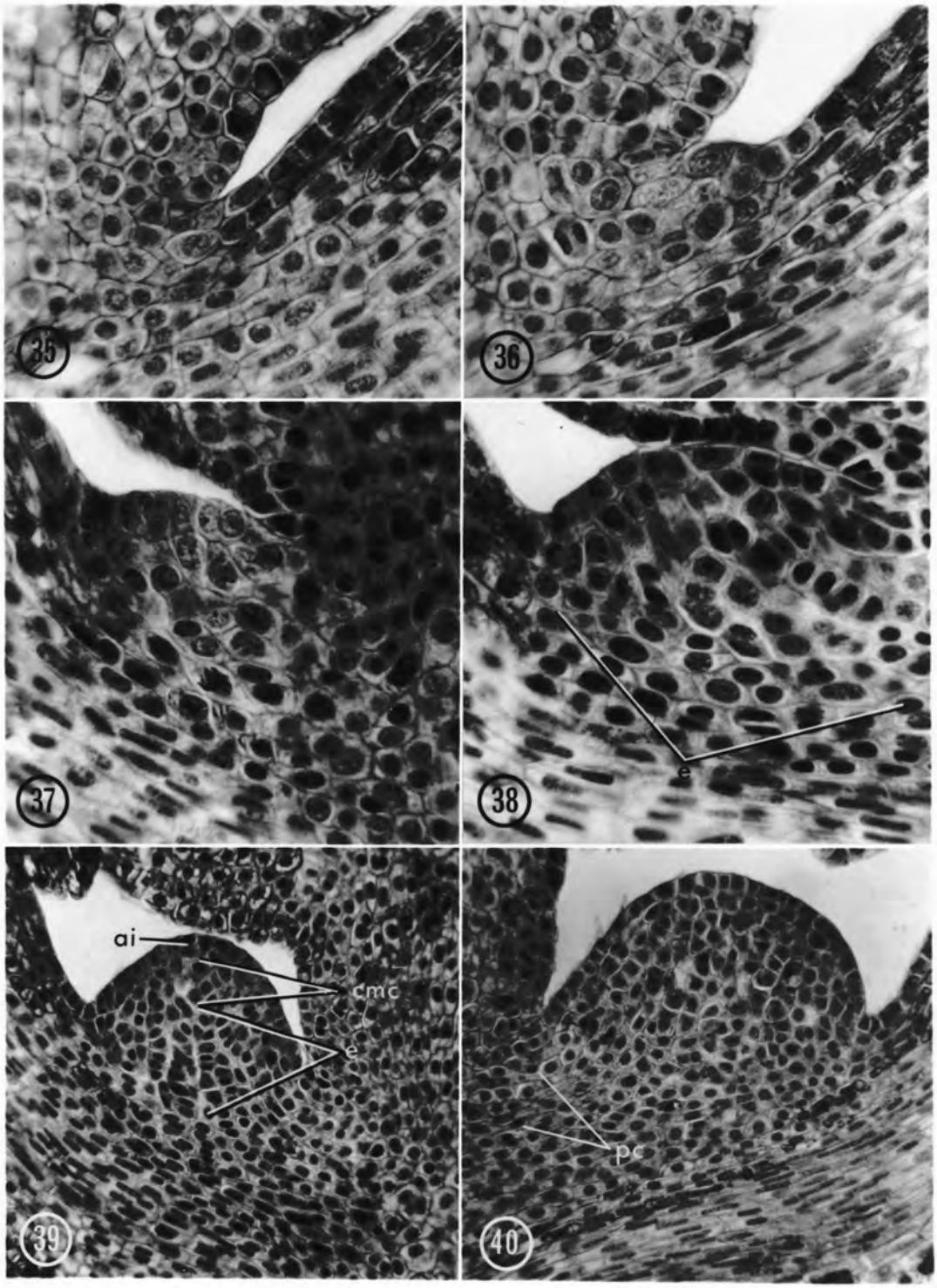
Figure 37. Extension of scale primordium along the adaxial bract surface by activities of epidermal cells of the bract. Cone axis is to the right.

Figure 38. Enlargement of scale primordium and initiation of eumeristem. Cone axis is to the right.

Figure 39. Median longitudinal section of a scale primordium showing a rudimentary pattern of zonation. Cone axis is to the right. X200.

Figure 40. Non-median longitudinal section of a scale primordium showing no pattern of zonation. Cone axis is to the left. X200.

e - eumeristem
cmc - central mother cells
ai - apical initials
pc - procambial region


35
36
37
38
e
ai
cmc
e
39
pc
40

Figures 41-46

Figures 41-43. Longitudinal sections of megasporangiate cone showing scale development. X200.

Figure 41. Non-median portion of scale primordium, nearer the lateral margin than Figure 40. No zonation apparent.

Figure 42. Median portion of scale showing growth of scale primordium parallel to bract surface. Rudimentary apical zonation is lost and long rows of genetically related cells are produced.

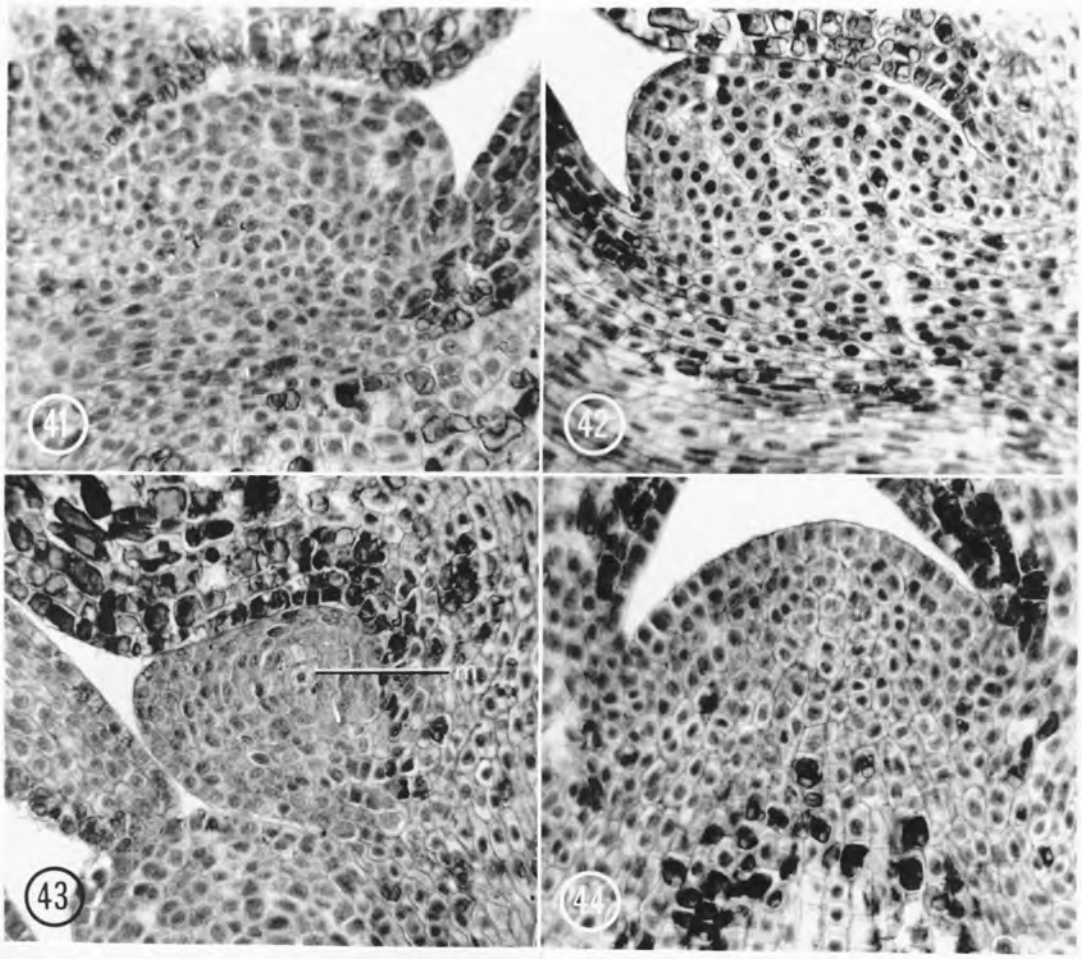
Figure 43. Lateral portion of scale primordium showing megaspore mother cell.

Figure 44. Median longitudinal section of the small apex of a dormant megasporangiate cone. Central mother cells are small and difficult to distinguish from other zones. X200.

Figure 45. Median longitudinal section through dormant megasporangiate cone. Note long bracts, scales and crown region. X17.

Figure 46. Dormant whole buds with bud scales removed. a - terminal vegetative, b - lateral vegetative, c - megasporangiate, d - microsporangiate. X3.5.

m - megaspore mother cell
cr - crown region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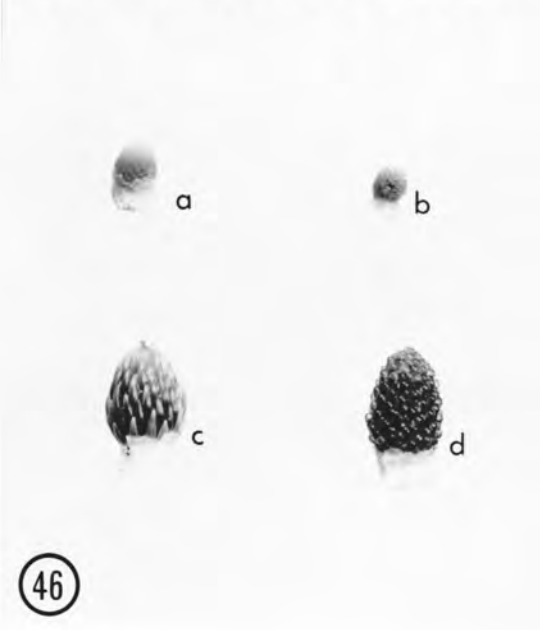
cr
45


a
b
c
d
46

Figures 47-50

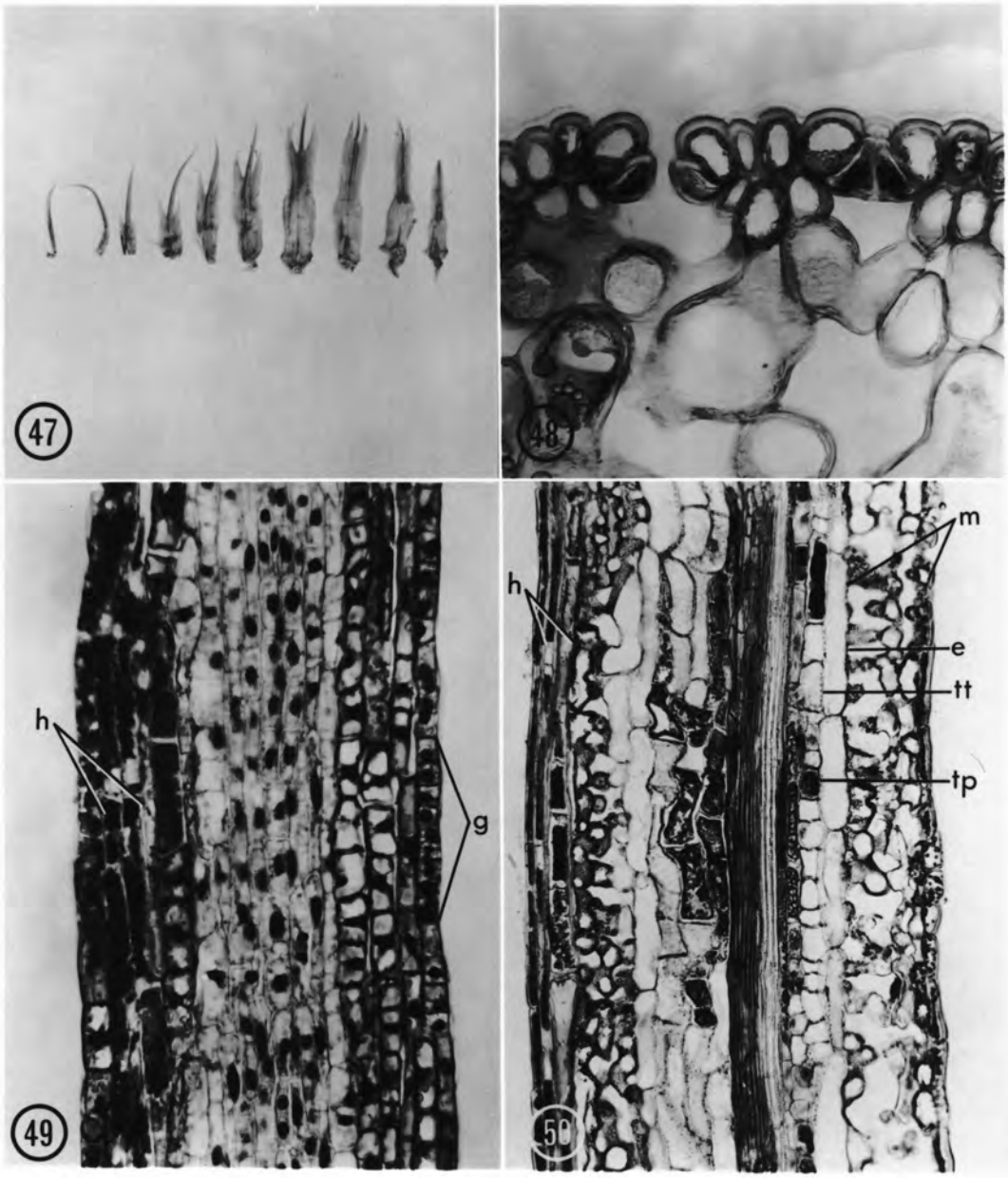
Figure 47. Bracts from mature cone arranged from left to right as they appear from the base to the top of the cone. X3/4.

Figure 48. Cross section of distal end of mature bract showing thick walled epidermis and guard cells overtopped by subsidiary cells. X430.

Figure 49. Median longitudinal section of bract at the onset of intercalary elongation in the spring. X200.

Figure 50. Median longitudinal section of distal portion of a mature bract. Xylem is to the right and phloem to the left in the vascular bundle. X100.

g - guard cell initials
h - hypodermal layer
e - endodermis
tt - transfusion tracheid
tp - transfusion parenchyma
m - mesophyll


47
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h
g
49
h
m
e
tt
tp
50

Figures 51-56. Cross sections of mature bracts. In all, the xylem lies above the phloem.

Figure 51. Distal portion of bract with distinct mesophyll, endodermis and transfusion tissue similar to the leaf. Note hypodermis on both abaxial and adaxial sides of bract. X100.

Figure 52. Vascular bundle of bract (Figure 51) showing transfusion parenchyma, and albuminous cells. X200.

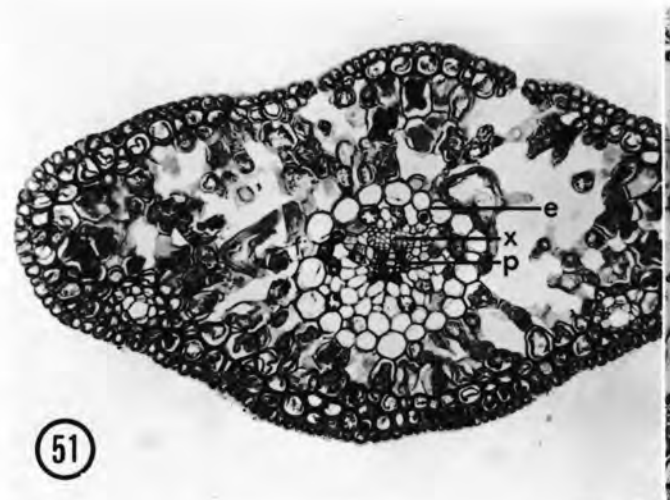
Figure 53. Mid-rib below the forked portion of the bract. No distinct endodermis is present and the hypodermis is most evident on the lower (abaxial) side of the bract. X100.

Figure 54. Vascular bundle of bract (Figure 53) showing pitted transfusion tracheids, transfusion parenchyma and albuminous cells. X200.

Figure 55. Mid-rib of basal, covered portion of the bract with cylindrical mesophyll cells, no endodermis or distinct transfusion tissue. X100.

Figure 56. Vascular bundle of basal, covered portion of the bract (Figure 55) separated into two strands by uniseriate ray-like plate of xylem and phloem parenchyma. X200.

a - albuminous cells
e - endodermis
x - xylem
p - phloem
tp - transfusion parenchyma
tt - transfusion tracheids



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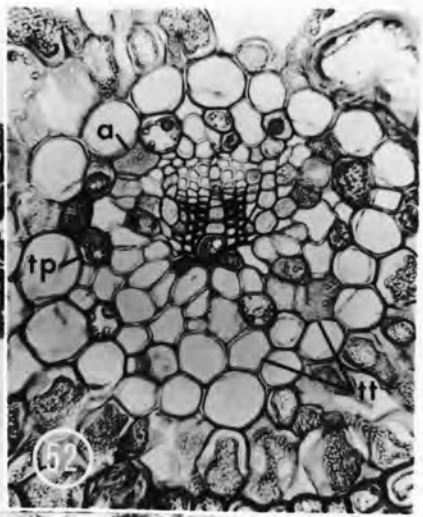

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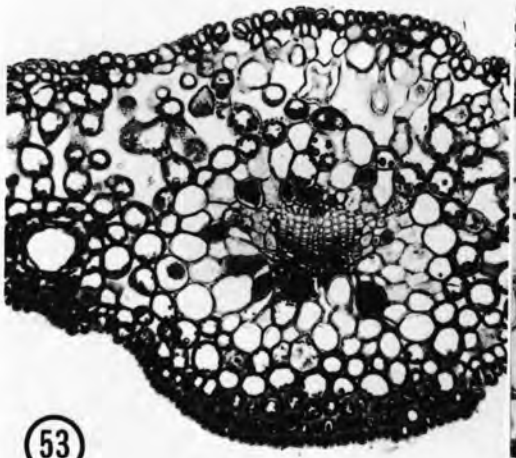

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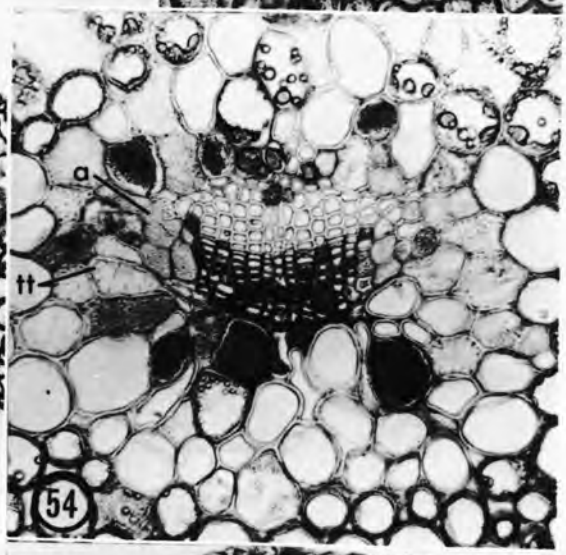

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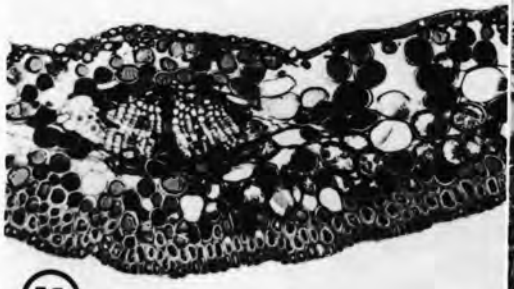

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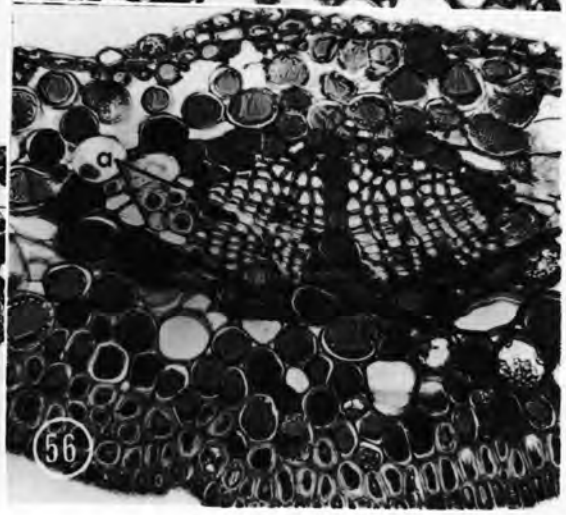


56

Figures 57-60. Median longitudinal sections of developing scales following dormancy. Cone axis is to the right in all figures.

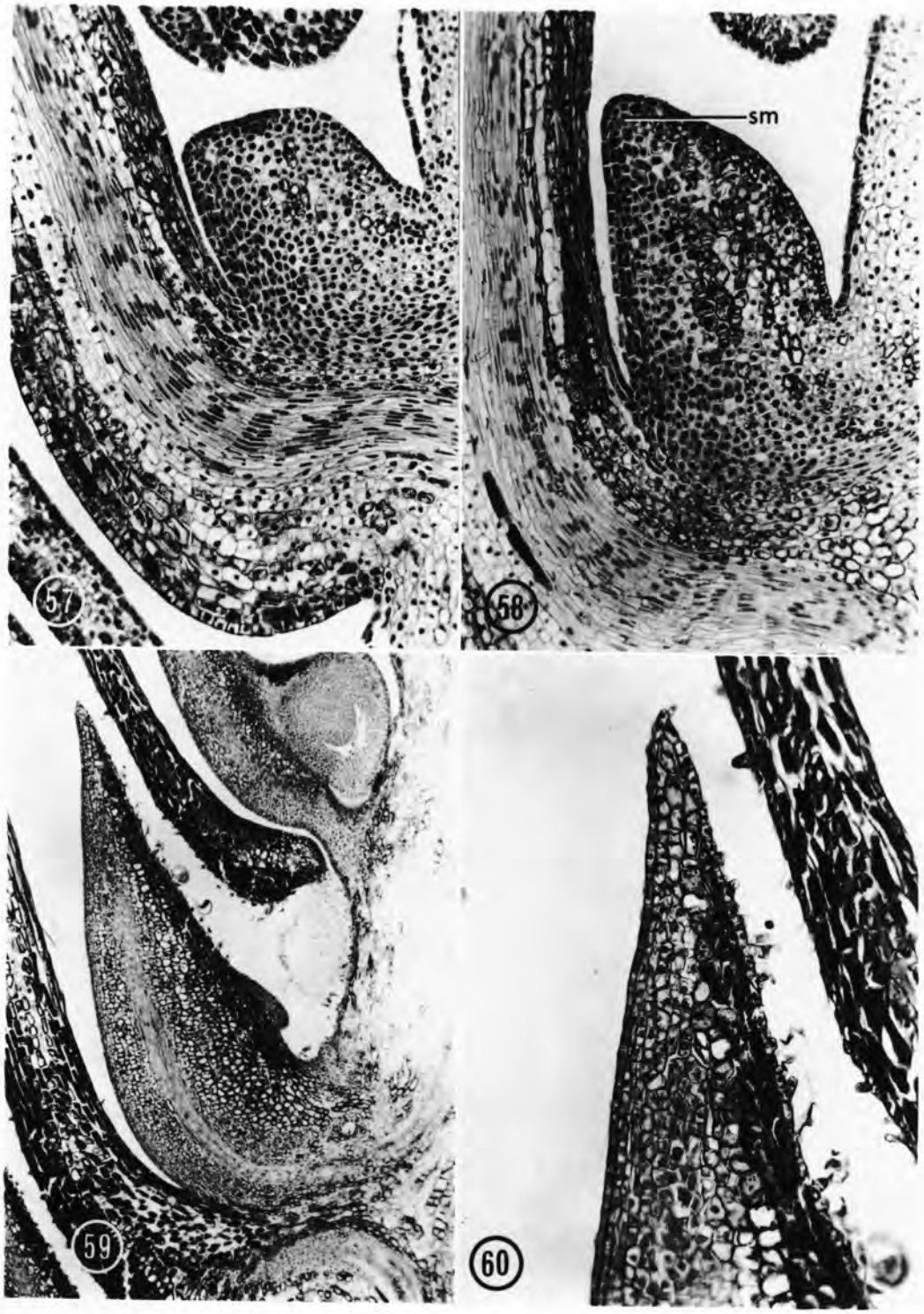
Figure 57. Scale at the onset of marginal growth. Cells in abaxial part of scale divide more frequently than those in the adaxial portion. X100.

Figure 58. Scale just before the time of bud burst with abaxial, adaxial and central procambial zones and submarginal meristem. X100.

Figure 59. Scale about 2 mm long during time of pollination. Abaxial, adaxial and central procambial zones are distinct. X35.

Figure 60. Pointed margin of scale (Figure 59) showing differentiated cells of the submarginal meristem. X100.

sm - submarginal meristem


sm
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Figures 61-66. Cross sections of developing scales showing vascular bundle differentiation. Xylem differentiates on lower (abaxial) side of the scale.

Figure 61. Scale just before time of bud burst (Figure 58) with concentrically arranged provascular strands in the band of meristematic cells. X100.

Figure 62. Developing vascular bundle with primary xylem and phloem and radially arranged rows of cells in the cambial region. X200.

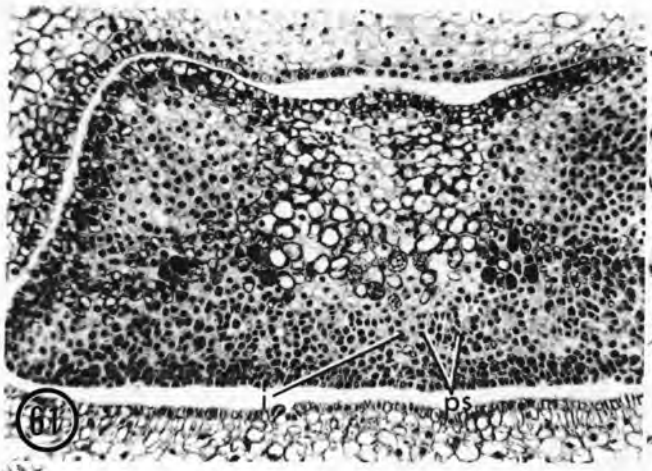
Figure 63. Half of the scale showing provascular strands and interfascicular areas in the abaxial side. X100.

Figure 64. Developing vascular bundle with primary xylem and phloem and broad zone of undifferentiated xylem and phloem mother cells between. Note small undifferentiated cells around the vascular bundle. X200.

Figure 65. Fully differentiated wide vascular bundle at the base of the scale. Note small interfascicular area of crushed cells and the developing macrosclereids on the abaxial side of the bundle. X200.

Figure 66. Fully differentiated vascular bundle from the central part of scale with broad area of secondary fiber-tracheids and narrow area of phloem. Note crushed phloem on adaxial side and macrosclereids on abaxial side. X200.

ps - provascular strand
i - interfascicular region
x - xylem
p - phloem
m - macrosclereid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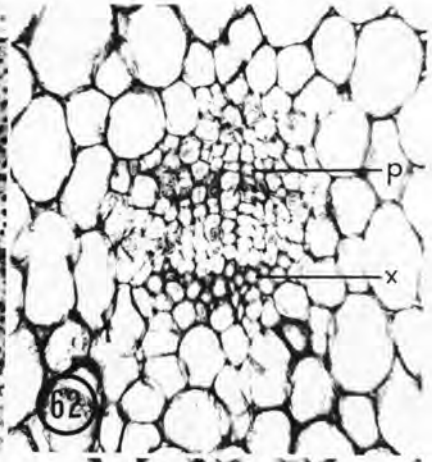
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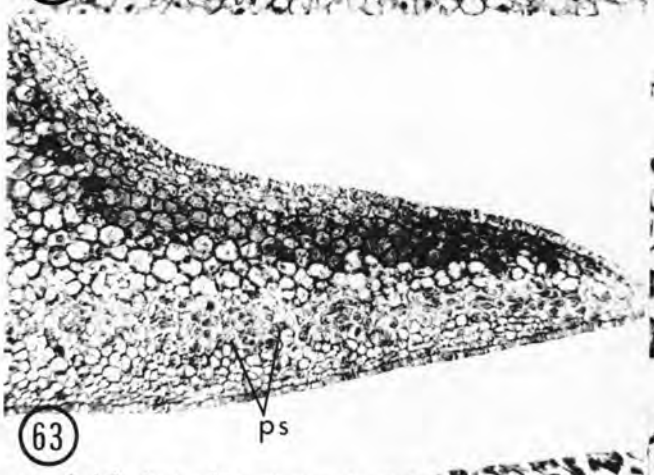
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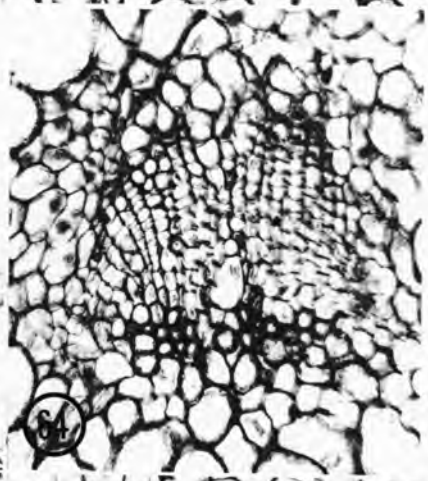
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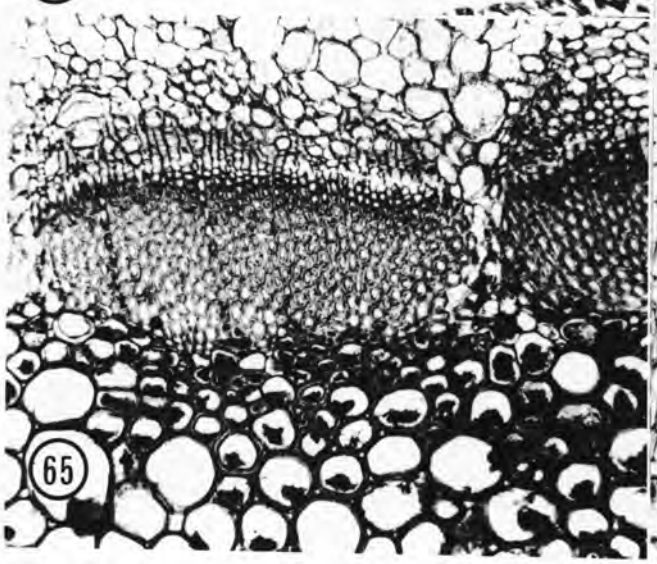
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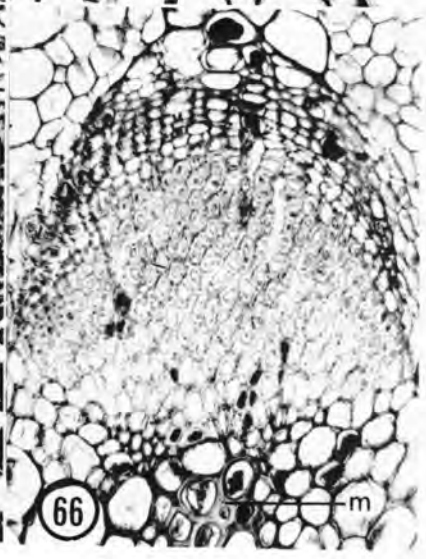

m
66

Figures 67-72

Figure 67. Scales and bracts from a mature cone. Those from distal portion are on the left. X3/4.

Figure 68. Fully elongated cone collected in early August. X1/2.

Figure 69. Non-median longitudinal section through a mature scale showing the layout of the various tissues. Numbers 1, 2 and 3 indicate approximate positions of sections shown in Figures 70, 71 and 72, respectively. X3.5.

Figures 70-72. Cross sections through a mature scale. The lower side is the abaxial side. X3.5.

Figure 70. Base of scale showing band of vascular tissue, large zone of macrosclereids on abaxial side and parenchyma tissue on adaxial side.

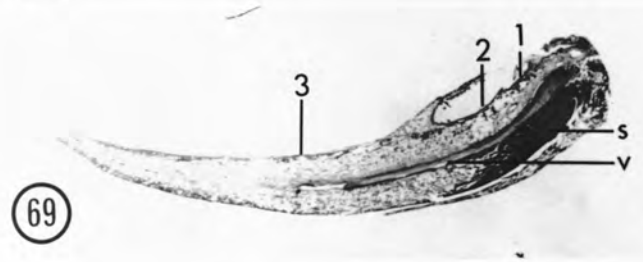
Figure 71. Proximal portion of scale with many branched vascular bundles.

Figure 72. Median portion of scale with numerous small vascular bundles and no macrosclereids.

v - vascular bundle
s - macrosclereids

67

68



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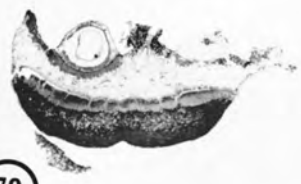

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72

Figures 73-78

Figure 73. Longitudinal section through the base of a scale at the time of pollination. Outer zone of elongated cells and inner zone of isodiametric cells all differentiate into macrosclereids. X100.

Figure 74. Longitudinal section through the base of a scale about 1 cm long showing inner and outer layers of cells which differentiate into macrosclereids. Lower side is the abaxial surface of the scale. X100.

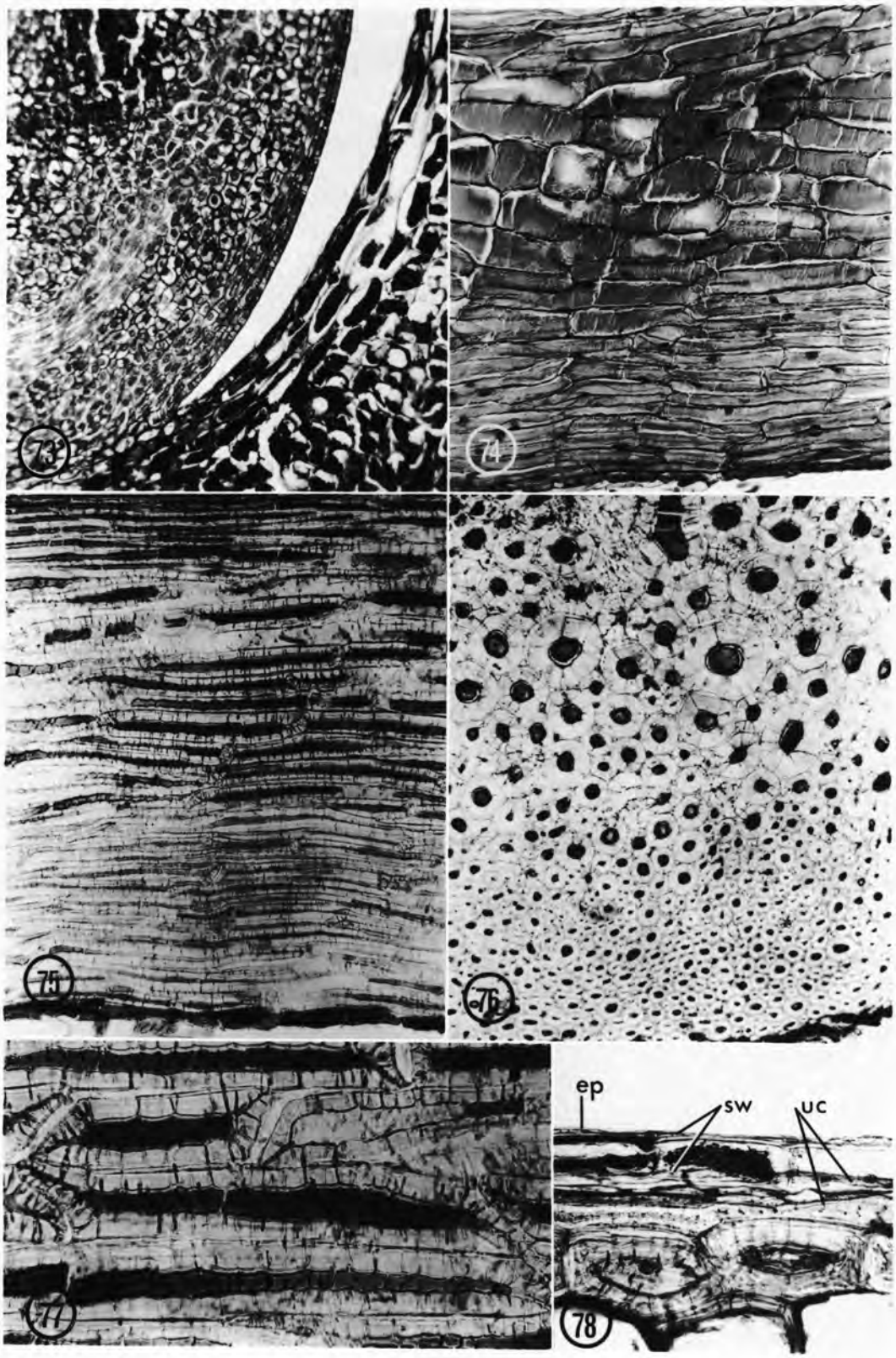
Figure 75. Longitudinal section of mature macrosclereids in the abaxial base of the scale. X100.

Figure 76. Cross section of mature macrosclereids in the abaxial base of the scale. Note branched pits. X100.

Figure 77. Longitudinal section of mature macrosclereids in abaxial base of the scale showing branched pits and overlapping end walls. X200.

Figure 78. Longitudinal section of adaxial side of the scale showing seed wing and underlying thick walled brachysclereids. Note thin outer epidermal layer and undifferentiated cells between seed wing and the scale. X200.

ep - epidermis
sw - seed wing
uc - undifferentiated cells


ep
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Figures 79-84

Figure 79. Longitudinal section of scale and developing ovule. Three layers of the integument can be distinguished which differentiate into the layers of the seed coat. X35.

Figure 80. Longitudinal section through the nearly mature seed coat. The upper layer is the inner layer and the thick walled brachysclereids form the middle layer. The outer layer is not well developed and is indistinguishable from the underlying layer of undifferentiated cells responsible for separation of the seed from the scale. X200.

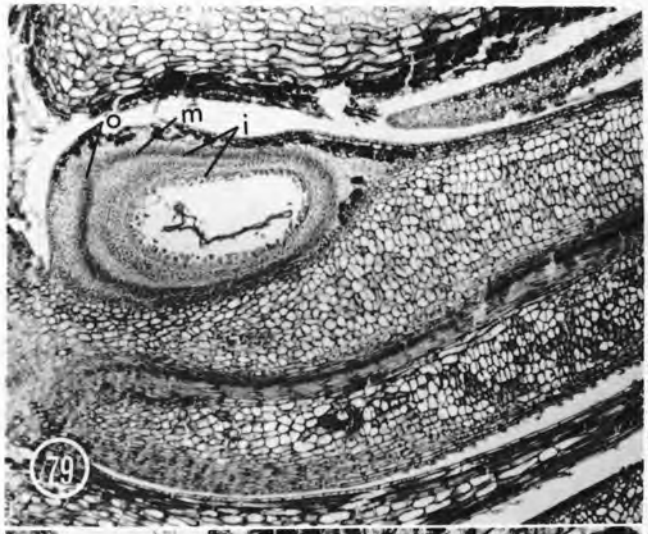
Figure 81. Longitudinal section of a young scale showing the developing seed wing and undifferentiated cells forming under the ovule. X100.

Figure 82. Longitudinal section of scale showing the hypodermal layer developing below the epidermis to form the seed wing. X100.

Figure 83. Cross section of adaxial side of the mature scale with the attached seed wing. Note undifferentiated cells below the seed wing. X200.

Figure 84. Longitudinal section of the distal abaxial portion of scale with thick walled hypodermal and epidermal cells and epidermal hairs. X200.

i - inner layer of integument and seed coat
m - middle layer of integument and seed coat
o - outer layer of integument and seed coat
uc - undifferentiated cells
h - hypodermis
sw - seed wing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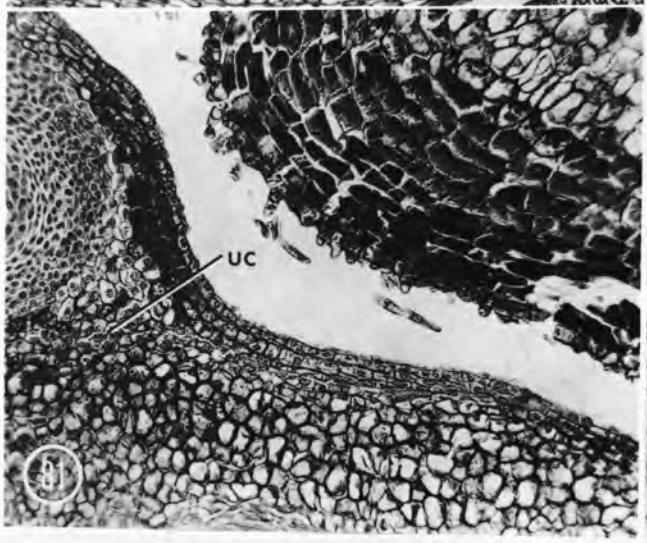
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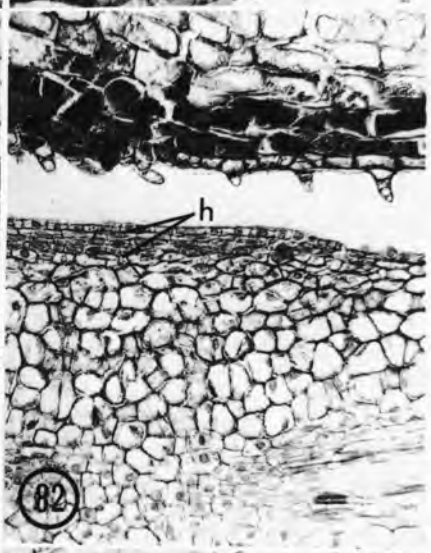
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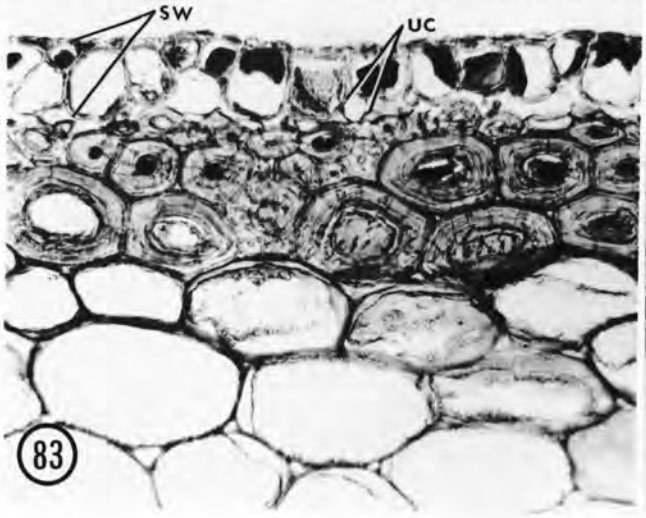

SW
UC
83

84

Figures 85-90

Figure 85. Median longitudinal section of an elongating megasporangiate bud following dormancy. Frequent divisions occur in rib meristem and pith mother cells. X100.

Figures 86-88. Cross sections of developing procambium and vascular bundles of the cone axis.

Figure 86. Concentrically arranged cells forming an indistinct procambium. X430.

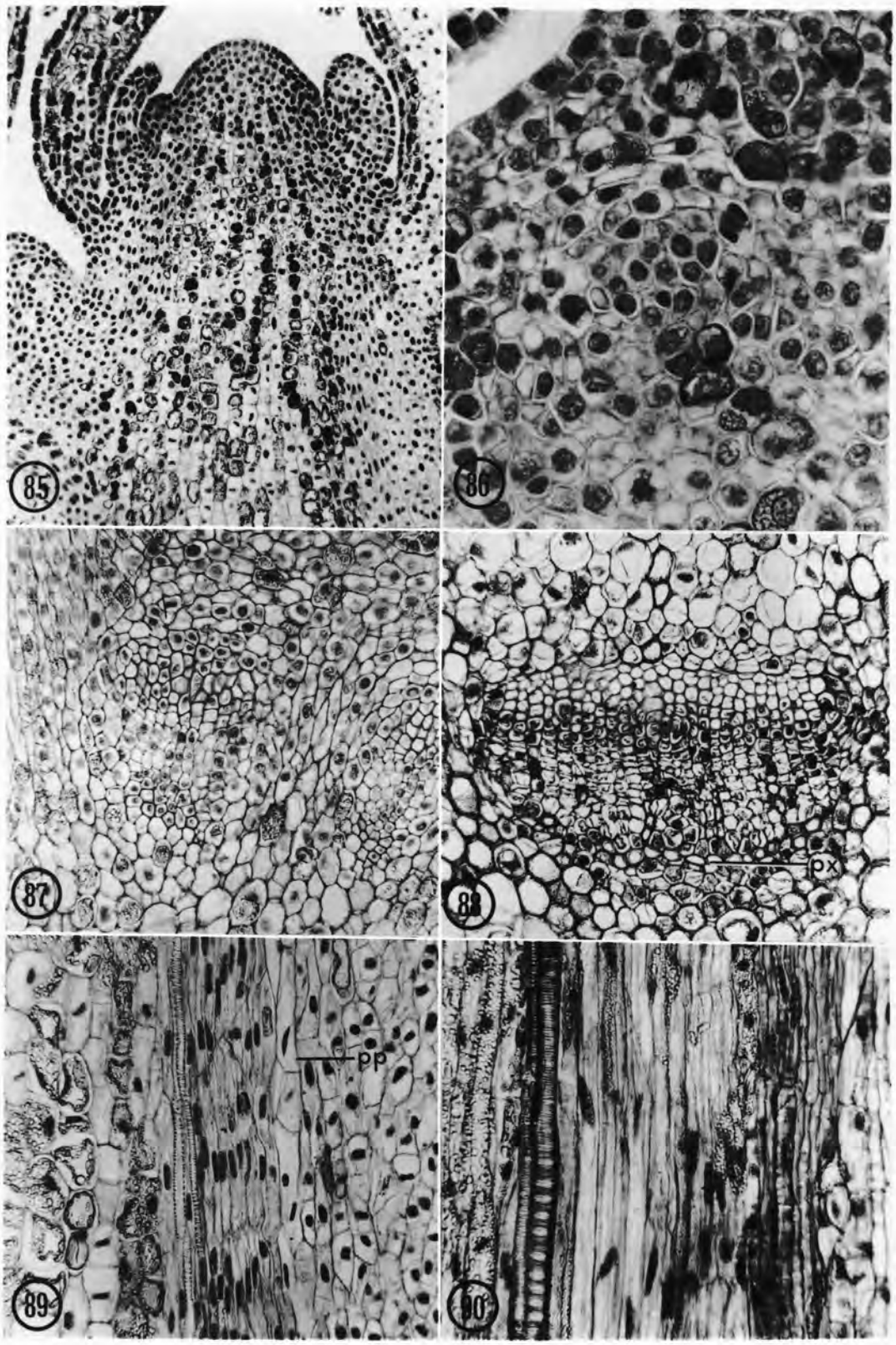
Figure 87. Enlarged vascular bundle with a cambial region of radially arranged cells between the primary xylem on the lower side and primary phloem on the upper side. X200.

Figure 88. Vascular bundle with deep tiers of primary phloem and a few thick walled primary tracheids. X200.

Figure 89. Longitudinal section of vascular bundle with primary xylem and primary phloem. X200.

Figure 90. Longitudinal section of vascular bundle with large metaxylem tracheids, phloem with faint sieve areas and broad zone of undifferentiated cells. X200.

px - primary xylem
pp - primary phloem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Figures 91-96

Figures 91-94. Cross sections of cone axes during elongation and maturation with formation and differentiation of xylem mother cells. Pith is at the bottom of each figure.

Figure 91. Vascular bundle in the axis of an elongating cone. X100.

Figure 92. A portion of the axis of a fully elongated but not yet mature cone. Xylem mother cells have just begun to differentiate. X31.

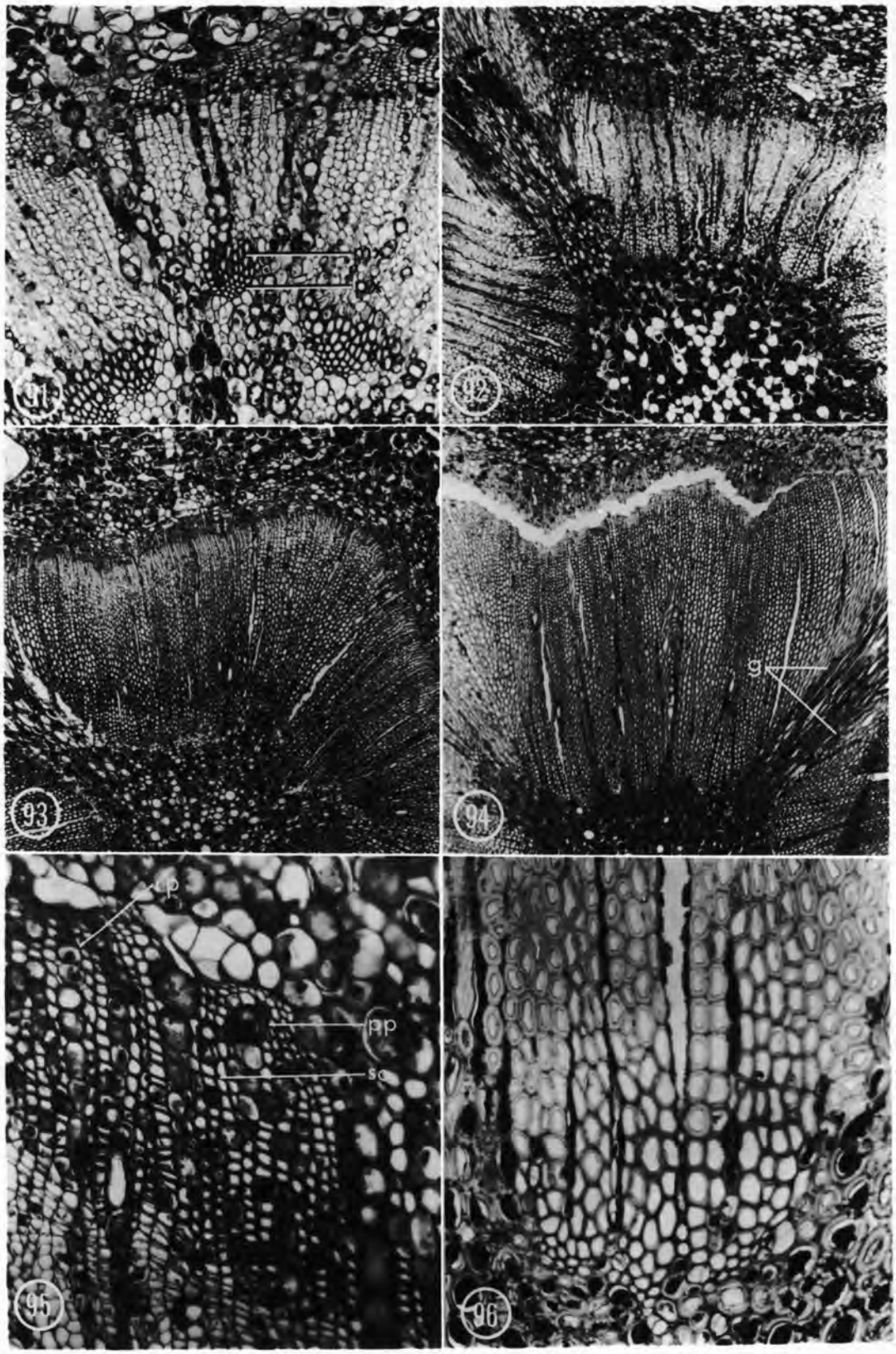
Figure 93. A portion of the axis of a nearly mature cone. X31.

Figure 94. A portion of the axis of a fully mature cone. All tracheids have differentiated. X31.

Figure 95. Cross section of phloem in the axis of a mature cone. X200.

Figure 96. Cross section of xylem in the axis of a mature cone. Note thick lignified walls of pith cells at the bottom of the figure. X200.

mx - metaxylem
px - protoxylem
g - fused leaf and branch gap
sc - sieve cells
pp - phloem parenchyma
rp - ray parenchyma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Figures 97-103

Figure 97. Cleared megasporangiate cone at the time of pollination showing the primary vascular system consisting of leaf and branch traces. X30.

Figure 98. Median longitudinal section of a megasporangiate cone at the time of pollination. X3.5.

Figure 99. Cleared scale from an elongating megasporangiate cone before macrosclereid differentiation showing dichotomous branching of vascular bundles. X3.

Figure 100. Cleared scale from a mature megasporangiate cone. Note large zone of macrosclereids at the base and numerous astrosclereids throughout the distal half of the scale. X3.

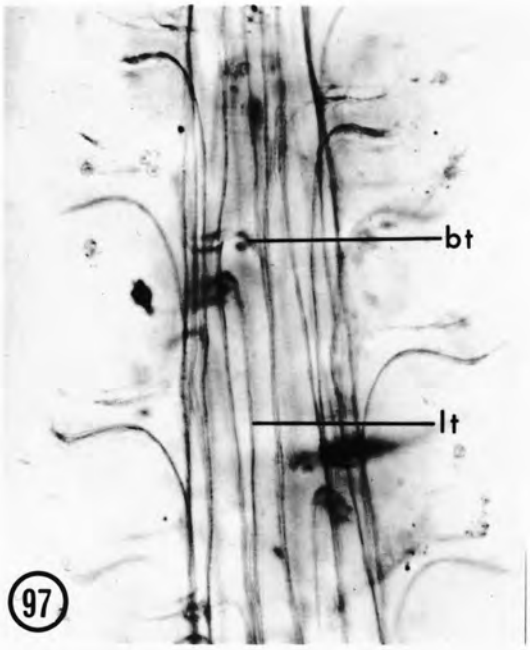
Figures 101-103. Mature scales from which all but the vascular tissue has been removed. X4.

Figure 101. Mature scale from distal part of cone with complete separation of scale and bract vascular tissue and long fused leaf and branch gap.

Figure 102. Mature scale from center of cone with shorter fused leaf and branch gap.

Figure 103. Mature scale from base of cone with very short fused leaf and branch gap and nearly fused vascular tissues of bract and scale.

lt - leaf trace (bract)
bt - branch trace (scale)
ms - macrosclereids
g - fused leaf and branch gap
s - scale vascular tissue
b - bract vascular tissue



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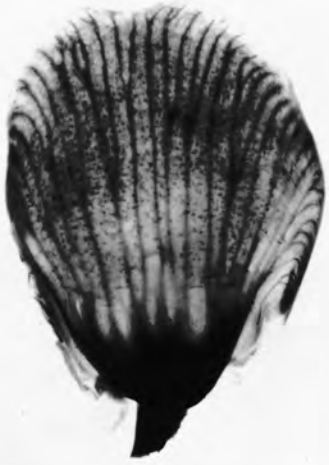

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