VEGETATIVE BUD DEVELOPMENT, CONE DIFFERENTIATION AND DEVELOPMENT IN PICEA ENGELMANNII PARRY.

by

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ABSTRACT

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The microanatomical development of vegetative and reproductive buds in Picea engelmannii Parry (Engelmann spruce) is described. Vegetative buds of Engelmann spruce from the Prince George Forest District (British Columbia) were collected and studied. In mid-April growth was resumed as determined from leaf mitoses two weeks later mitotic activity was observed in the apex. Bud-scale initiation began in terminal buds by the end of May. Bud-scale initiation began in axillary buds two weeks later than terminal buds when terminal bud shoot elongation began. Bud-burst occurred in late June and both shoot elongation and bud-scale initiation were complete by late July. In early August terminal vegetative buds became anatomically differentiated by the initiation of leaves. Axillary bud leaf initiation began one week later.

In terminal and axillary vegetative buds leaf initiation was completed by late September and buds ceased growth by mid-October.

Putative pollen-cones initiated microsporophylls after bud-scale initiation. Two abaxial microsporangia per sporophyll were initiated by late August. Microsporangial enlargement began in mid-September and ceased by mid-October. Pollen mother cells did not undergo meiosis before growth cessation.

Putative seed-cones initiated bracts directly after bud-scale initiation. Bract primordia were initiated as a group of darkly staining cells within the peripheral zone. In mid-August, just above these cells another group of darkly staining cells began to develop into the ovuliferous scales. Two developing ovules formed adaxially and on each side of the median longitudinal axis of each ovuliferous scale. These ovules formed one central megaspore mother cell which enlarged and overwintered in a pre-meiotic stage.

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DEDICATION

I wish to thank my mother and father for their forbearance, understanding and belief in an estranged son so obsessed that he could only see the light at the end.

Chapter I

INTRODUCTION

With the depletion of easily accessible lowland conifers such as Picea glauca, P. sitchensis, Pseudotsuga menziesii, Thuja plicata, Pinus contorta, Tsuga heterophylla and even the higher mid-elevation Chamaecyparis nootkatensis, it has become necessary to log in sub-alpine regions. The subalpine region of the British Columbia, 760 to 3660m, is comprised of the Engelmann spruce-subalpine fir forest type (Fig. 1).

In British Columbia there are over 84 million cubic meters of mature conifer tree volume. Of this, the spruces (represented by Picea sitchensis, P. glauca and P. engelmannii) make up over 18 million m^3. This is second only to the hemlocks (Tsuga mertensiana and T. heterophylla). The interior spruces of British Columbia (P. glauca and P. engelmannii) account for 90 percent or over 16 million m^3 of merchantable conifer volumes (Young and Bruce 1975). In 1980 spruce accounted for over 21 percent of the total conifer species cut in British Columbia (Anonymous 1980). Distribution maps (Horton 1959; Fowells 1975: Hosie 1969) suggest that approximately one-third of this volume results from P. engelmannii.

Logging at higher elevations demands rapid reforestation because the growth season is short and conifer growth rates are slower than at lower altitudes. An intricate knowledge of the species development in terms of vegetative growth, bud set, breakage of dormancy, anatomical differentiation of bud types (i.e. vegetative and reproductive) etc. is needed in seed production and seedling propagation. In particular, the period of sexual differentiation is important since silvicultural practices such as fertilizer application, high temperatures, low moisture regimes, elevated plant carbohydrate levels, gravity, plant hormones and root prunning may enhance this differentiation (Matthews 1963; Pharis and Kuo 1977).

The purpose of this study was to (1) document microanatomical vegetative bud development and define the interval of anatomical differentiation; and, (2) document the development of the resulting vegetative and reproductive buds in P. engelmannii.

Chapter II

LITERATURE REVIEW

2.1 TAXONOMY

Engelmann spruce is a high elevation species inhabiting the Rocky Mountain subalpine regions from 720 to 3660m elevation. In the northern Rocky Mountains the species occurs at elevations as low as 760m, whereas, in the southern Rocky Mountains it is usually found in isolated pockets above 2900m (Fig. 1) (McSwain *et al.* 1970).

The distribution of *P. engelmannii* (Fig. 1) shows complete sympatric overlap with subarctic *P. glauca*. In 1882, along the decreasing elevation gradient between the Peace River Plateau and the Athabasca river, Asa Gray (cited in Daubenmire 1973) recorded an intergradation of *P. engelmannii* into *P. glauca*. Since that time, extensive introgressive hybridization has been noted (Wright 1955; Moss 1955; Horton 1959) and in these areas, overlap of morphological characters (eg. ovuliferous scale shape, cone length, leaf length etc.)(Garman 1957; Wright 1955; Horton 1959) and chemical characters (eg. terpenes, sesquiterpenes etc.) (Ogilvie and von Rudloff 1968; Habeck and Weaver 1969) has been described.

The simplest, quickest method of distinguishing the two species is based primarily on cone morphology (Garman 1957; Horton 1959; Taylor 1959; Ogilvie and von Rudloff 1968; Daubenmire 1973).

Picea engelmannii	Picea glauca
-barely rounded ovuliferous scale	-rounded ovuliferous scale
-crisped ovuliferous scale margin	-smooth ovuliferous scale margin
-thin ovuliferous scale	-thick ovuliferous scale
-pubescent twigs	-glabrous twigs
-seed wing edge to the end of scale greater than 2.9 mm.	-seed wing edge to end of scale less than 2.8 mm.
-widest part of ovuliferous scale is at mid-position	-widest part of ovuliferous -scale is back 1/3 from the scale apex

While the above characteristics show a difference between the species, it must be noted that they represent pure Engelmann and white spruce. With gradations in elevation there is extensive hybridization; hence, intermediate characters between the two species occur with the Engelmann spruce cone morphology becoming predominant at the higher elevations (Daubenmire 1973). Clearly there is some ques-

tion as to whether or not two distinct species exist, for in many instances both are collectively regarded as interior spruce. La Roi and Dugle (1967) concluded that differences in needle extract chromatography as well as the morphological index (based on 15 characters of cones and branches) (Horton 1959) were not significant. However, many distinguishing terpenes were not considered. Taylor (1959) regards and Habeck and Weaver (1969) suspect Engelmann spruce to be a subspecies of white spruce, whereas, others accept the chemotaxonomic work of Ogilvie and von Rudloff (1968) as evidence in support of two distinct species.

In this study, P. engelmannii sample trees were identified on the basis of cone scale morphology at all three sites. Chemotaxonomic terpene analysis was not carried out. However; terpene analysis in the nearby Purden lake area (elev. appr. 1000m) has shown P. engelmannii x P. glauca hybridization "...leaning towards P. engelmannii and that higher elevation regions are undoubtedly Engelmann spruce" (von Rudloff pers. comm. 1981). The present study was made on species from these higher elevations.

Engelmann spruce is monoecious. Seed cones are generally restricted to the upper four to six whorls and pollen cones usually occur below to 30 percent of the tree height as per P. glauca (Eis 1967; Owens and Molder 1977a). However, in years when cones are abundant, pollen cones in open grown trees may occur to ground level. P. engelmannii be-

gins to bear cones at about 20 years and production increases for 200 to 250 years (McSwain et al. 1970). The first cones are seed cones, followed by pollen cones borne in the zone beneath as the tree grows taller. Good seed cone crops are cyclic, occuring every two to six years, with light intervening crops (Fowells 1958 ; Eis 1967).

2.2 GROWTH AND DORMANCY

Romberger (1963) defined several states of dormancy and concluded that "...in spite of some progress, confusion and vagueness about the nature and meaning of dormancy is still present".

Two interpretations of dormancy are generally used for conifers. Dormancy is often used in the general sense for that period when a living tissue that is predisposed to grow no longer does so (Nienstaedt 1966; Schopmeyer 1974; Leopold and Kriedemann 1975; Wareing and Phillips 1978). Growth is normally defined as an increase in volume by cell division and/or cell enlargement (Esau 1977) associated with elevated respiration rates and nucleic acid levels (Leopold and Kriedemann 1975). Since altered chemical concentrations (growth substances) generally are considered responsible for dormancy induction and breakage (Perry 1970; Bachelard 1980) changes in these substances also might be delimiters of the dormant state.

Others (Romberger 1963; Lavender 1980) consider bud-burst to be the zero point of the annual growth cycle and consider it to be equivalent to the end of dormancy, perhaps because it is convenient to measure. This is based on the definition of dormancy advanced by Doorenbros in 1953 (in Lavender 1980) as any case in which a tissue predisposed to elongate does not do so. Using these criteria Romberger (1963), Perry and Simon (1967) and Kozlowski (1971) consider that the terminal bud may enlarge at a very slow rate and/or develop inner bud scales even in a dormant state. This tends to refute the concept of dormancy as conveying resistance to injury during stressful periods such as low temperatures by growth impedement (Vegis 1964; Kozlowski 1971; Perry 1970; Wareing and Phillips 1978).

The first interpretation of dormancy applies to vegetative buds of most conifers where the presence of cell divisions is used as the criterion. The second interpretation does not apply to conifers where detailed anatomical studies have been made. This is because: (1) a few weeks before bud-burst, bud swelling occurs and is caused by early shoot elongation yet by the first interpretation this would not be considered as elongation or growth; and, (2) shoot organogenesis in terms of axillary bud initiation, bud-scale initiation, and mitotic activity increases also occur many weeks before bud break. Therefore, it is difficult to accept that such buds, or in fact any such system should be considered dormant.

2.3 VEGETATIVE BUD DEVELOPMENT

At the tip of each branch there is an apical meristem which originated from the embryonic stage of the plant (Romberger 1969; Steeves and Sussex 1972; Esau 1977). The apical meristem is enclosed by brown leathery bud scales in many conifers. The activity of this primary meristem leads to the formation of a telescoped shoot consisting of undifferentiated cells. The result of this epigenetic accretion of structure is observed when the green shoot elongates the following spring. Shoot apical meristems are the most active determiners of tree growth and form (Rhomberger 1969) and thus all aspects of development (growth, differentiation, histogenesis and organogenesis) may be related to the activity of these formative regions.

The shoot apical meristem of conifers is not a simple collection of dividing cells but a composite of four cytohistologically distinct regions. Popham (in Wardlaw 1968) established several types of histological organization to describe the apices of vascular plants. Only one of these types (type VII) accurately described the conifer apex. Based on the literature, there is little question that the model is accurate (Gifford and Corson 1971; Wardlaw 1968; Esau 1977). It should be noted that in the past, other histological models to describe vascular plant apices have been proposed. For example Hansteins' Histogen theory and the tunica-corpus concept advanced by Schmidt have various ap-

plications but fail to adequately describe conifer apices (Esau 1977). These models have been treated extensively elsewhere (Foster 1939a; Gifford 1954; Romberger 1963). In this study, Pophams' type VII cytohistological zonal patterns will be recognized as the: (1) apical initials; (2) central mother cells; (3) peripheral zone; and, (4) rib meristem (Fig. 2).

Because this study deals with internal shoot tip changes with time, it is necessary to clarify the concept of the shoot apex. Parke (1959) first used the term shoot apex to describe the terminal region of the shoot above the last formed primordia. He also accepted the term shoot tip as defining the apical promeristematic tissues and the subtending primary meristematic tissues. Earlier, Foster (1939a, 1939b) regarded the apex as the shoot tip to the point of the central tissue differentiation (which in Cycas revoluta occured below the site of leaf insertion). This approach was valid but did not account for fluctuations in apical shape. As such, the usefulness of the term is limited in descriptive apical development (Parke 1959; Gifford and Corson 1971).

Parke (1959) recognized that the apical meristem did not regularly contain all meristematic regions, at dormancy the rib meristem would be excluded (Fig. 2a), whereas, during leaf initiation it would be included (Fig. 2b). This tendency was overlooked since the terminology allowed for

more accurate descriptions of bud development in Abies concolor. Still, many regard the shoot apex variously as the apical meristem and underlying primary meristem tissue (Wardlaw 1968; Steeves and Sussex 1972; Esau 1976) or equivalent to the apical initials and central mother cells (promeristem) (Esau 1958; Foster and Gifford 1974).

As long as changes in zonal pattern, function and shoot development are well understood, an absolute definition of the shoot apex, although helpful, is not essential in shoot organogenesis and histogenesis. This study will refer to the apex as that region above the last initiated primordia (Johnson 1951; Parke 1959).

2.4 BUD DIFFERENTIATION

Before the appearance of vegetative or reproductive structures (anatomical differentiation) most conifers exhibit bud-scale initiation (rather than leaf initiation as do many angiosperms). During bud-scale initiation, the shoot apex is often termed undifferentiated since bud-scale initiation is followed by the initiation of definite vegetative or reproductive structures. Although there is an association between bud-scale initiation and differentiation (Owens et al. 1977; Owens and Molder 1976), the demarcation between them is difficult to define. This is because the early stages of vegetative and reproductive bud development appear quite similar i.e. early buttress initiation and development).

In angiosperms, this differentiation (often called transition to flowering) is usually accompanied by characteristic changes usually in the ordered sequence (Steeves and Sussex 1972): (1) increased RNA and protein levels, (2) increased mitotic activity, (3) DNA duplication, (4) increased apical size, (5) termination of leaf initiation and (6) initiation of floral parts (Bernier 1971).

2.4.1 Differentiation and apical mitotic activity

Conifers are thought to display biochemical similarities to angiosperms (Doorenbos and Wellensiek 1959; Gifford and Corson 1971). As a result of this close parallel certain of the above transitional steps might define this period, particularly mitotic activity, based on apical size. In fact, elevated mitotic activity has been associated with differentiation in P. glauca (Owens and Molder 1977a), Abies amabilis (Owens and Molder 1977c) and Larix occidentalis (Owens and Molder 1979). However these observations often represented small sample sizes involving apices selected as reproductive. Ideally, mitotic counts using a large number of buds should demonstrate a peak at the time of differentiation even if the vegetative : reproductive ratio is 1:1.

2.4.2 Differentiation and apical measurements

Differentiation has also been associated with various apical parameters such as height (Owens et al. 1977; Owens and Molder 1977c), diameter (Owens and Molder 1977c), depth and shape. Apical height is defined as the distance from the apical tip to the level of the last initiated lateral primordia, and width is the distance across the apex at this level. Depth refers to the distance from the apical tip to the base of the rib meristem. The appearance of an apex may then be indicative of the developmental pathway of the apex.

2.4.3 Differentiation and ergastic substance accumulation

Recently there has been the observation and documentation of an apical feature associated with vegetative differentiation. This character termed ergastic substance accumulation has been noted in P. glauca (Fraser 1962), P. mariana (Fraser 1966), P. sitchensis (Owens and Molder 1976), Pseudotsuga menziesii (Owens, unpublished data), Abies amabilis (Owens and Molder 1977c), Tsuga heterophylla (Owens and Molder 1973). This character results from the synthesis and accumulation of polyhydroxyphenolic compound (Hejenowicz 1979) in vegetative apices.

Such cellular biochemical alterations are not unfounded, indeed Bernier (1971) cites evidence of increased RNA and protein levels during evocation of flowering. Similarly, Barber and Steward (1968) working with Tulipa apices ob-

served that certain soluble proteins disappeared from vegetative apices during evocation, while other soluble proteins normally found in the floral structures began to appear. It is probable that these changes represent characteristic ultrastuctural modifications, but data of subcellular changes are lacking (Hicks 1980). On the other hand, Halperin (1978) could cite no evidence of altered protein levels before the floral transition stage, suggesting that protein level changes followed transition and directed the development of the lateral primordia.

2.5 PATTERNS OF SEED AND POLLEN-CONE INITIATION

Owens and Molder (1977b) described five characteristic coning patterns in the Pinaceae family:

Pseutosuga and Abies

Pollen and seed cones develop from axillary undifferentiated apices.

Tsuga

Seed cones differentiate from previously vegetative terminal apices, whereas, pollen cones develop from terminal and undifferentiated axillary apices.

Pinus

Both pollen and seed cones normally differentiate from buds initiated in the axils of cataphylls within the long-shoot terminal bud. Pollen and seed-cone buds appear in proximal and distal position, respectively.

This genus is further complicated by the appearance of polycyclic shoots, dwarf shoots and a delayed period of seed cone differentiation in the soft pines.

Larix

Pollen and seed cones differentiate from one and two year old, previously vegetative short shoot apices.

Picea

Both pollen cones and seed cones differentiate from previously vegetative terminal apices or undifferentiated axillary apices.

Chapter III

MATERIALS AND METHODS

3.1 SAMPLES AND COLLECTIONS

Three vigorous, mature Engelmann spruce trees were selected on the basis of past good annual seed and pollen cone production at each of three sites in the Prince George Forest District. Sites 1 and 2 were situated 83 Km SE of Prince George at 1400m and 1300m, respectively, whereas, site 3 at 1670m was located 42 Km ESE of Prince George.

On each tree, branches were sampled from the female bearing region of the crown (the upper four to six whorls) and male region (between the sixth and ninth whorl). Terminal buds from second order branches were collected begining in late March 1979 then weekly from May 19 until bud-burst in late June. Thereafter, developing terminal and axillary buds were collected weekly until September 1, then biweekly collections continued until November 6. Supplemental collections of terminal vegetative buds were made the first two weeks of April 1980.

For collections from March through June, the vegetative buds were dissected and fixed in Navashins (Craf) solution (Berlyn and Miksche 1977). After July buds were fixed in formalin-acetic acid (FAA) (Johansen 1940; Berlyn and

Miksche 1977). Samples were shipped to the University of Victoria where they were dehydrated in a tertiary butyl alcohol series (Johansen 1940) and embedded in Tissue-Prep for longitudinal microtomy. Serial sections of 6 um were made on an "AO" rotary microtome, then stained with iron-hematoxylin and safranin as per Johansen (1940).

Destructive counts of seed cones and pollen cones were made in early July (1980) to characterize the cone-bearing nature of each tree from which buds were collected in 1979. Seed cone tree productivity was assessed by removing the terminal six whorls of the tree and carefully lowering them to the ground. All seed cones and vegetative shoots were then counted enabling tree productivity to be expressed as seed cones per 100 potential buds. By noting individual positions of the seed cones and vegetative shoots (i.e. terminal or axillary) it was possible to quantify positional variability as the percent of terminal and axillary buds that became reproductive.

Pollen cone productivity was assessed by selecting three branches from the ninth whorl of each tree and counting all buds. Again tree productivity was expressed as pollen cone number per 100 potential buds. Counts were further fractioned into terminal and axillary positions for calculation of positional variability.

3.2 TEMPERATURE MEASUREMENTS

In April 1979, a hygrothermograph mounted in a Steveson screen was placed at site 1 and a second at site 3. Both were established at a height of approximately two meters above the ground and within 70m of the nearest collection tree.

3.3 VEGETATIVE SHOOT ELONGATION

Four terminal buds per collection tree were tagged at mid-tree height beneath the collection regions to monitor shoot elongation. These buds were located close to the crown periphery and on the compass points N, S, E and W. When these vegetative buds burst in late June, vegetative shoot lengths were measured weekly until mid-August.

3.4 ANATOMY, APICAL MEASUREMENTS AND LIGHT MICROSCOPY

Only median longitudinal sections of apical buds were selected and used in the description of apical development. Many of these sections were drawn through a KENA VISION Tech 'A' microprojector from which measurements of apical width, height and area were made. Zonation patterns were noted and mitotic counts (based on the number of 6 um sections which contributed 10 percent of the apical diameter) were made microscopically and superimposed upon the apical tracings. This provided apical mitotic frequencies from mid-May through to winter dormancy.

Some slides could not be used. This resulted from several factors including insect damage, microtomy damage, non-median sections, poor fixation, etc. which precluded certain observations. As a result, the sample size (n) is shown next to the plotted mean 95 percent confidence limits.

The number of bud scale and leaf primordia were counted in the most median section of each apex. Primordia attached to either the receptacle (bud scale primordia) or the bud axis (leaf primordia) were included in these counts.

A Reichert Microphotometer was used to quantify the apical concentration of ergastic substances accumulated in the pith region. Since the safranin was taken up by certain cells, the degree of ergastic substance accumulation was estimated from the safranin uptake. Absorbance was then set to zero on a group of cells appearing similar to the ergastic containing cells but lacking ergastic substances. Transmittance was determined after positioning the section so that the narrow field of view was located slightly above the last formed primordium and in line with the central bud axis. The transmittance recorded was then converted to absorbance. Increase absorbance values were associated with increased concentration of ergastic substances.

Light micrographs were made using a Reichert compound microscope fitted with a Riechert PHOTO AUTOMATIC camera system. A Leitz Aristophot was used to photograph macroscopic bud scales.

3.5 ELECTRON MICROSCOPY

In late August (1980) branch samples were collected from each tree at each site and frozen (-5° C) for subsequent scanning electron microscopy. Several months later, all bud scales were removed, and the frozen vegetative and reproductive buds were fixed in Zirkles fixative (Conn *et al.* 1960). Material was dehydrated along an ethanol gradient to 100 percent ethanol, then along a gradient to 100 percent iso-amyl acetate and dried with a BOMAR carbon dioxide CRITICAL POINT DRYER. Specimens were mounted on stubs and gold coated with a TECHNICS HUMMER sputter coater and viewed with a JEOL-35U scanning electon microscope (SEM) operating at 20 Kv. Scanning electron micrographs were used to determine the phyllotaxy and give comparisons of vegetative and reproductive development rates.

The ultrastucture of the apical zones of terminal vegetative buds from the similar species *Picea glauca* was characterized. *P. glauca* was used because of the remoteness of the *P. engelmannii* sites and lack of electron microscope facilities in the vicinity. Material was collected in mid-May, during bud-scale initiation, from a juvenile tree at the University of Victoria. Vegetative buds were dissected, and the apices were removed, trimmed and double sequentially fixed in glutaraldehyde and osmium tetraoxide (Weakley 1972). Specimens were then embedded in Spurr's Low Viscosity Embedding Medium and sectioned on a Reichert "OmU2" ul-

tramicrotome to approximately 500 angstroms. Ultrasections were post-stained in uranyl acetate and lead citrate (Weakley 1972) then observed with a Philips E300 (type PW6001/00) transmission electron microscope (TEM) operating at 60 Kv.

Chapter IV

OBSERVATIONS

4.1 CONING CHARACTERISTICS

Reproductive buds differentiated from both terminal and axillary buds after completion of bud-scale initiation. Seed cone counts for each tree indicated that the percent of reproductive buds differentiated in 1979 ranged from 6 to 58 percent (Table I) with a mean of 29 percent. Because the original data base was small it was beneficial to include counts from seven more reproductively mature trees in the immediate area (approximately 1200meters) as presented in Table I. The number of cones in terminal position was quite variable; however, on a percent basis a few trends became apparent (Fig. 3). Seed cones were confined to the upper four to six whorls and were preferentially located in terminal positions furthest from the tree bole. This relationship was associated with an apparent increase in branch vigor with increasing distance from the bole. The above observation held for trees with light cone crops. Trees with heavy cone crops exhibited the same tendency but in addition more axillary seed cones developed nearer the tree bole as tree productivity increased.

The percent of terminal seed cones that developed was always greater than the percent of axillary seed cones. The overall ratio of terminal to axillary seed cone position in the fifteen trees was 2.9:1. This ratio decreased with increasing tree productivity but the terminal position was always predominant (Fig. 15).

Trees 1 and 3 at site 3 demonstrated the highest tree productivity (57 and 58 percent, respectively). However, the number of developing buds capable of becoming either seed cones or vegetative buds averaged 48 percent that of the lower elevation trees. This represents a tree productivity of only 20 to 25 percent.

The number of pollen-cone buds on three-branch samples per tree ranged from 0 to 77 percent with a mean of 55 percent of all buds becoming reproductive (Table I). Over 90 percent of the pollen cone buds were axillary and were spaced intermittently along the shoot (Fig. 16) or in proximal clusters (Fig. 17).

4.2 WEATHER

Hygrothermographs located at sites 1 and 3 were damaged in 1979, precluding complete temperature profiles at either elevation. The incomplete weather records indicated that cooler maximum daily temperatures (approximately 4° C) occurred at the higher elevation. However, by late June the temperatures at both sites were equal.

4.3 VEGETATIVE BUD ANATOMICAL DEVELOPMENT

The apical anatomical development of P. engelmannii was inadequately described by Lewis and Dowding (1924). Apical development in this species parallels that for P. glauca (Fraser 1962; Owens et al. 1977; Owens and Molder 1977a), P. mariana (Fraser 1966), P. sitchensis (Owens and Molder 1976; Tompsett 1978), and P. smithiana (Pillai and Chacko 1978).

The developmental findings of this study will be briefly outlined followed by a more detailed account of vegetative bud development in P. engelmannii throughout one growth season.

Following a period of mitotic growth inactivity during the winter (in this study equated with the dormant condition), the first morphological change that occurred in the spring was the initiation of bud scales by the shoot apical meristem. This was followed by the elongation of the preformed shoot which can be divided into 2 phases: bud swelling; and, bud-burst. Shoot elongation and bud-scale initiation ceased late in July resulting in an organogenic pause when no foliar organs were being initiated. After this pause apices became anatomically differentiated as vegetative, female or male. Because of this the organogenic pause may be associated with anatomical differentiation. Bud development until the time of anatomical differentiation has been termed growth I and is followed by a second growth

phase termed growth II (Korody in Parke 1959). During growth II, terminal and axillary apices initiated either vegetative or reproductive structures until late October when apical mitotic activity ceased and buds became dormant. The last growth phase represents the development of the shoot axis which will elongate after dormancy the following spring.

4.4 BUD MITOTIC ACTIVITY

Because collections were not made in April 1979, the end of bud dormancy could not be determined for that year. However, the supplemental 1980 collections indicated that dormancy ended and bud growth began by the second week of April, based on the appearance of mitotic activity within the leaves of vegetative buds from both male and female regions of the crown. Mitotic rates of leaf primordia increased for 2 weeks before mitotic activity was observed in the apex.

No mitoses were ever observed in apical initials or central mother cells. The adjacent peripheral zone showed high mitotic activity, and the rib meristem below showed an intermediate activity.

Terminal bud mitotic counts (from early July to dormancy) were pooled with axillary bud counts and plotted resulting in a mitotic profile from May to October (Fig. 10). Peripheral zone mitotic activity increased until late June,

remained at the same level until mid-July, then rapidly increased before there was an abrupt decrease followed by a second peak in early August. There was then a gradual drop in mitotic activity until late October. Although not statistically significant, what appears as two peaks in figure 10 can be explained by fractioning the samples into sex (male and female) and position (terminal and axillary). The first peak in mid-July is associated with axillary and female positions only (Figs. 11a and c), whereas, the trough and the second peak are characteristic of all four types (Figs. 11a, b, c and d).

The first peak is correlated with rapid bud-scale initiation in axillary buds. Terminal buds exhibit no period of rapid bud-scale initiation (Figs. 10, 13) and no corresponding peak (Fig. 11b).

The second peak (Fig. 10) occurs at the begining of leaf initiation in terminal and axillary buds and apices from female regions showed increased activity before those from male regions (Figs. 11a, b, c and d).

Rib meristem mitotic frequency was low from May to September (Fig. 10).

In early October (1979) the mitotic frequency of the apical zones was low (Fig. 10). Two weeks later no apical mitoses were observed in terminal or axillary vegetative buds from male or female regions of the crown at either the high or low elevations. Low mitotic activity occurred with-

in leaf primordia. Mitotic activity was terminated within two weeks (late October) indicating that vegetative bud growth had ceased and bud dormancy had begun.

4.5 SHOOT ELONGATION

Vegetative bud-burst occurred during the last week of June at sites 1 and 2 and one week later at site 3 (Fig. 4). Shoot elongation ended by the last week of July at sites 1 and 2 and one week later by site 3. However, the rates of shoot elongation between the three sites were similar.

The delay in bud-burst at the higher elevation site 3 was accompanied by an apparent reduction in final mean shoot length (Fig. 4). Although this feature was not significant a trend of increased elevation and decreased shoot length was evident.

Because the pattern of shoot length increases at sites 1 and 2 were not significantly different, these values were pooled and plotted as percent total shoot elongation while the one week delay at site 3 justified a separate plotting. Further, the addition of microscopic pre-flush lengths yielded a set of logistic growth curves (Fig. 5).

4.6 BUD DEVELOPMENT

4.6.1 Bud-scale initiation

Bud-scale initiation in terminal buds began about mid-May and continued at a constant rate through to mid-July (Fig. 6). Axillary buds, with two bud scale buttresses, were observed in many leaf axils of the elongating shoot by late May, but further initiation of bud scales did not occur for another four weeks. All bud scales were initiated by July 11 in terminal buds and late July in axillary buds. Axillary buds initiated 33 percent fewer bud scales than did the terminal buds.

Bud-scale initiation occurred from a localized group of cells in the peripheral zone below the level of the central mother cells. These cells were conspicuous because they had both anticlinal and periclinal division planes in comparison to the predominantly anticlinal division plane in other regions of the peripheral zone (Fig. 18). Random division produced a swelling of the outer peripheral zone and protoderm (Fig. 20). Prior to this swelling, protodermal cells were normally compressed indicating recent anticlinal division (Fig. 19); however, just after buttress appearance, the division plane of the protoderm changed to periclinal producing two layers of cells (Fig. 20). These divisions continued until a distinct buttress formed.

4.6.2 Bud scale development

The transition from buttress to bud scale primordium was marked by the formation of a distinct zonation pattern in the primordium apex similar to that of leaves. Development of the bud scales resulted from different mitotic rates in the subapical, apical initials and the marginal meristems (Figs. 22, 23) and resulted in a broad flat primordium.

Two different types of bud scales were observed in both terminal and axillary buds. Bud scales were initiated for a short period during early shoot elongation. These looked like leaf primordia but did not develop as leaves, rather they elongated and broadened, loosely enclosing the apex. These primary bud scales became deltoid with a hairy margin and a terminal longitudinally oriented, thickened, abaxial rib of supportive tissue (Figs. 26, 27, 28). Below this tissue was a single procambial strand which showed differentiation only where it joined the receptacle of the bud axis. Secondary bud scales were initiated after primary bud scales. Secondary bud scales became thin, broad and ovate with smooth tapered margins and lacked supportive or conductive tissue (Figs. 23, 24, 25). However, just below the base of each secondary bud scale there was a single procambial strand extending through the receptacle (Fig. 33). Secondary bud scales were initiated until the cessation of shoot elongation.

In dormant buds these two bud scale types were distinct. The inner secondary bud scales represented the majority of the bud scales enclosing the apex. The primary bud scales made up the outer basal 1 - 2 spirals of bud scales (Fig. 29). After bud-scale initiation began in the spring, both types of bud scales accumulated ergastic-staining substances in the cytoplasm of the abaxial and adaxial epidermal cells. As scale growth slowed the adaxial cells of the primary bud scales were cleared of these substances (Fig. 30) but the cytoplasm still stained darkly. The abaxial cells retained these compounds unless they were located on the outer most surface of the bud, where a very thick cuticle developed (Fig. 31).

Successively initiated primordia were not confined to the apical flanks, rather they were displaced along a broad flattened receptacle. The receptacle contained numerous resin ducts and large numbers of longitudinally oriented procambial strands diverging from the shoot vasculature below (Figs. 32, 33). These strands showed basipetal differentiation from meristematic procambial cells to secondarily thickened xylem cells (Fig. 34, 35, 36).

As the end of bud-scale initiation approached, the apex was completely overarched and tightly enclosed within the overlapping bud scales (Fig. 21). The end of bud-scale initiation was identified by a cessation in the development of the most recently initiated primordia and the appearance of

more than one buttress on either flank of the apex. At this time a number of rudimentary primordia were observed in many of the sections. These primordia were positioned midway between the vertical plane of the bud and the horizontal surface of the receptacle. These rudimentary primordia were similar to the bud scales but had the size, shape and cytoplasmic staining characteristics of the early foliar organs (leaf, microsporophyll or bract).

Receptacle formation occurred throughout bud-scale initiation. Once a bud scale primordium had been initiated it was displaced from the apical flank by underlying periclinal divisions in the peripheral zone. When the bud scale showed no attachment to the apex these cells began to enlarge and large horizontally oriented resin ducts formed, further displacing the bud scale from the apex. These resin ducts were arranged spirally from the central pith of the bud. Two sets of opposing spirals were observed, one clockwise (composed of 8 spirals) the other counter-clockwise (composed of 13 spirals). Between the 13 counter-clockwise spirals, uniform spirals of procambial strands penetrated the receptacle to each primary and secondary bud scale. The bud scale phyllotaxy was 8 plus 13.

The formation of spiral resin ducts was observed only in the receptacle. Once bud-scale initiation was complete cell division, cell enlargement and resin duct formation halted, terminating receptacle generation.

4.6.3 Anatomical differentiation

During bud-scale initiation terminal and axillary buds differed only in the rate and number of bud scales initiated. Development did not vary with putative sex of the bud or crown region from which the bud was collected. After bud-scale initiation was completed differences occurred between putative vegetative and reproductive buds.

Unlike bud scales, foliar organs (leaves, bracts and microsporophylls) were initiated without displacement to the receptacle. In the early stages i.e. lateral primordium initiation) two types of apices were observed. The first type was low and conical with a typically small height-width ratio, a narrow peripheral zone and diverging flanks (Fig. 37). The second was tall and rounded with a larger height-width ratio, a wide peripheral zone and somewhat parallel flanks (Figs. 38, 39). By observing buds at different stages during the summer, it was determined that the first type invariably resulted in a vegetative bud while the second became reproductive. As a result, reproductive buds were the first to form a broad apex by increased peripheral zone division (late July). Within 2 - 3 weeks, the same shape could be observed in vegetative apices during later leaf initiation.

Reproductive apices elongated and broadened before bract or microsporophyll initiation began, whereas, vegetative apices broadened only after several leaf primordia had been initiated.

A mammillar apex was observed in both reproductive and vegetative apices where its development was associated with broad, rounded apices and rapid lateral primordial initiation. Although the apical broadening associated with the mammillar shape was usually observed one to two weeks earlier in reproductive than vegetative buds, both aquired the feature in the same manner. Increased cell division of the peripheral and transition zones broadened the apex, but the infrequently dividing central mother cells and apical initials prevented a similar expansion at the apex summit. These quiescent cells consequently appeared as a protruding bump (Figs. 40, 41).

The occurence of mammillar apices disappeared before winter bud dormancy, during the period of both decreasing mitotic frequencies and decreasing foliar initiation rates. The occurence of mammillar apices disappeared in pollen-cone buds by mid-August, in seed-cone buds by late August, and in vegetative buds by mid-September

4.6.4 Leaf initiation and buttress development

4.6.4.1 Leaf initiation

Near the completion of bud-scale initiation, terminal and axillary buds showed what appeared as a one to two week stop or slowing of bud-scale initiation (Fig. 6) prior to leaf initiation (Fig. 13). It was during this "organogenic pause" that anatomical or bud-type differentiation occured.

Leaf initiation began the final week of July in terminal buds and one week later in axillary buds (Fig. 7). At this time the developing vegetative buds could be identified by their low, sharply conical apex and mode of ergastic substance accumulation (Fig. 37).

Although the one week delay between terminal and axillary buds had extended to two weeks near the end of leaf initiation the rates of initiation were similar. By mid-September when leaf initiation was completed, equal numbers of leaves had been initiated in median longitudinal sections of both terminal and axillary buds.

Leaf initiation began in the same manner as bud scale initiation, with the randomly oriented division of a number of peripheral zone cells. This localized activity disrupted the mantled appearance of the peripheral zone (Fig. 42). Mitotic activity continued yielding a slight lateral protrusion (Fig. 43). The foliar buttresses formed were at first an unorganized group of homogenous isodiametric cells (Fig. 44). The buttresses then became heterogenous first by differentiation of apical and subapical initials then by procambial cells (Fig. 45). The apical and subapical initials, had a larger volume of diffuse nuclear material, and were therefore lighter staining than the surrounding protodermal and epidermal cells. Apical initials were similar in cell size and shape to protodermal and epidermal cells (i.e. periclinally elongate) while subapical initials appeared in

serial longitudinal sections as a distinct group of three to five larger isodiametric cells. The appearance of these organized regions (Fig. 45) signalled the end of the buttress-like growth habit and the begining of leaf primordial development.

4.6.4.2 Early leaf development

During buttress development abaxial and adaxial subepidermal cells showed similar rates of division; however, as the buttress became about eight cells in length abaxial subepidermal regions showed increased cell density and reduced cell size, presumably because of increased mitotic rates. As a result the terminal end of the developing primordia grew upward. At the same time the depressed mitotic activity of apical and subapical initials constricted the primordium tip to a taper.

During leaf initiation, a SEM view of a vegetative bud with bud scales removed revealed a spiral phyllotaxy of leaves (Fig. 46). This phyllotaxy can be expressed in terms of contact parastichies. Where leaf primordia are arranged with either 8 short clockwise parastichies and 13 long counterclockwise parastichies resulting in an 8 plus 13 phyllotaxy or 13 clockwise and 21 counterclockwise parastichies (13 plus 21 phyllotaxy). These patterns reflect a constant angle of successive leaf initiation known as the angle of divergence. This angle was estimated as 138° (SE) 0.307

based on the measurements of thirty successive leaf primordia of three different buds. This angle deviates slightly from the ideal angle of 137° 30' (Steeves and Sussex 1972) also known as the Fibonacci angle (Cannell and Bowler 1978).

The procambium was first identified as two adjacent rows of slightly elongate sister cells, with darkly staining nuclei confined to the central longitudinal axis of the primordium. These cells extended from just beneath the subapical initials deep into the peripheral zone of the apex (Fig. 47) where they became continuous with the vasculature of the bud axis. The procambial cells divided anticlinally and centrifugally (Fig. 48) aligning nuclei into bands across the procambial strands (Fig. 47). This banding was accentuated by elongation of the procambial cells until dormancy.

Although leaf initiation was completed by mid-September, primordial growth continued until dormancy. During that period primordial length increased from approximately 280 to 380 um. Cell counts made from longitudinal sections of leaf primordia indicated that ground tissue cell numbers per 75 um^2 were not significantly different in early September and at dormancy ($P(X_{31,0.50} > 3.793)=0.0609$). This indicated that mitosis and not cell elongation is primarily responsible for pimordium elongation. By late October, the terminal vegetative buds (Fig. 55) averaged 4 mm in height and 3 mm in diameter while the distal axillary vegetative buds were usually the same size or slightly smaller regardless of position.

By dormancy, leaf primordia contained a central core of procambial tissue which lacked any cellular differentiation (Fig. 48). Surrounding this tissue was a presumptive endodermis of larger cells lacking the characteristic Casparian strips. Around this central structure, varying densities of future mesophyll cells gave the primordium its four sided cross-sectional appearance. After dormancy, the leaf primordia developed and tissues differentiated (Figs. 56, 57).

4.6.5 Pollen cone initation and early development

4.6.5.1 Pollen-cone initiation

Microsporophyll initiation began the final week of July in both terminal and axillary buds. During microsporophyll initiation, microsporangiate strobili (pollen cones) could be microscopically identified by their tall, broad, bullet-shaped apices and their mode of ergastic substance accumulation (see section 2.4.3 and 4.8.2.) (Fig. 38). The apical initials and central mother cells of the developing pollen cones became less defined as the cone grew taller and became obscured as the apex was completely utilized in microsporophyll initiation (Fig. 58). The acropetal initiation of the microsporophylls and microsporangia was complete by late August (Figs. 51, 52) being more rapid than comparable leaf (Figs. 49, 50) or bract (Figs. 53, 54) initiation.

Microsporopyhyll initiation varied from leaf initiation in that the first formed buttresses did not appear basally

but almost midway up the apex resulting in a stalk-like structure (Fig. 38). In addition, these buttresses exhibited a growth perpendicular to the cone axis with little of the upward growth typical of leaf primordia.

4.6.5.2 Pollen-cone bud development

When the buttresses were approximately eight cells in length, elongate procambial cells containing darkly staining nuclei were observed extending from the buttress to the procambium of the inner bud axis of the shoot tip. At the same time, the buttress appeared as a homogenous group of cells in longitudinal section (Fig. 59). All buttresses appeared to halt development at this eight-celled stage so that the flanks of the apex were enveloped with a series of uniform buttresses (Fig. 60).

In early August, resin ducts appeared in the cortex (derived from the peripheral zone) beneath each microsporophyll base. Resin ducts often surrounded the procambial strand (Figs. 59, 61, 61). The development of the resin duct occurred as the procambial strands elongated. That is, the cortical region derived from the peripheral zone failed to elongate or undergo mitotic activity at a rate comparable to shoot tip elongation, resulting in a tearing action. This was greatly emphasized during microsporophyll and microsporangial enlargement (Fig. 63).

Microsporangia began to be intiated in early August as two distinct groups of randomly dividing, abaxial, subepidermal cells positioned below and to each side of the microsporophyll procambium. Observation of serial longitudinal sections indicated these areas to be ellipsoidal and longitudinally oriented (Fig. 64). The abaxial surface of the primordium became slightly distended with large (20 um), uninucleate, multinucleolate (usually 4 - 6 nucleoli), thin walled and darkly cytoplasmic staining cells. The tapetum was then identified as two layers of adjacent cells enclosing the larger central, angular sporogenous cells. The sporogenous tissue arose shortly before the apex was completely used in sporophyll initiation. At the same time, epidermal and subepidermal cells in the distal adaxial region directed growth of the primordium upwards forming a pointed tip (Figs. 65, 66).

After the formation of the tapetum, division of the sporogenous cells within continued to cause the microsporangia to enlarge. Microsporophylls enlarged by elongation of the cells underlying and adjacent to the microsporangia as well as procambial and epidermal cells (Figs. 67, 68, 69). The only cells that did not elongate were the tapetal cells and cells above the microsporangia (excluding the procambial cells). This elongation growth continued until dormancy when mitotic activity ceased.

Sporogenous tissue did not undergo meiosis before dormancy. During the winter these cells contained darkly staining cytoplasm and an intensly staining fibrous network of nuclear material containing several even darker staining nucleoli. These cells were essentially the same as the sporogenous tissue described during early microsporangial development (compare Figs. 65 and 66). At this point pollen-cone buds were smaller than vegetative buds (approximately 3 x 3 mm) and appeared ovate with the broadest region at the mid-point of the bud axis (Figs. 51, 52). In pollen-cone buds the apex was completely utilized in microsporophyll initiation and no zonal pattern was observed (Fig. 58).

4.6.6 Seed-cone initiation and early development

4.6.6.1 Seed-cone initiation

Bract initiation began in the final week of July in both terminal and axillary buds. At this time the developing megasporangiate strobili (seed cones) could be identified by their tall, broad, bullet-shaped apices and the manner of ergastic substance accumulation (Fig. 39) (see sections 2.4.3 and 4.8.2) The apex grew larger and initiated lateral primordia along the apical flanks (Fig. 70).

Initially, the peripheral zone appeared as 10 to 15 ensheathing layers of anticlinally dividing cells. This pattern was then disrupted by cytoplasmically darkly staining, conspicuously nucleated subepidermal cells along the apical

flanks. These areas demonstrated increased mitotic activity both anticlinal and particularly periclinal. Continued mitotic activity produced a localized tangentially oriented row of meristematic cells which would become the bract buttress. Just above this region another, smaller localized group of cells was observed which would become the ovuliferous scale. Ovuliferous scale meristems were sequentially initiated above the cells of the newly initiated bract meristems (Figs. 71, 72).

4.6.6.2 Seed-cone bud development

Development of bract procambial tissue occurred within the proximal bract buttresses by the second week of August after approximately eight bract buttresses had been initiated (as seen in longitudinal section). Procambial development then began in successively more distal bract buttresses when they became about eight cells in length. At no time were cortical resin ducts observed in association with the procambial strands of the bract.

The lightly staining, vacuolated cells surrounding the procambium comprised the ground tissue. As the bract grew larger, mitotic activity in this region decreased but was active in the darkly staining marginal meristem. The margin of the bract grew sharply upwards almost enclosing the developing ovuliferous scale (Figs. 73, 74, 75, 80).

Cells which would form the ovuliferous scale were present in early seed cone development, but the scale did not become conspicuous until after the formation of the subtending bract procambium (Fig. 73). Then there was increased randomly oriented ovuliferous scale mitotic activity leading to a buttress in the bract axil. The buttress was basally attached both to the bract and the cone axis. The buttress became low and broad (90 x 250 um, respectively) with a three dimensional central plate, usually six files of cells and approximately 70 um thick and 120 um in breadth. These cells appeared to arise from a group of subepidermal cells (similar to apical initials and subapical initials of most leaf primordia) (Fig. 74). Surrounding this plate and comprising the periphery of the ovuliferous scale primordium was a region 7 to 10 cells deep which arose from the margin of the primordium. Above and below this plate was another non-layered region three to four cells deep.

By late August the ovuliferous scales were about two-thirds the bract length and the differentiated central plate region had disappeared (Fig. 75). At the same time two ovule primordia developed on either side and slightly below the median longitudinal axis of each ovuliferous scale. Each ovule primordium arose within the ovuliferous scale and formed a slight adaxial swelling before cone buds became dormant in late October (Fig. 77). The overwintering ovuliferous scale was cross-sectionally larger than the bract

(Fig. 76), and still about two-thirds as long. Two longitudinally oriented resin ducts were observed, one on each side of the bract procambium, extending for half the bract length. One conspicuously large megaspore mother cell was situated at the center of each ovule primordium (Figs. 77, 78).

Dormant seed-cone buds were much larger (5 x 3 mm) than the dormant pollen cone (Figs. 55, 66, 80).

Although seed cone apices initiated bracts and ovuliferous scales until dormancy much of the apex remained distinct. The following spring, the apex initiated more bracts and rudimentary ovuliferous scales, but the latter failed to develop ovules (Singh and Owens 1981). The cone apex remained distinct and was observed until the period of pollination (Fig. 79).

4.7 APICAL CROWN DEVELOPMENT AND BUD DAMAGE

4.7.1 Crown development

Before the end of bud-scale initiation (mid-July) several layers of cells extending across the basal pith region of vegetative and reproductive apices accumulated starch granules. These starch granules accumulated acropetally through the future pith to the level of the developing receptacle (Fig. 81) where the crown region ultimately developed. During leaf, bract and microsporophyll initiation, the granules continued to accumulate acropetally in the pith

cells but remained below the last initiatied primordium (Fig. 82). Accumulation continued until the bud ceased growth at which time starch granules disappeared within one week.

In early August the crown region began to differentiate as a layer 10 to 15 cells thick, forming a diaphragm across the pith at the base of the bud and separating the pith of the developing bud from the pith of the subtending shoot (Figs. 55, 66, 80, 83). In fixed material these cells were initially plasmolyzed then the cell walls thickened until dormancy (Fig. 84).

The crown region stained with ruthenium red (Johansen 1940) indicating that pectic substances were contained throughout the cell walls and particularly the middle lamella. The IKI (Johansen 1940) and phloroglucinol (Johansen 1940) staining tests proved negative indicating starches and lignins, respectively, were not present in the crown region. A Zinc-Chloro-Iodide reaction for cellulose (Jensen 1962) showed a positive test suggesting that either cellulose or hemicellulose was also a major component of the inner cell walls.

A crown cavity began to form below the crown region in all terminal and axillary bud types (vegetative and reproductive) by early October. It formed in the pith of the subtending shoot but did not disrupt the procambial communication between that region and the overlying bud (Figs. 55, 66, 80).

4.7.2 Bud damage

Two types of bud damage were observed microscopically at the time of leaf, bract and microsporophyll initiation (mid-August). The first type appeared to be insect damage by the larval form of the genus Argyresthia (family Yponomeutidae) (Furnis and Carolin 1977) where the axis of the bud and eventually the subtending shoot was hollowed out (Fig. 85). The crown provided little resistance to the penetrating larva. Although this genus represented by A. picea has been reported to infest P. glauca in the Yukon Territory, it has not previously been observed in P. engelmannii.

The second type of damage was a spontaneous degeneration and abortion of axillary buds after bud-scale initiation. Usually the more proximal the bud the greater the tendency to abort while the more distal buds continued to develop. Abortion of proximal axillary buds left a central pillar of collapsed pith tissue. This pillar then collapsed further (Fig. 86) and a meristematic plate of cells (corresponding to the lower crown region) formed a zone structurally and functionally analogous to a periderm or abscission zone (Figs. 87, 88). This zone extended horizontally into the receptacle on each side of the axis severing procambial communication and separating the underlying shoot from the deteriorating bud. The cause of bud abortion was not determined. In some instances there was no external damage, in

others the bud scales were disrupted and collapsed as if external damage was a factor.

Latent apices were observed but the development was distinct from bud abortion. Here the apex normally initiated bud scales but no or only a few rudimentary lateral primordia (Fig. 89), and an apical zonation pattern was not evident. These rudimentary primordia lacked procambial strands and appeared somewhat displaced from the apical flank (Fig. 89). Buds may remain in this latent condition for several years, then initiate more lateral primordia and elongate to form epicormic shoots.

Latent and aborted buds were common. Counts of buds from the upper six whorls of three mature trees in late August showed that 66 percent of all buds developed the previous spring failed to expand into mature reproductive or vegetative structures. This phenonmenon could be explained by either bud abortion, bud latency or both. Based on the observed relative frequency of apical latency (as determined by frequency of epicormic shoots) it was felt that the majority of the undeveloped buds were aborted buds. It is felt that the above 66 percent estimate is conservative since axillary apices might have been reabsorbed at a very early developmental stage shortly after initiation and therefore not included in the counts.

4.8 APICAL MODIFICATIONS

4.8.1 Apical dimensions

Apical dimensions changed in terminal vegetative buds after dormancy and continued throughout the summer.

Apical height increased from about 75 um in late March to 190 um just after bud-scale initiation began at the end of June (Fig. 8). Following the stage of rapid leaf initiation the apical height gradually decreased to the mean dormant height of 75 um.

Apical width exhibited a continuous increase from the start of bud-scale initiation (230 um) throught the early slow phase of leaf initiation (430 um) (Fig. 8). Width increases occurred during bud-scale initiation and subsequent generation of the broadening receptacle. As rapid leaf initiation occurred along the flanks of the apex apical width decreased to a mean dormancy value of 230 um.

Apical area, in longitudinal section (Fig. 9a) increased after dormancy (70 um^2) and during bud-scale initiation when the mean area stabilized at about 210 um^2. Rapid decreases in apical area coincided with rapid leaf initiation and reached a minimum (70 um^2) by the end of leaf initiation.

The apical height-width ratio (Fig. 9b) increased rapidly after the resumption of growth (0.40) to a maximum (0.73) at the end of May. From early June until dormancy there was a gradual decrease to 0.40.

4.8.2 Apical ergastic substance accumulation

In mid-July ergastic substances began to accumulate in the pith cells of many developing buds. These substances (Fig. 37) (presumably acidic compounds because of their preferential safranin uptake) were extra-vacuolar and confined to the cell protoplasm as deduced from transmission electron microscopy (TEM) in the similar species P. glauca. The accumulation of these compounds occurred in two modes: (1) total infiltration of the pith region (Fig. 37); and, (2) incorporation into diffuse longitudinal files of pith cells (Fig. 38).

The accumulation was gradual as illustrated by photometric measurements (Fig. 12). It was not apparent until after foliar initiation had begun that vegetative apices exhibited the first mode of accumulation and reproductive apices the second. Mode one apices were responsible for the plotted values due to their preponderance; whereas, mode two apices occurred at such a low frequency as to have a negligible effect on the curve.

These ergastic substances remained in the extra-vacuolar protoplasm until early September when they began to locate in numerous smaller vacuoles. By mid-September the ergastic substances could be found only within the vacuoles and in October these vacuoles began to coalesce with the newly formed, large central vacuole. This coalescence of vacuoles was associated with the mid-September decrease in ergastic substances (Fig. 12).

The ergastic substances occupied the central vacuole throughout dormancy. Because these pith cells were located in the elongating shoot (and not the apex) further microphotometric changes were not recorded. However, continued observation revealed that shortly before the resumption of mitotic activity there was a fragmentation of the central vacuole into numerous small vacuoles. Further, in late June and early July the ergastic substances began to reappear in the extra-vacuolar protoplasm of the pith cells associated with shoot elongation.

4.8.3 Apical zonation

4.8.3.1 Light microscopy

During dormancy the four apical zones of the vegetative buds (apical initials, central mother cells, peripheral zone and rib meristem) stained similarly (Table IIa) and were indistinct as noted in other species (Owens and Molder 1973; Owens and Molder 1977c; Owens *et al.* 1977). However, zonal patterns could be delimited by variations in cell size and arrangement (*i.e.* files or randomly grouped cells).

Central mother cells appeared as a small, randomly oriented group of large cells subtending the smaller apical initial cells. The underlying rib meristem cells were smaller and arranged in longitudinal files while the peripheral zone cells were even smaller (Fig. 90).

As shoot elongation and bud-scale initiation began, the central mother cells and apical initials appeared as one zone because they possessed the same cellular detail (Fig. 91), whereas, the cells of the peripheral zone and rib meristem were similar and showed characteristics indicative of high mitotic activity (eg. small cells with condensed nuclear material). Two types of interphase nuclei were observed. Peripheral zone nuclei at interphase had small dense nucleoli with diffuse chromatin. The large apical initial-central mother cell interphase nuclei had less distinct nucleoli and diffuse chromatin (Table IIb).

When the rate of bud-scale initiation in terminal vegetative buds slowed (mid-July) the apical initials and central mother cells became distinct and four zones could be recognised (Fig. 92). The nuclei of the apical initials were smaller and more darkly staining than nuclei in the central mother cells. Peripheral zone and rib meristem cells appeared very similar except for the lighter staining cytoplasm of the latter.

Just prior to leaf initiation (late July), ergastic substances began to accumulate in the pith subtending the rib meristem (see section 2.6.4). Cytoplasmic and nuclear details of the rib meristem and remaining apical zones did not vary from those noted at the end of bud scale initiation (Table II). During leaf initiation (early August to mid-September) the nuclear volume of apical initials and central

mother cells decreased so that by dormancy, the nuclei appeared the same size as nuclei in the peripheral zone and rib meristem (Fig. 93). Concomitantly, the zonation became more subtle and was distinguishable only by cell size and arrangement. There was no change in apical zonation during dormancy.

Unlike vegetative apices (Fig. 92), reproductive apices did not have distinct apical initials and central mother cell regions at the end of bud-scale initiation. These two regions remained indistinguishable, except by position, throughout bract and microsporophyll initiation (Figs. 60, 70).

4.8.3.2 Electron microscopy

Thick (0.2 um) cross-sections of terminal buds of P. glauca (Fig. 94) were observed in order to identify the cells of the; apical initials (Figs. 94 level AA, 95), central mother cell region (Figs. 94 level BB, 96), rib meristem (Fig. 94 level CC), pith and peripheral zone (Figs. 94 level DD, 97). Ultrasections (approximately 500 Å) made at these levels (Fig. 94 AA, BB, CC, DD) show the ultrastucture of each cell type (Figs. 98 - 103, Table III).

When an ultramicrograph of pith and peripheral zone tissue is observed (Figs. 94 level DD, 97, 98) it can be seen that the pith region is dominated by cells having large central vacuoles and an electron dense extra-vacuolar proto-

plasmic substance. Extra-vacuolar protoplasmic location is specified since the substance is found within both the nucleus and cytoplasm yet outside the vacuole. This material represents the ergastic substance accumulation noted in P. glauca and P. engelmannii light microscopy. Because of the masking effect of the electron dense material, a full description of the organellar composition is not possible. However, a few cells in the pith region did not concentrate this substance and in these cells normal sized nucleoli (approximately 2 um in diameter) were observed within the rather diffuse chromatin of the nuclei.

The rib meristem cells located just beneath the central mother cells (Figs. 94 level CC, 99) show many small vacuoles, compared to the large central vacuoles of the pith. Mitochondria and differentiated chloroplasts were observed interspersed among the vacuoles. Dictyosomes and rough and smooth endoplasmic reticula were also present. The nucleus contained rather condensed chromatin and normal sized nucleoli.

The central mother cells contained no conspicuous nucleoli (Figs. 94 level BB, 96, 100). Larger isodiametric cells containing large nuclei with extremely diffuse chromatin were present, as compared to normal interphase conditions showing varying degrees of chromatin condensation in the adjacent peripheral zone cells. These cells showed a high density of ribosomes, mitochondria and a low density of vacuoles and differentiated plastids.

The peripheral zone cells (Figs. 94 level CC, 96, 101) had the highest density of ribosomes and mitochondria. The chloroplasts were less numerous but as differentiated as those in the rib meristem and central mother cells. Small vacuoles were a prominant feature yet they were not as abundant as in the rib meristem cells. Nucleoli were obvious and nuclear chromatin appeared condensed in most instances, possibly indicating high mitotic division rates.

The apical initial cells (Figs. 94 level AA, 95, 102) located just above the central mother cells were characterized by high ribosomal, chloroplastic, vacuolar and dictyosomal densities but low mitochondrial densities. Nuclear chromatin was slightly more compact than in the central mother cells (Fig. 100) but never as dense as the peripheral zone cells(Fig. 101). Nucleoli were persistent in nuclear cross-sections.

Protodermal cells (Figs. 94 level CC, 103) located above the region of lateral primordium initiation had a high density of mitochondria. The nuclei of these cells were often large and surrounded by a darkly staining, granular cytoplasm.

Chapter V

DISCUSSION

5.1 GROWTH AND DORMANCY

In any study, the plant structure and the criteria for dormancy must be clearly stated since different structures and regions of the tree may show varying periods of apparent dormancy. While this study did not deal with physiological dormancy, it did deal with apical growth and development as defined by the onset of mitotic activity. As such, mitotic division is probably the most precise and easily measurable criterion of dormancy for use in microanatomical developmental studies.

For example, this study showed that leaf primordial mitotic activity began in mid-April no matter if buds were sampled from the upper (female) or the lower (male) region of the crown. Mitotic activity also began on the same date in seed cone ovuliferous scales and pollen cone tapetal cells on the same trees (Singh and Owens 1981).

Vegis (1964) notes that dormancy and the cessation of growth may show '...geographical variation of the same species, with differences in temperature and daylength in their place of origin...". Even though this point is based on physiological observations the statement might be extended

equally well to microanatomical studies where growth is defined by mitotic activity. For example, Owens et al. (1977) showed that the resumption of apical mitotic activity in Picea glauca is variable with geographical location and elevation differentials of 500m. Brown and Pollack (1981) observed only a four day delay in resumption of reproductive bud mitotic activity with a 400m elevation increment. However progressively longer delays in the latter stages of bud development also were associated with the increased elevation.

This study showed that the cessation of apical cell divisions in P. engelmannii was not influenced by an elevation difference of 260m. But it is possible that the elevation differential was not great enough to influence mitotic activity.

5.2 ANATOMICAL DEVELOPMENT

Intraspecific developmental phenologies often vary with climatic and genotypic factors. Bud development, including bud burst and shoot elongation, is assumed to be modified by climatic factors such as fluctuations in temperature (Sarvas 1965; Fraser 1966; Eriksson et al. 1978) resulting from elevation and latitude variations. This might be the case when the phenologies at the three sites of this study are considered. There was a tendency for decreased total shoot elongation with increased elevation. As well, development

at the high elevation site 3 lagged one week behind that at the low sites 1 and 2 despite the fact that there were no obvious delays in the begining and end of apical dormancy. Genotypic adaptations are also important, Roche (1969, 1970) and Eriksson et al. (1978) have shown that even spruce seedlings from high elevation or northern latitudes grown at lower elevations or southern latitudes retain their inherent slow growth characteristics.

5.2.1 Predifferentiation anatomical development:a correlative mechanism

The anatomical development of a vegetative bud is an integrated complex of developmental processes. These processes include activties of the apex (eg. mitotic activity, bud-scale initiation, receptacle formation, leaf initiation and procambial development) and the subtending shoot axis (eg. axillary bud initiation and development, leaf and shoot elongation, ergastic substance accumulation and crown formation). It is unresonable to presume that vegetative bud development represents a simple, ordered collection of independent processes without some form of interaction. Bud development must be viewed as a complex of these interacting processes.

5.2.1.1 Shoot elongation

Anatomical differentiation in P. engelmannii coincided with the completion of rapid shoot elongation. Shoot elon-

gation extended past the period of anatomical differentiation in the species Tsuga heterophylla (Owens and Molder 1973) and a similar trend was noted in P. abies (Dunberg 1979), Larix occidentalis (Owens and Molder 1979), Abies concolor (Parke 1959), Pseudotsuga menziesii (Allen and Owens 1972), Picea glauca (Fraser 1962; Owens and Molder 1977), P. mariana (Fraser 1966) and P. sitchensis (Owens and Molder 1976).

Pollard and Logan (1979) and Ekberg et al. (1979) speculate that the final shoot length for species with preformed winter buds is limited through the photoperiodic control on the number of stem units and leaf primordia developed the previous year. However, this growth potential is seldom realized, as evidenced by shoots that fail to totally elongate leaving the leaves in an tightly spaced, semi-telescoped condition (Roche 1970;Dunberg 1979). This suggests that although the number of preformed leaf primordia and internodes determines the maximum shoot length, other factors influence the begining, duration and end of shoot elongation (Fraser 1962, 1965; Dunberg 1979).

Only recently have the endogenous implications of shoot elongation been studied but not in conjunction with bud development (Dunberg 1976). In P. abies, gibberellin-like substances rapidly increased with continued shoot elongation then dramatically decreased about one week after maximum elongation (Dunberg 1976). In the same study it was noted

that the variety of gibberellin-like substances, based on bioassay techniques, was greater in shoot than leaf extracts. In Pseudotsuga menziesii seedlings, rapid shoot elongation was associated with the metabolism of H-GA4 into several other gibberellins (Wample et al 1975).

The above investigations imply an increase of gibberellin-like substances and possibly a degradation or change in quantities of growth inhibitor substances with shoot elongation. It is not yet possible to ascertain if this endogenous condition is a result or cause of shoot elongation.

5.2.1.2 Bud-scale initiation

Although the rate of bud-scale initation in Picea engelmannii varied between terminal and axillary buds, both show a decrease and, in terminal apices, a cessation of bud-scale initiation in mid- to late July. This interval has not been noted in other spruces because microscopic bud scale and leaf primordial counts have not been attempted. Even so, Owens (pers. comm. 1980), working with P. glauca speculates that near the end of bud-scale initiation there is a slight increase in apical height which may be a consequence of reduced bud-scale initiation. In P. smithiana (Pillai and Chacko 1978), Abies concolor (Parke 1959), Pseudotsuga taxifolia (Sterling 1946), Torreya californica (Kemp 1943) and Tsuga heterophylla (Owens and Molder 1973) there was a notable increase in apical height at the end of bud-

scale initiation. Data presented here show that increases in apical height were not significant but decreased bud-scale initiation rates were.

The cessation of bud-scale initiation and development appears to be abrupt. During active bud-scale initiation, buttress development normally follows, producing a large laminar bud scale. However, once active initiation was completed, bud-scale development halted leaving the last initiated bud scales in a partially developed state and not totally displaced to the receptacle. These formed rudimentary primordia intermediate in structure between bud scale and leaf primordia.

The pause following terminal bud bud-scale initiation is significant since it not only preceded anatomical differentiation of the bud but appeared to synchronize the development of axillary and terminal buds perhaps prepatory to this differentiation. By the end of terminal bud bud-scale initiation (early July) only approximately half of the axillary bud bud scales had been produced. However by late July axillary buds had "caught up" and both axillary and terminal buds exhibited an "organogenic pause" at a common developmental point when both bud types could be influenced by a differentiation process.

These observations imply that there is a decrease in the rate of bud scale intiation and an organogenic pause before leaf initiation, suggesting that leaf initiation is not

merely a continuation of bud-scale initiation with a altered developmental pattern.

Subsequent to anatomical differentiation, development of axillary vegetative apices lagged behind the terminal buds in leaf initiation rates.

When the interaction between shoot elongation and the rate ot bud scale initiation is considered, terminal apex development shows a positive correlation with the grand phase of shoot elongation. It should be noted that indole-3-acetic acid showed increases during shoot elongation in Picea abies (Dunberg 1976). Since indole-3-acetic acid is reportedly synthesized in the apex and moves mainly down the stem (Bidwell 1974), auxins may be affecting both terminal bud development and shoot elongation, and this may partially explain this correlation in bud development (rate of bud-scale initiation) and shoot elongation.

5.2.1.3 Receptacle generation and crown formation

Receptacle generation occurs throughout bud scale initiation but does not occur during leaf initiation. Because of this, the cessation of receptacle generation may be associated with anatomical differentiation.

The receptacle forms as a result of a short period of peripheral zone periclinal cell division followed by cell enlargement and the formation of a large spirally arranged resin duct system. As the rate of bud scale initiation and

shoot elongation decreased, so did the formation of the receptacle. However, receptacle generation continued for a brief period after bud-scale initiation. This point is well illustrated by the observation of conspicuous rudimentary primordia at the base of vegetative and reproductive apices. The interpretation is that these structures were the last initiated bud scales and the decrease in receptacle formation did not fully displace them from the bud axis.

Receptacle development involves few mitoses relative to the receptacle size and receptacle generation is primarily by cell enlargement and resin duct formation. I speculate that this may be a response to possible depressed bud nutrient levels during bud scale initiation, and shoot and leaf elongation. This integrated system would be further energetically taxed if mitotic activity was the sole source of receptacle generation.

The cessation of receptacle generation coincides with the early formation of a crown region at the level of the receptacle. The cellular processes (cell differentiation and wall thickening) of this formative crown region might concievably terminate the early periclinal mitoses of the receptacle, halting receptacle generation.

5.2.1.4 Ergastic substance accumulation

The term "ergastic substance" is used very generally to include vacuolar inclusions such as tannins, crystals, fats, oils, starch and protein bodies (Jensen and Salisbury 1972; Esau 1977). Of these compounds, tannins appear to be most important in terms of growth regulation and development (Bidwell 1974; Hejenowicz 1979). The presence of ergastic substances in the pith region of developing vegetative buds has been supported by many conifer studies (Gifford and Wetmore 1957; Fraser 1966; Owens and Molder 1973, 1976, 1977c; Pillai and Chacko 1979; Hejnowizc 1979).

While tannin accumulation might be regarded as a simple storage of metabolic by-products, conifer tissue culture studies indicate that the accumulation in the central vacuole may result from synthesis in the endoplasmic reticula and dictyosomes (Hall et al. 1972; Baur and Walkinshaw 1974). This synthesis is thought to occur via the Shikimate pathway, followed by various coupling and condensation reactions mediated by the polyphenol oxidase enzyme system (Taylor and Battersby 1967). As such, synthesis and primary localization in small vesicles might require significant metabolic energy and this requirement might depress mitotic activity. In the present study this period of presumptive tannin synthesis coincided with the end of bud-scale initiation, and cells in the tannin synthesis stage were never observed in mitotic division. However, once the vesicles had

coalesced with the central vacuole mitotic activity again was observed. Similar observations have been illustrated in the work of Chafe and Durzan (1973), Baur and Walkinshaw (1974) and Hejnowicz (1979).

Corcoran *et al.* (1972) and Green and Corcoran (1975) working with angiosperms concluded that tannins depress GA induced growth, although the inhibition may be relieved by application of exogenous GA. This is noteworthy since a rise in gibberellin-like substances has been correlated with increased shoot elongation in *P. abies* (Dunberg 1976). It might be speculated that the endogenous peak in gibberellin-like substances (in the final stages of shoot elongation) releases the apical inhibitory effect of the tannins allowing growth of the bud axis and leaf initiation to begin.

It is further postulated that in *P. engelmannii* the initial synthesis and presence of the extra-vacuolar protoplasmic ergastic substances in the pith region is associated with the cessation of bud-scale initiation and the subsequent organogenic pause in the adjacent peripheral zone. It is possible that the abrupt cessation in bud-scale initiation and the subsequent organogenic pause are required in order to generate a lateral primordium initiation stimulus leading to vegetative or reproductive bud formation.

5.2.2 Physiological differentiation

5.2.2.1 The differentiation process

In P. engelmannii, anatomical differentiation is a short process occuring after bud-scale initiation when the apex initiates either vegetative or reproductive structures. It is likely that physiological-biochemical differentiation processes preceed this interval by two to three weeks coinciding with the organogenic pause (Fig. 13). In most hormone enhancement studies, hormones have been applied over a long intervals in order to successfully bracket the period of physiological-biochemical differentiation (Pharis and Owens 1966; Owens and Pharis 1967; Ross and Pharis 1976a, 1976b; Ross 1977; Puritch et al. 1979; Ross and Greenwood 1979; Wheeler et al. 1980). In fact, Dunberg (1979) suggests that the period of physiological-biochemical differentiation occurs at 80 to 100 percent of the final shoot elongation. However, recent hormonal timing investigations (Tompsett 1978; Luukkanen 1980; Ross et al. 1981) have indicated that the physiological-biochemical differentiation occurs very early in bud development. For example Tompsett (1978) working with P. sitchensis, recommends that chemical applications aimed at influencing bud differentiation should be applied at the begining of bud-scale initiation.

In light of these studies anatomical differentiation may be regarded only as a final point, beyond which cultural treatments would not be successful in enhancing reproductive

differentiation. Cultural treatments should be applied at an earlier time and continue through anatomical differentiation.

5.2.2.2 Differentiation and mitotic frequency

In the present study, collections were made weekly, and the notable increase in apical mitotic frequency (which is often considered to be an approximation of physiological differentiation (Bernier 1971)) occured over a two week period. However, there are problems in approximating differentiation using only mitotic frequency data: (1) the dual component nature of the mitotic peak; and, (2) the low percent of reproductive apices. These are explained below.

Although the time of anatomical differentiation was not observed to vary with the type of cone produced, the mitotic peak was composed of two peaks so that the upper tree whorls (female region) peaked one week before the lower whorls giving two peaks in mitotic activity rather than one. This is in conflict with other investigations which showed that pollen cones differentiated before seed cones (Giertych 1967; Owens and Molder 1977b).

The interpretation of the mitotic frequency data is further complicated by the low percentage of reproductive apices. Although the samples included a large number of apices, most became vegetative (approximately 80 percent) and only a small proportion (approximately 20 percent) became

reproductive. However, if the concept of differential morphogenesis is valid, i.e. the process of lateral primordium initiation in vegetative and reproductive apices is essentially the same (Cutter 1959; Jackson and Sweet 1972; Romberger 1974; Lindgren et al. 1977)) one might expect the period of differentiation to be the same for reproductive and vegetative apices. Thus a peak in mitotic frequency proceeding anatomical differentiation might be an estimate of the time of bud type differentiation. This study illustrates that a mitotic frequency burst does occur, but only after the period of anatomical differentiation, during early primordium initiation.

In P. glauca (Owens et al. 1977) mitotic peaks could not be correlated with anatomical differentiation, however, in a later paper (Owens and Molder 1977c) a rise in mitotic activity was observed but only in buds selected as presumptive seed cones. For practical purposes, the mitotic peak is a fair index of anatomical differentiation. However in morphogenetic studies the peak in conifers occurs later in the differentiation process than in most angiosperms.

From the data presented in the above reports and the present study it appears that increased mitotic activity alone is not a reliable nor precise indicator of anatomical differentiation. Other criterion must be used as well and these are discussed below.

5.2.2.3 Differentiation and apical measurements

Owens and Molder (1977c) demonstrated increases in apical height and width in Abies amabilis during the period of anatomical differentiation. Later, Owens et al. (1977) working with P. glauca oberved a similar increase in apical width at anatomical differentiation but a peak in apical height was not apparent.

In this study, apical measurements (height, width, area and height-width ratio) show no inflection points corresponding to the observation of anatomical differentiation and appear to be poor indicators of differentiation.

5.2.2.4 Differentiation and ergastic substance accumulation

The only apical measurement that associates well with observed anatomical differentiation is ergastic substance accumulation (see section 2.4.3 and 4.8.2). It coincides with the differentiation of vegetative and reproductive bud types and furthermore, the percentage of pith cells containing ergastic substances may reflect the sexual status of that apex, such that high levels are associated with vegetative apices while lower levels are associated with reproductive apices.

5.3 SEED-CONE POSITION AND PRODUCTIVITY

5.3.1 Seed cone productivity

There are two important components in determining the number of seed-cone buds produced: (1) the number of axillary apices initiated and developed to differentiation; and, (2) the number of axillary and terminal apices differentiating as reproductive.

Several studies have shown that particularly cool temperatures during axillary bud initiation, are correlated with abundant seed cone crops (Lowry 1966; van Vrendenburch and La Bastide 1969; Lindgren *et al.* 1977), suggesting an increased number of axillary buds which are capable of becoming reproductive. However, an increased number of axillary buds alone does not convey a greater cone crop. Warm temperatures and dry moisture regimes during bud differentiation have been correlated with large cone crops (Matthews 1963; Brondbo 1968; Puritch 1972; Lindgren *et al.* 1977). In *P. glauca* (Fraser 1962) not only were these elevated temperatures correlated with increased cone crops, but cool temperatures at the same time were associated with poor crops.

Since all trees in the present study were similar in terms of full crown, good growth and a history of cone productivity, their full potential for high productivity should be realized in an environmentally favorable year. However, this was not the case, tree productivities varied considerably even though environmental conditions were essentially

homogenous. Because of this wide variability one must presume an interaction between environment and the inherent genotypic, biochemical and physiological factors.

It is probable that pollen-cone differentiation and development reflects the same physiological alterations of seed-cone buds but involves different endogenous factors (Giertych 1967). Still, large scale seed cone production is usually of greater importance in tree improvement and reforestation programs due to the marked periodicity of seed cones. On the other hand, pollen cones show less of a periodicity and generally larger crops posing less difficulty in pollen collection.

5.3.2 Seed cone position

In P. engelmannii when cone production is low (less than 30 percent of buds) seed cones develop primarily from the reproductive differentiation of previously vegetative terminal buds, whereas high tree productivity results from increases in the proportion of reproductive buds in both terminal and axillary positions. The number of seed cones in axillary position never exceeded those in the terminal position (mean terminal - axillary ratio 2.9:1). The predominantly terminal positional relationship has been noted but without quantification by Owens and Molder in P. glauca (1977) and P. sitchensis (1976).

5.3.3 Tree periodicity

Seed cone productivity of vigorous growing trees may be quite variable and in this study ranged from 6 percent to 52 percent of potential buds. The formation of reproductive buds from previously vegetative terminal buds and undifferentiated axillary buds terminates the vegetative capacity of the apex. For example, in most conifers (with a similar manner of reproductive differentiation to P. engelmannii), a seed cone productivity of 60 percent means that 60 percent of the active growth points are lost and the potential of upper crown growth might also reduced by 60 percent (Lindgren et al. 1977). As well, the nutrient sink implications of high cone productivity (Rooke and Sweet 1970; Dickmann and Kozlowski 1968, 1970, Kozlowski 1971; Powell 1977a, 1977b) would cause decreased vigor of future vegetative growth.

The formation of terminal seed-cone buds might stimulate many of the newly initiated potentially latent axillary apices to develop, sustaining a near constant number of vegetative growth points. However, observations made in this study suggest that the number of latent buds present is not sufficient to maintain these growth points over a number of years of maximal tree productivity. This observation along with the tendency towards decreased shoot elongation and bud development with increasing elevation might be responsible for the typical narrow spire-like upper crown in P.

engelmannii. In P. engelmannii, high tree productivities are seldom observed over two consecutive years, rather there is normally a two year interval between good seed cone production on a given tree.

Similar seed cone periodicities have been observed in crop estimates for entire stands of most native conifers (Fowells 1965; Franklin 1968; Ronco 1970; Dobbs 1972; Lindgren et al. 1977). In particular, stands of P. engelmannii show a three to six year periodicity (Ronco 1970; Dobbs 1972) which is in agreement with the above estimation based on crown observations of individual trees. Although individual tree productivity was calculated in the present study, it is not difficult to visulalize the synchrony in a stand every three to six years resulting in elevated seed cone crops in isolated regions as was described in Abies balsamea (Powell 1977a). This syncrhonized aspect is supported when environmental factors (temperature, rainfall, moisture regimes, etc.) are correlated with axillary bud initiation and anatomical differentiation (Matthews 1963; Puritch 1972). Because of this it is felt that environmental conditions may serve as a promotor and hence a synchronizing agent behind reproductive differentiation in forest stands.

5.4 APICAL ZONATION

5.4.1 Light microscopy

Changes in the apical zonation pattern have been noted in many studies. In P. engelmannii these changes are usually manifest as an indistinct pattern during dormancy, a distinct pattern during bud-scale initiation through to leaf initiation, and again an indistinct pattern at dormancy.

The presence of a distinct zonal pattern seems indicative of the readiness to produce and develop lateral foliar primordia. For example, in this study and Abies amabilis (Owens and Molder 1977c) latent buds lacked a distinct zonal pattern of apical initials and central mother cells. These observations might suggest that the apical initials and central mother cells are exerting a morphogenetic control on the development of the bud by mediating the peripheral zone activity. On the contrary, Cecich and Miksche (1970) noted that irradiation and subsequent losses of apical initials and central mother cell volume and zonal pattern did not affect the leaf production of P. glauca apices. This observation implies that those zones are not functioning in controlling leaf initiation.

Confusion over the presence or absence of zonation at any one time may result from our inability to resolve minute differences using the light microscope.

5.4.2 Electron microscopy

Of the five zonal cell types studied (pith, rib meristem, central mother cells, peripheral zone and apical initials), combinations of certain ultrastructural aspects (Table III) help to differentiate these zones under the light microscope.

Apical zonation patterns result primarily from differences of mitotic division planes, vacuolar and nuclear densities and sizes and cell size. That is, the central mother cells have the largest nuclear volume compared to any other cell and show the least mitotic activity. As such, light microscopy shows this region to be lightly staining when compared to the smaller darkly staining peripheral zone cells having smaller more dense nuclei. The peripheral zone cells which divide mainly anticlinally are distinguished from pith and rib meristem cells by decreased cell size and increased optical density. Rib meristem cells are distinquished from pith cells because the former are smaller, with numerous small vacuoles and no darkly staining extra-vacuolar protoplasmic ergastic substances. The filed appearance of the pith is another distinctive feature in the delimitation of apical zones.

The above observations and interpretations should be treated with caution since apical zonation patterns change during different stages of development and specimens were collected only during late bud-scale initiation. For exam-

ple, the mergence of the apical initials and central mother cells during early bud scale initiation no doubt reflects ultrastructural modification. The same would be true for an apex approaching dormancy where zonal patterns become very indistinct.

Few studies have been conducted on the ultrastructural aspects of apical zonation. What literature exists is based upon apices from germinating seed (Cecich and Horner 1977) and young seedling of Pinus (Cecich 1977, 1979). The findings of these studies were similar to those for Picea glauca. Cecich (1977) notes that in six-month-old Pinus banksiana seedlings the apical initials and central mother cell nuclei stained lightly because they contained little or no heterochromatic clumping, however, the peripheral zone and rib meristem nuclei were heterochromatic. At the same time prominant nucleoli were observed in the apical initial and central mother cell nuclei. The later finding is not in keeping with the present study. The reason for this is probably that apical zonation is variable depending on the developmental stage of the apex and that Pinus and Picea (collected in mid-July and mid-May respectively) would not be developmentally synchronized. A second conflicting observation that arises in Pinus banksiana (Cecich 1977) is the appearance of mylen-like lipid spheres called spherosomes located in the apical initials and central mother cells. These spheres were reportedly extracted upon fixation with

Navashin Craf IV but remained when material was fixed for TEM in a glutaraldehyde-osmium tetroxide double fix using PIPES buffer. The reverse was noted in Picea glauca where the Navashin's fixative did not extract these bodies while the similar double fix buffered with cacodylate acid failed to retain the spherosomes. Perhaps the buffer requirement is essential to satifactory fixation. Otherwise the ultrastructural zonal aspects of Pinus banksiana (Cecich 1977, 1979, 1980) are similar to those of Picea glauca.

Chapter VI

CONCLUSIONS

In conclusion, the phenology of vegetative buds in P. engelmannii is the same as that described for the other native Picea (P. glauca and P. sitchensis) and may be outlined as in figure 13.

The developmental highlights may be summarized as:

(1) Growth begins in mid-April as determined by the appearance of leaf primordium mitoses.

(2) Anatomical differentiation occurs in late July to early August as determined by: (a) a completion of bud scale initiation and an associated organogenic pause; (b) the completion of shoot elongation; (c) the accumulation of a darkly staining ergastic substance in the pith region of the shoot tip; and, (d) a burst in apical mitotic activity shortly after differentiation. Apical changes such as height, width, area and height-width ratios are poor indicators of anatomical differentiation. This interval of anatomical differentiation does not represent that of physiological-biochemical differentiation which cannot be predicted from the present study. Anatomical differentiation represents the final point at which cultural practices may influence bud development.

(3) During the organogenic pause the apex may; (a) abort, (b) become latent, (c) become vegetative or (d) become reproductive.

(4) Seed cones tend to differentiate from previously vegetative and newly initiated, previously undifferentiated axillary apices in a mean ratio of 2.9:1.

(5) Pollen-cone buds develop almost exclusively from axillary, previously undifferentiated apices.

(6) Development and growth ceased by late October as determined by the end of mitoses within the telescoped, preformed vegetative and reproductive buds.

The gap between the physiological-biochemical and the anatomical differentiation of developing apices must be resolved in order to accurately identify the time at which a reproductive apex becomes determined. Such investigations will require a complete integration of physiology, biochemistry, histochemistry and microanatomical development (at both the light and electron levels). I feel that meaningful results cannot be realized by any one of these disciplines alone.

Chapter VII

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Chapter VIII

TABLES

Table I: 1980 seed and pollen cone counts of *Picea engelmannii* Table contains positions and numbers of reproductive cones as initiated in 1979. Female counts represent all buds in the upper six whorls while the male counts represent random three branch samples from the ninth whorl.

Site	Tree	Sex	TERMINAL POSITION Veg	TERMINAL POSITION Rep	TERMINAL (%) Tot	TERMINAL (%) Rep	AXILLARY POSITION Veg	AXILLARY POSITION Rep	AXILLARY (%) Tot	AXILLARY (%) Rep	FINAL SUMS Tot	FINAL SUMS (%) Rep
1	1	f	190	218	409	54	393	259	652	40	1061	45
1	2	f	260	40	300	13	444	8	452	2	752	6
1	3	f	142	94	236	40	428	1	429	0	665	14
2	1	f	155	104	259	40	281	103	384	27	643	32
2	2	f	165	180	325	55	514	14	528	3	853	23
2	3	f	62	32	94	34	202	14	216	7	310	15
3	1	f	52	126	178	71	130	113	243	47	421	57
3	2	UPPER SIX WHORLS BLOWN OFF NATURALLY IN 1980										
3	3	f	24	79	103	77	94	81	175	46	278	58
s	m	t f	372	156	528	30	245	0	245	0	773	20
u	e	r f	139	193	332	58	223	7	230	3	562	36
p	n	e f	444	71	515	14	348	1	349	0	864	8
p	t	e f	198	512	710	72	459	186	645	29	1355	52
l	a	s f	415	202	617	33	888	51	939	5	1556	16
e	l	f	249	77	326	24	373	53	426	12	752	17
-		f	196	215	411	52	520	66	586	11	997	28
1	1	m	NO POLLEN CONES ON TREE: CONE CROP ZERO									
1	2	m	33	4	37	11	5	124	129	96	166	77
1	3	m	91	13	104	13	16	131	147	89	251	57
2	1	m	102	19	121	16	37	350	386	90	508	73
2	2	m	114	8	122	7	27	101	128	79	250	44
2	3	m	179	2	181	13	23	60	83	72	264	23
3	1	m	62	0	62	0	37	110	147	75	209	53
3	2	m	50	0	50	0	14	38	52	72	102	37
3	3	m	64	1	65	2	54	160	214	75	279	57

Table II: Changes in apical zone cellular detail of *P. engelmannii* during vegetative bud development.

ZONE	CYTOPLASM	NUCLEI
(a) Cellular details during apical dormancy		
Peripheral	-dark	-interphase, dense, dark
Rib meristem	-light	-interphase, dense, dark
Apical initials	-light	-interphase, dense, dark
Central mother cells	-light	-interphase, dense, dark
(b) Cellular details during early bud-scale initiation		
Peripheral	-dark	-interphase to telophase
Rib meristem	-light	-interphase, dense
Apical initials	-light	-interphase, diffuse
Central mother cells	-light	-interphase, diffuse
(c) Cellular details during late bud-scale initiation		
Peripheral	-dark	-interphase to telophase
Rib meristem	-light	-interphase to telophase
Apical initials	-dark	-interphase
Central mother cells	-light	-interphase

Table III: Ultrastuctural characters of the five zonal cell types studied in vegetative terminal apices of *Picea glauca*.

Character	Pith	Rib meristem	Central mother cells	Peripheral zone	Apical initials
Mito. density	-*	+++	++++	++++	
Chloro. density	-	++++	+	++	
Vacuole volume	++++	++	+	++++	
Cell size	++	+	++++	+	++
Nuclear size	+	++	++++	++	++
Nucleolar size	-	+	-**	+	+
Chromatin condensation	-	++++	+	++++	
Mitotic activity	++	++	+	++++	

-* no observations due to ergastic substances

-** nucleoli not identified

Note; all +'s represent increasing magnitude of character.

Chapter IX

FIGURES

Figure 1.

The distribution of *Picea engelmannii* Parry, (after McSwain *et al.* (1970)).

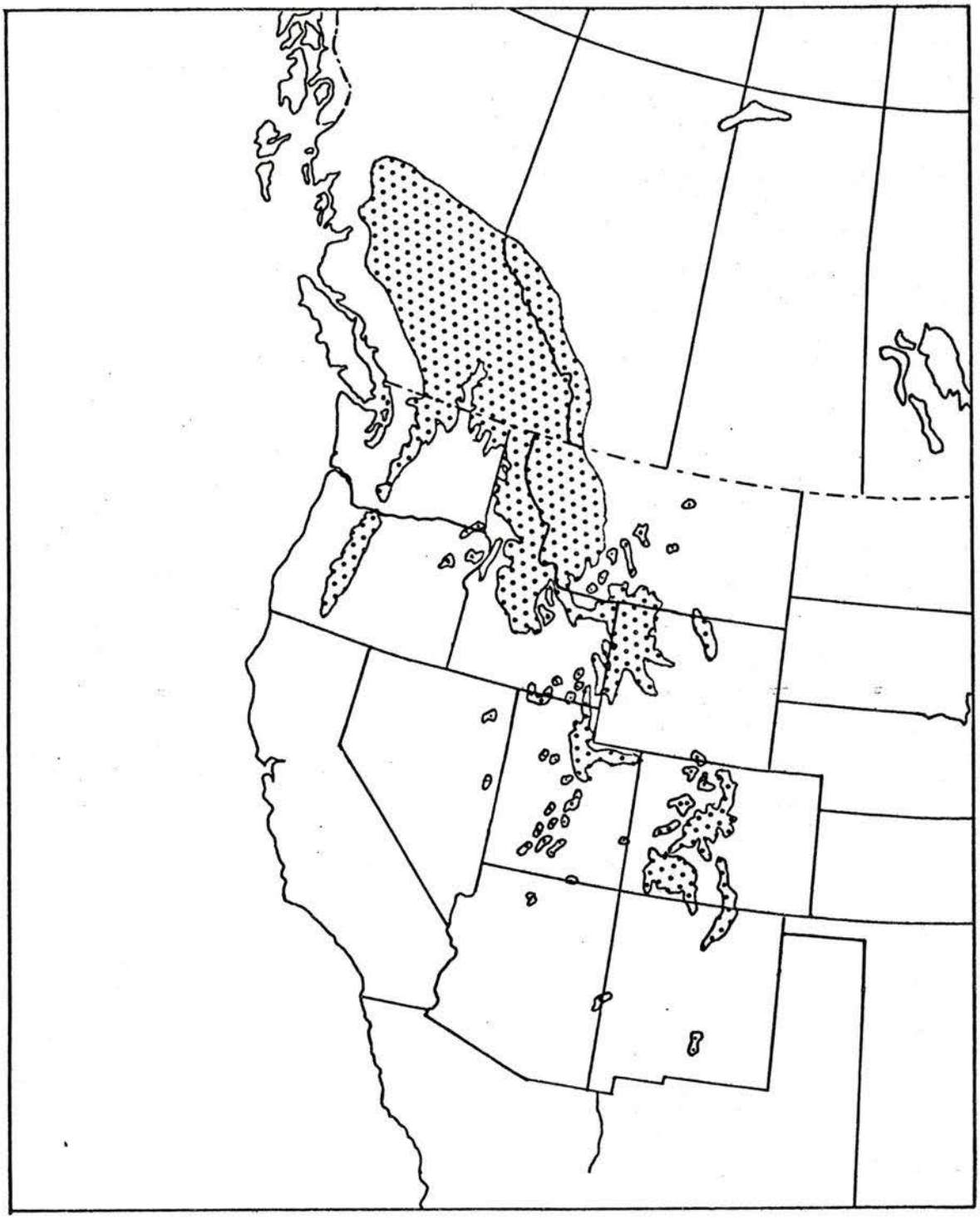
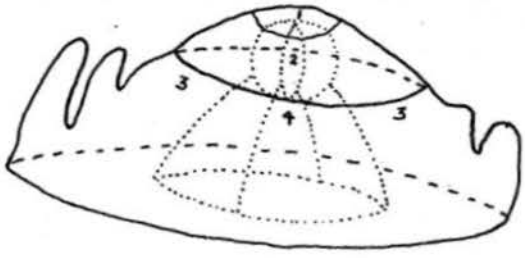


Figure 2.

The shoot tip of Abies concolor during dormancy (a) and leaf initiation (b). The apex defined as the area above the last initiated primordia (above level CC) varies at each developmental stage. Note that all four zones do not necessarily lie within the apex. apical initials, 1; central mother cells, 2; peripheral zone, 3; rib meristem and central tissue zone, 4. (after Parke (1959)).


3
2
4
3

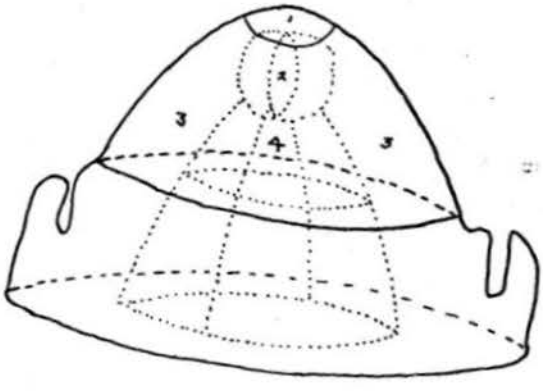

1
2
3
4
3

Figure 3.

A plot of seed-cone position showing the corresponding percents of terminal and axillary seed-cones versus tree productivity. Upper graph region (●) is represented soley by terminally located seed-cones while the lower region (O) is composed of axillary seed-cones. Note, in no instance does the number of axillary seed-cone on any one tree exceed the number of terminal seed cones on the same tree.

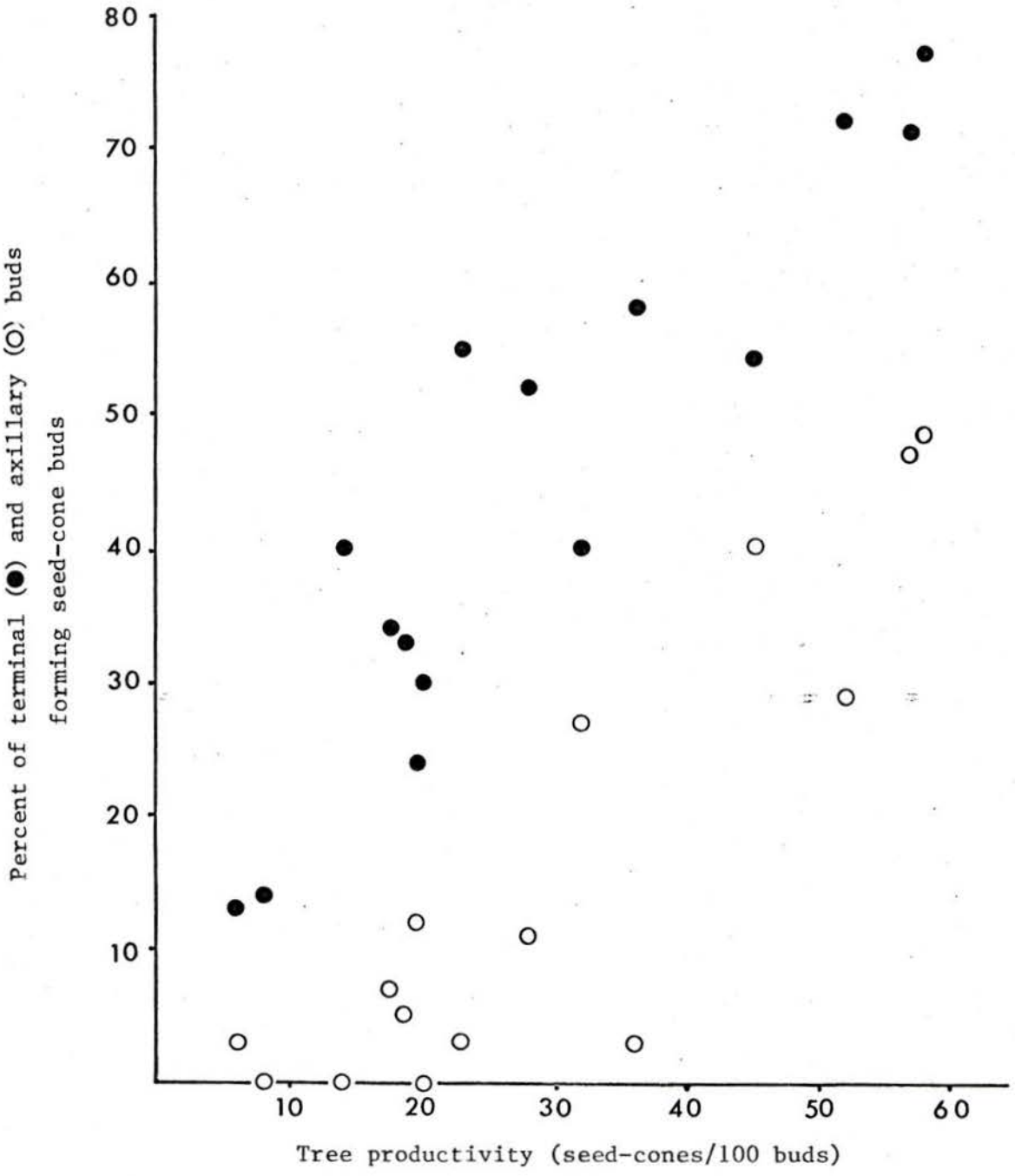
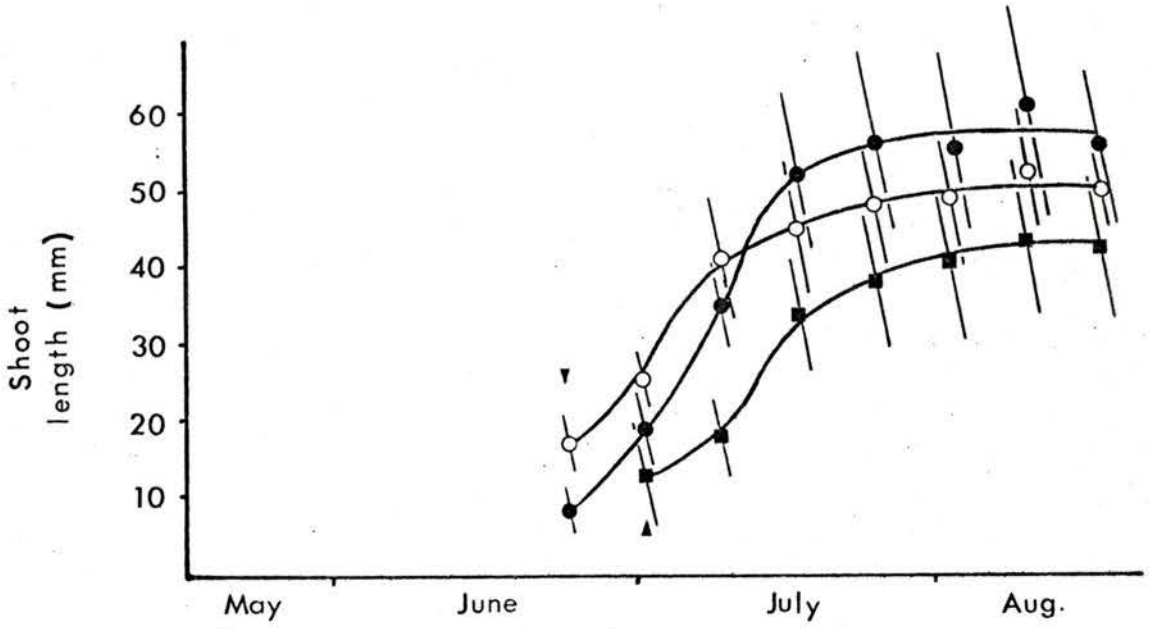

Percent of terminal (●) and axillary (○) buds forming seed-cone buds
80
70
60
50
40
30
20
10
10
20
30
40
50
60
Tree productivity (seed-cones/100 buds)

Figure 4.

Shoot elongation of terminal vegetative buds at three different elevations from the end of dormancy to mid-August. Plotted values (representing the mean lengths of four elongating shoots in the peripheral mid-crown region) are fitted with 95% confidence limits. (●), site 1; (○), site 2; (■), site 3. Note: arrows mark the point of bud-burst.

Figure 5.

Percent of total shoot elongation in terminal vegetative buds versus time. Data from sites 1 and 2 have been pooled therefore each point (●) represents a sample of size n=8 whereas the plot of site 3 data (■) represents a sample of size n=4. Shoot lengths from dormancy to mid-June have been obtained from prepared slides and made up to the appropriate sample sizes (i.e. n=8 and n=4) for continuity. (■), pooled sites 1 and 2; (●), site 3. Note: arrows mark the point of bud-burst at each site.


Shoot
length (mm)
60
50
40
30
20
10
May
June
July
Aug.

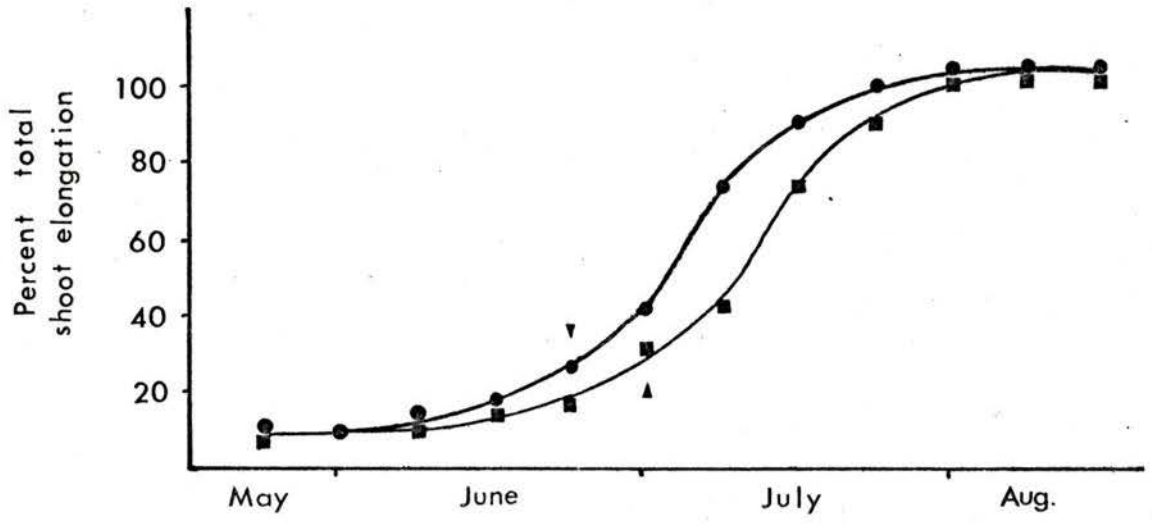
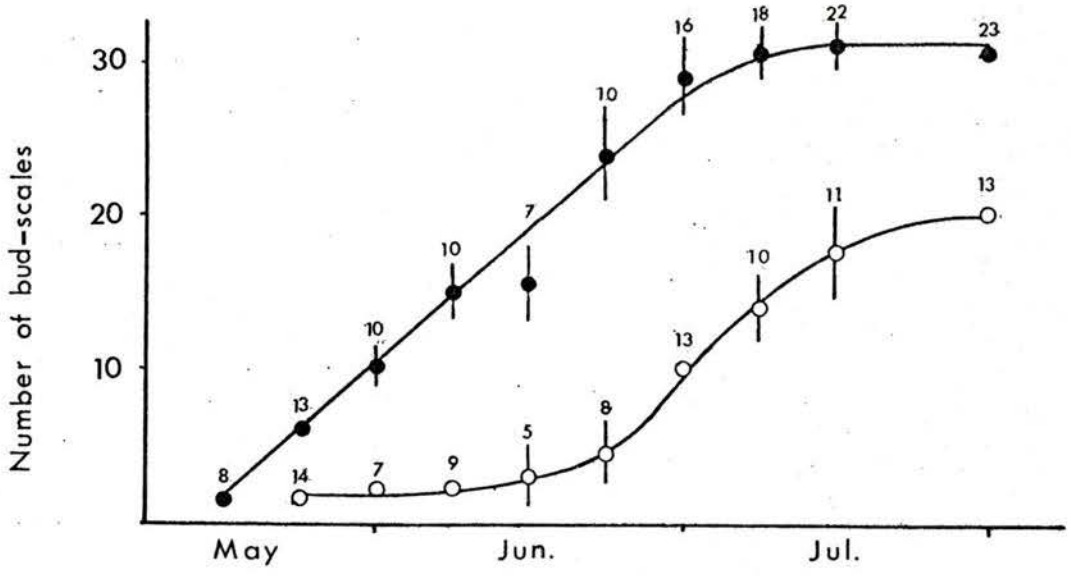

Percent total
shoot elongation
100
80
60
40
20
May
June
July
Aug.

Figure 6.

Bud scale initiation in terminal and axillary buds from dormancy to early August. Bud scale number is derived microscopically from the counts of primordia along the apical flanks and upon the receptacle as observed in the most median longitudinal section. Sample size (n) is plotted along with 95% confidence limits. (●), terminal buds; (O), axillary buds.

Figure 7.

Leaf initiation in terminal and axillary buds from the end of bud scale initiation to early October. Leaf primordia number is derived microscopically from the number of primordia retained along the apical flanks in median longitudinal sections. Sample size (n) is plotted along with 95% confidence limits. (●), terminal buds; (O), axillary buds.


Number of bud-scales
30
20
10
May
Jun.
Jul.
8
13
10
10
7
16
18
22
23
14
7
9
5
8
13
10
11
13

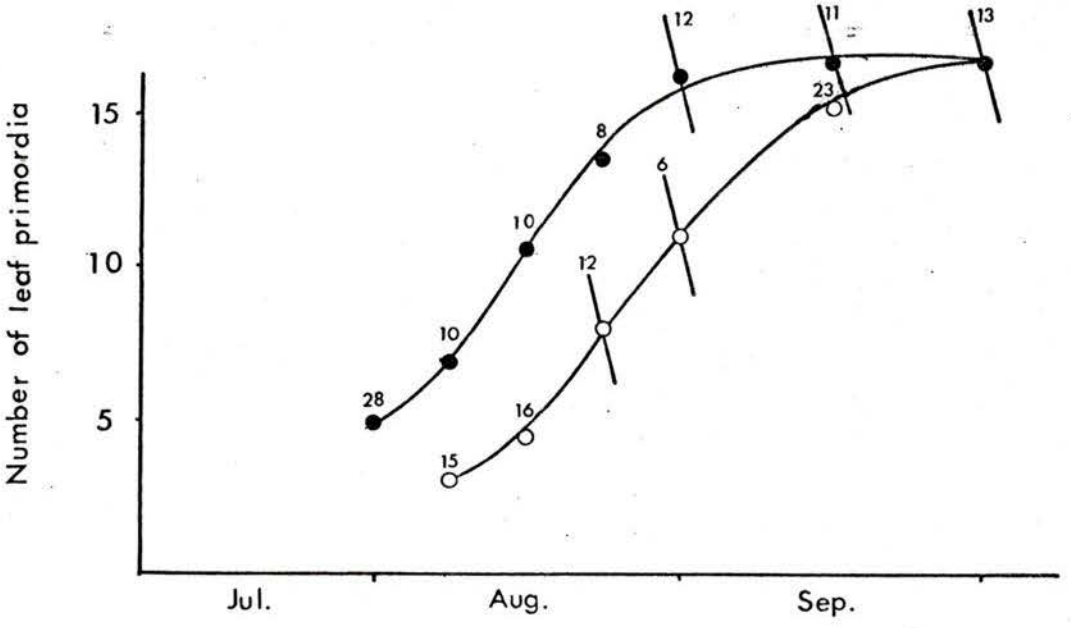

Number of leaf primordia
15
10
5
Jul.
Aug.
Sep.
28
10
10
8
12
11
13
15
16
12
6
23

Figure 8.

A plot of height and width (as determined from median longitudinal section of terminal vegetative buds) from early April to early November. Vegetative developmental phenology (upper graph region) has been included. Sample sizes (n) are the same for both plots and appear with 95% confidence limits. (●), apical width in um; (O), apical height in um.

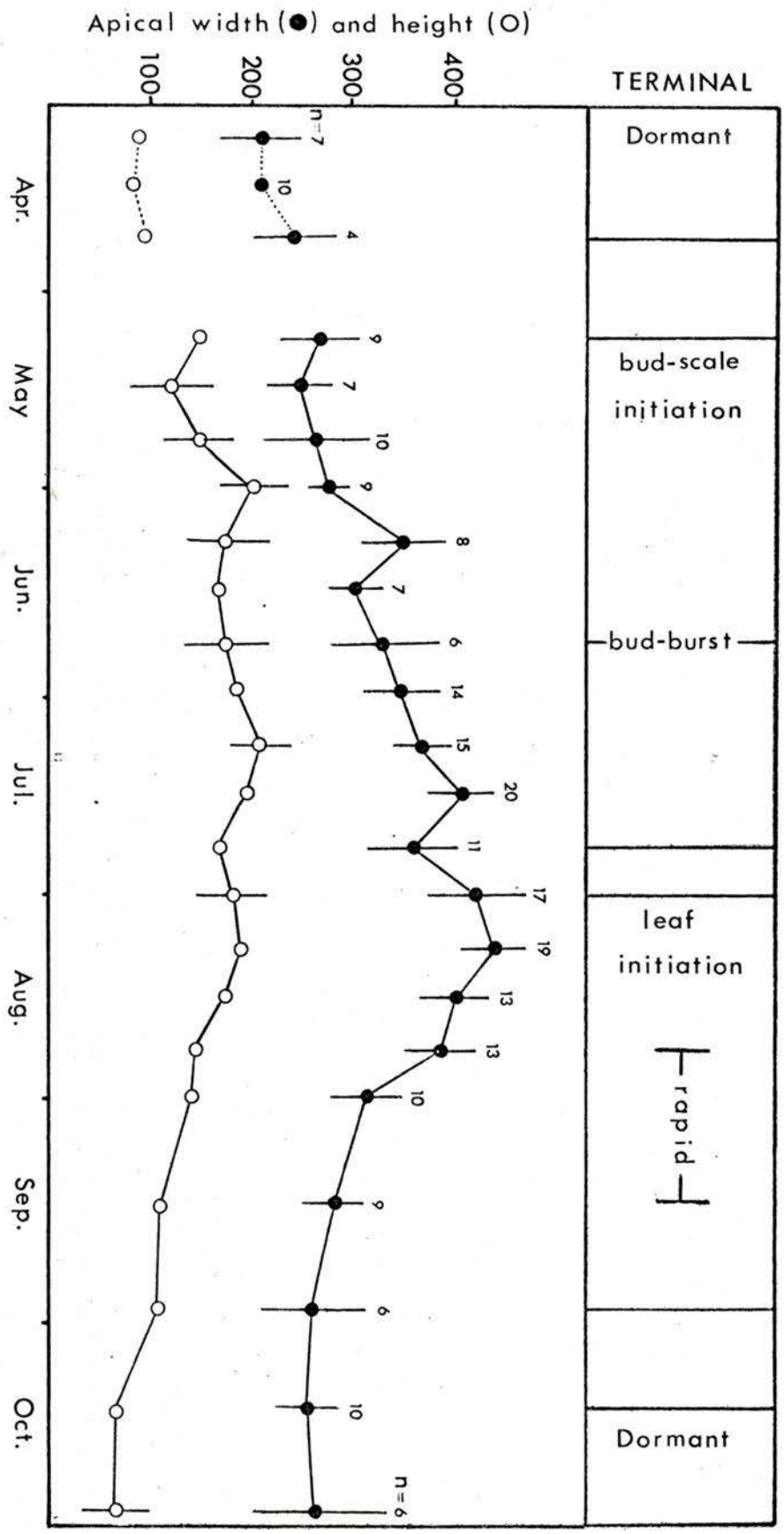

Apical width (●) and height (O)
100
200
300
400
TERMINAL
Dormant
bud-scale initiation
bud-burst
leaf initiation
rapid
Dormant
Apr.
May
Jun.
Jul.
Aug.
Sep.
Oct.
n=7
n=6

Figure 9.

(a) The apical area of the most median longitudinal section of terminal vegetative buds from early April to early November. (b) The height-width ratio of terminal vegetative buds as determined from the median most longitudinal sections during the early April-early November interval. Note, the upper graph region represents vegetative developmental phenology. Both plots represent the same sample size (n) and are fitted with 95% confidence limits.

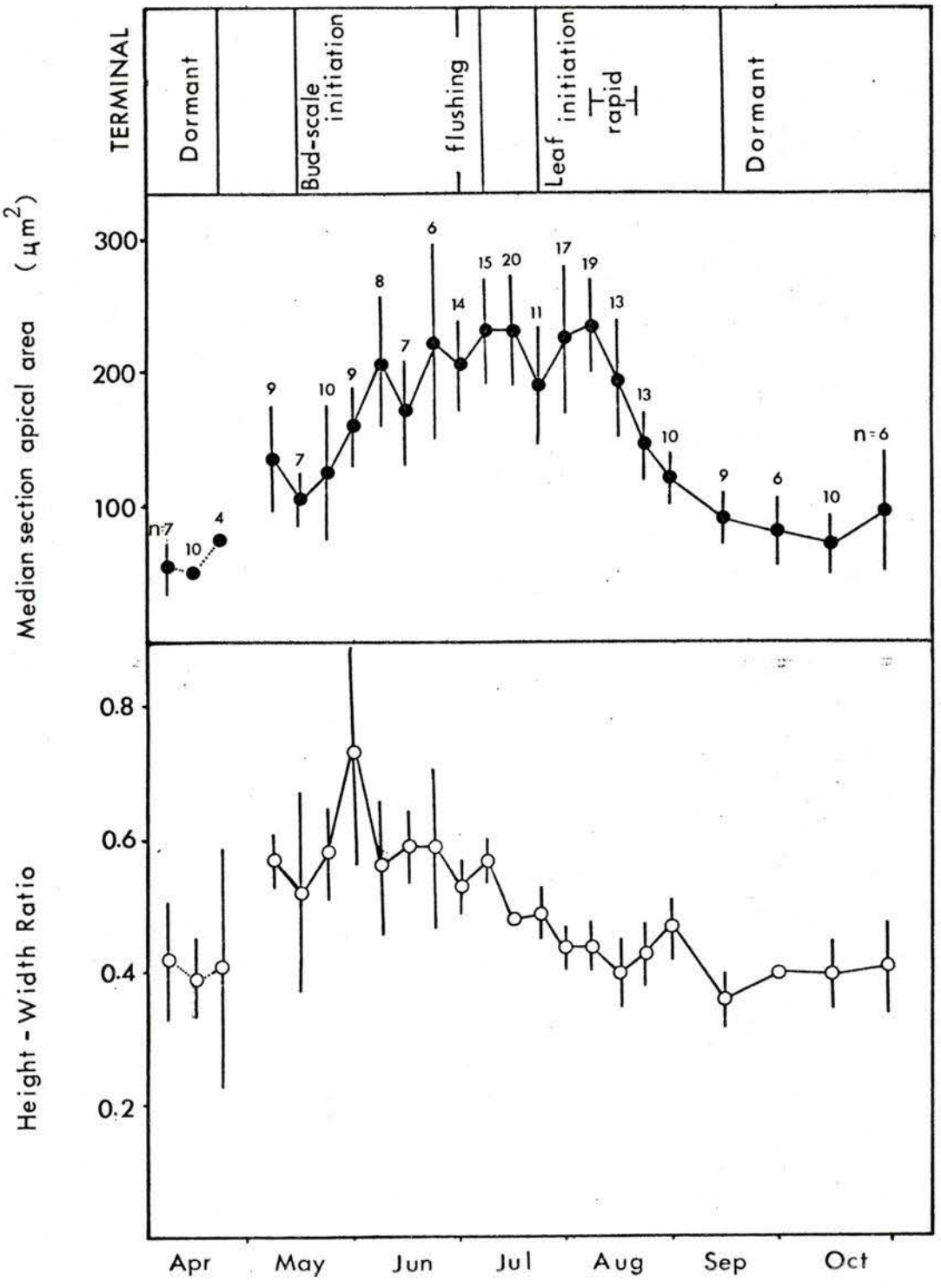

TERMINAL
Dormant
Bud-scale initiation
flushing
Leaf initiation
rapid
Dormant
Median section apical area (μm²)
300
200
100
n=7
10
4
9
7
10
9
8
7
6
14
15
20
11
17
19
13
13
10
9
6
10
n=6
Height - Width Ratio
0.8
0.6
0.4
0.2
Apr
May
Jun
Jul
Aug
Sep
Oct

Figure 10.

A plot of apical mitotic frequencies versus time. Mitotic frequencies of terminal and axillary buds (based on 10% of the apical diameter) have been pooled and plotted for each date begining in early July and continuing to dormancy, values prior to this date represent mean terminal bud mitotic counts only. Apical mitotic counts have been fractioned into peripheral zone activity and rib meristem activity. Note, the vegetative bud developmental phenologies of terminal and axillary buds have also been drawn. (O), peripheral zone mitotic frequency; (●), rib meristem mitotic frequency.

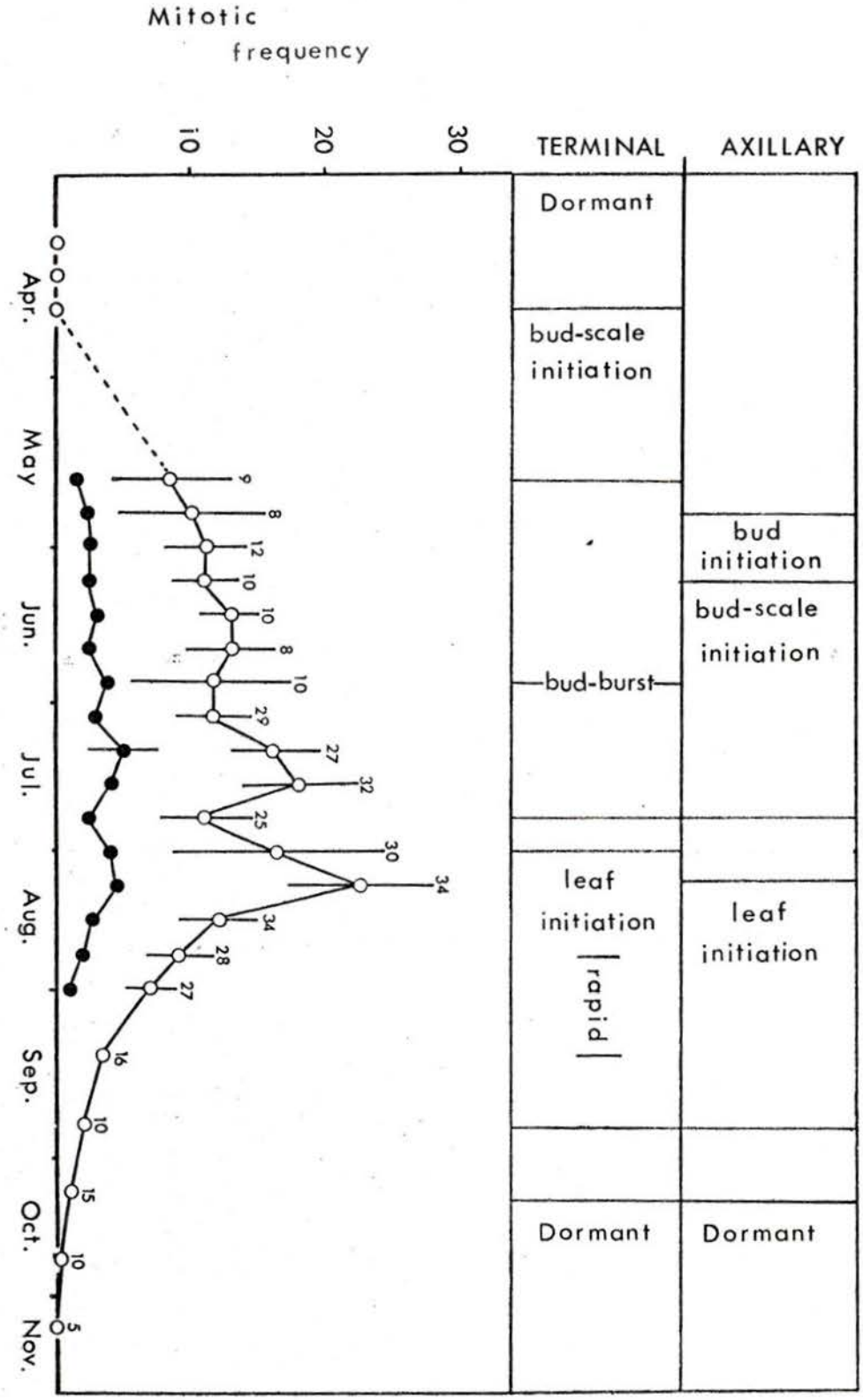

Mitotic
frequency
10
20
30
TERMINAL
AXILLARY
Dormant
bud-scale
initiation
bud
initiation
bud-scale
initiation
bud-burst
leaf
initiation
rapid
leaf
initiation
Dormant
Dormant
Apr.
May
Jun.
Jul.
Aug.
Sep.
Oct.
Nov.
9
8
12
10
10
8
10
29
27
32
25
30
34
34
28
27
16
10
15
10
5

Figure 11.

Apical mitotic frequency plots of peripheral zones versus time as derived from Figure 10. The data has been fractioned into positions (terminal and axillary) and potential sex (female or male zone). Sample sizes (n) and 95% confidence limits have been plotted. (a), peripheral zone mitotic frequency of axillary buds; (b), peripheral zone mitotic frequency of terminal buds; (c), peripheral zone mitotic frewquency of buds from the female regions; (d), peripheral zone mitotic frequency of buds from the male region.

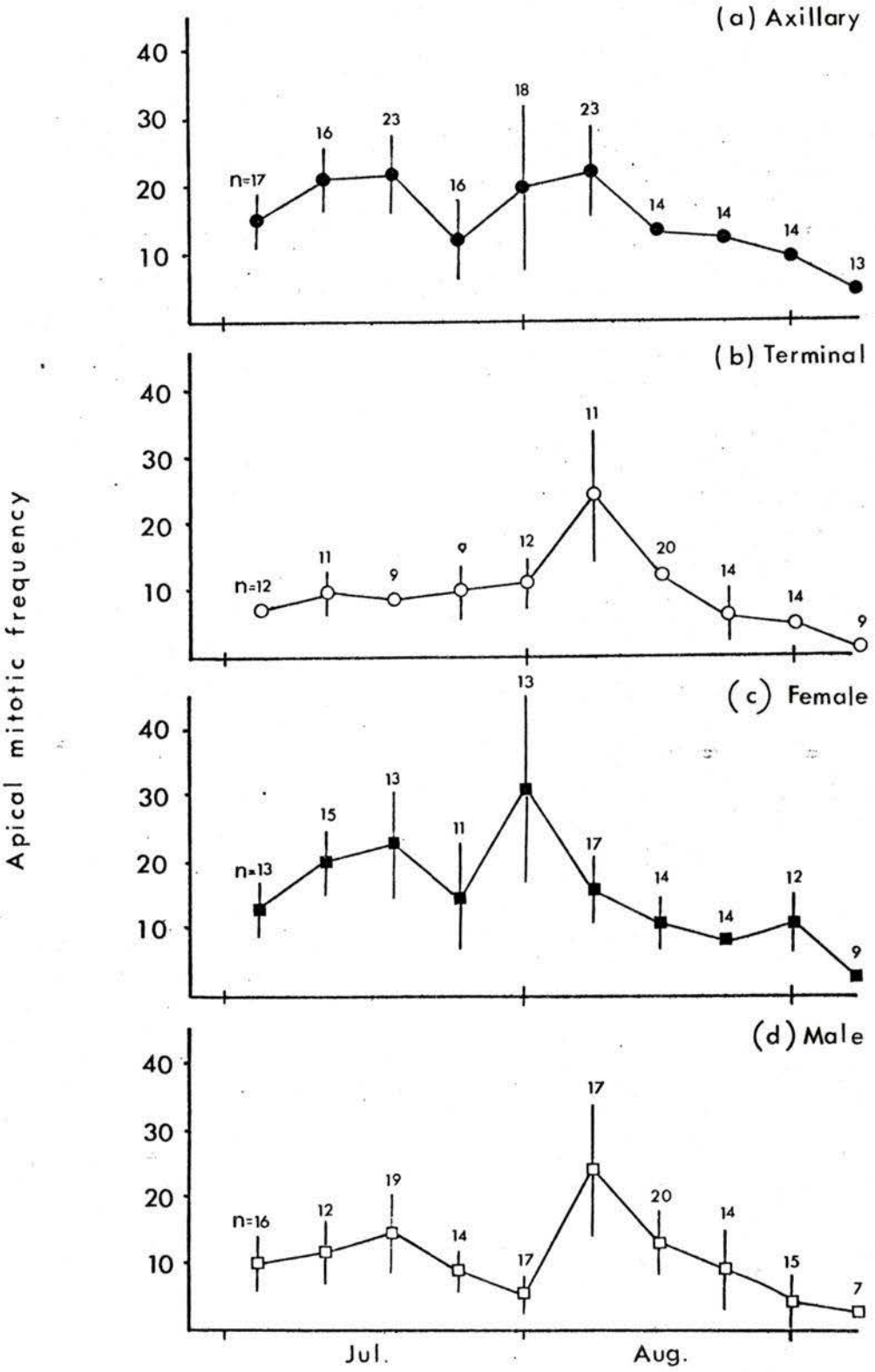

(a) Axillary
40
30
20
10
n=17
16
23
16
18
23
14
14
14
13
(b) Terminal
40
30
20
10
n=12
11
9
9
12
11
20
14
14
9
(c) Female
40
30
20
10
n=13
15
13
11
13
17
14
14
12
9
(d) Male
40
30
20
10
n=16
12
19
14
17
17
20
14
15
7
Jul.
Aug.
Apical mitotic frequency

Figure 12.

Ergastic substances in the apical pith area of Picea engelmannii buds (terminal and axillary). Plotted values represent the mean percent absorbance (of the pith region) at 540 nm fitted with 95% confidence limits.

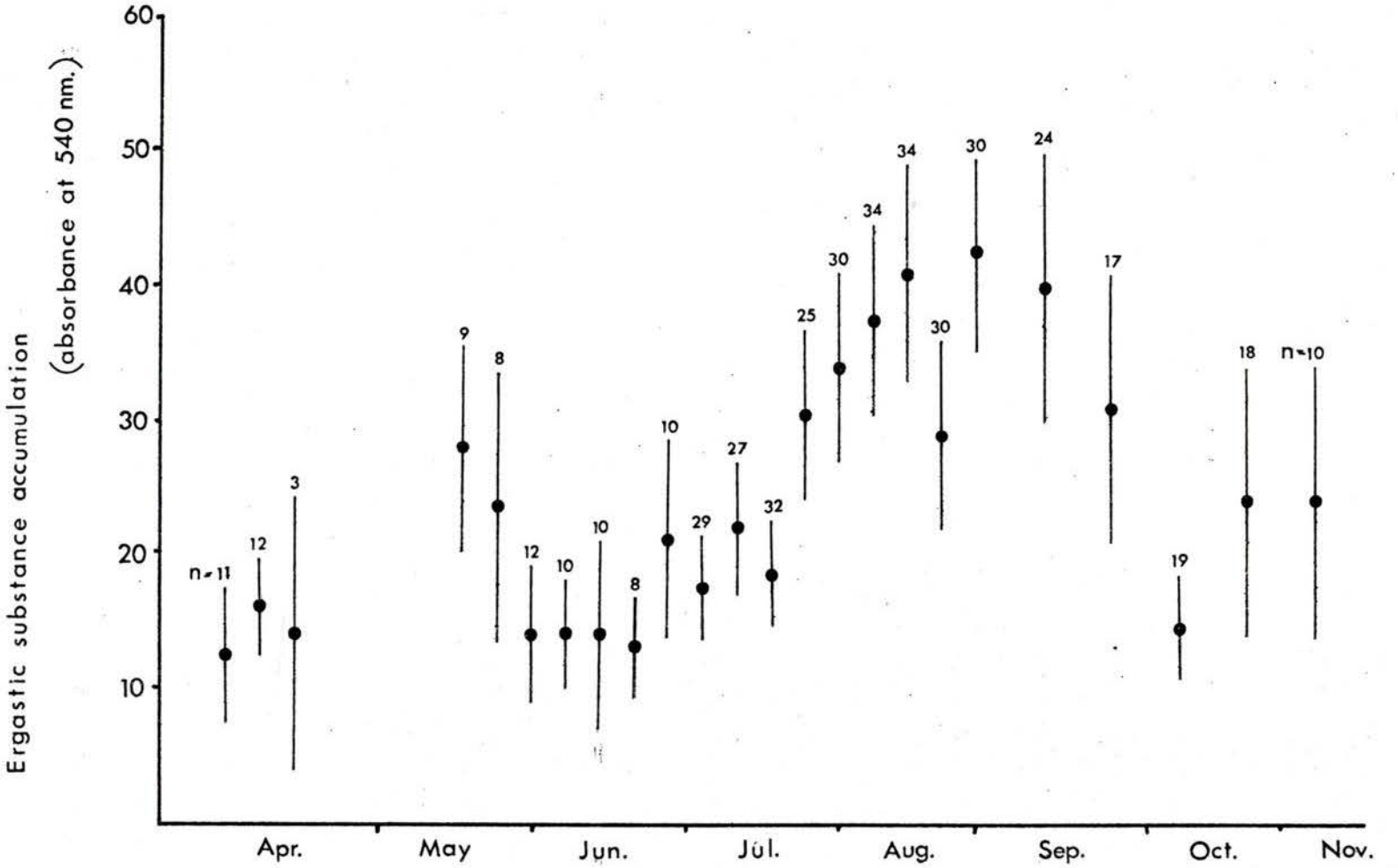

Ergastic substance accumulation
(absorbance at 540 nm.)
60
50
40
30
20
10
n=11
12
3
9
8
12
10
10
8
10
29
27
32
25
30
34
34
30
30
24
17
19
18
n=10
Apr.
May
Jun.
Jul.
Aug.
Sep.
Oct.
Nov.

Figure 13.

The phenology of vegetative bud development, cone differentiation and development in Picea engelmannii Parry collected from the Prince George Forest District (1979).

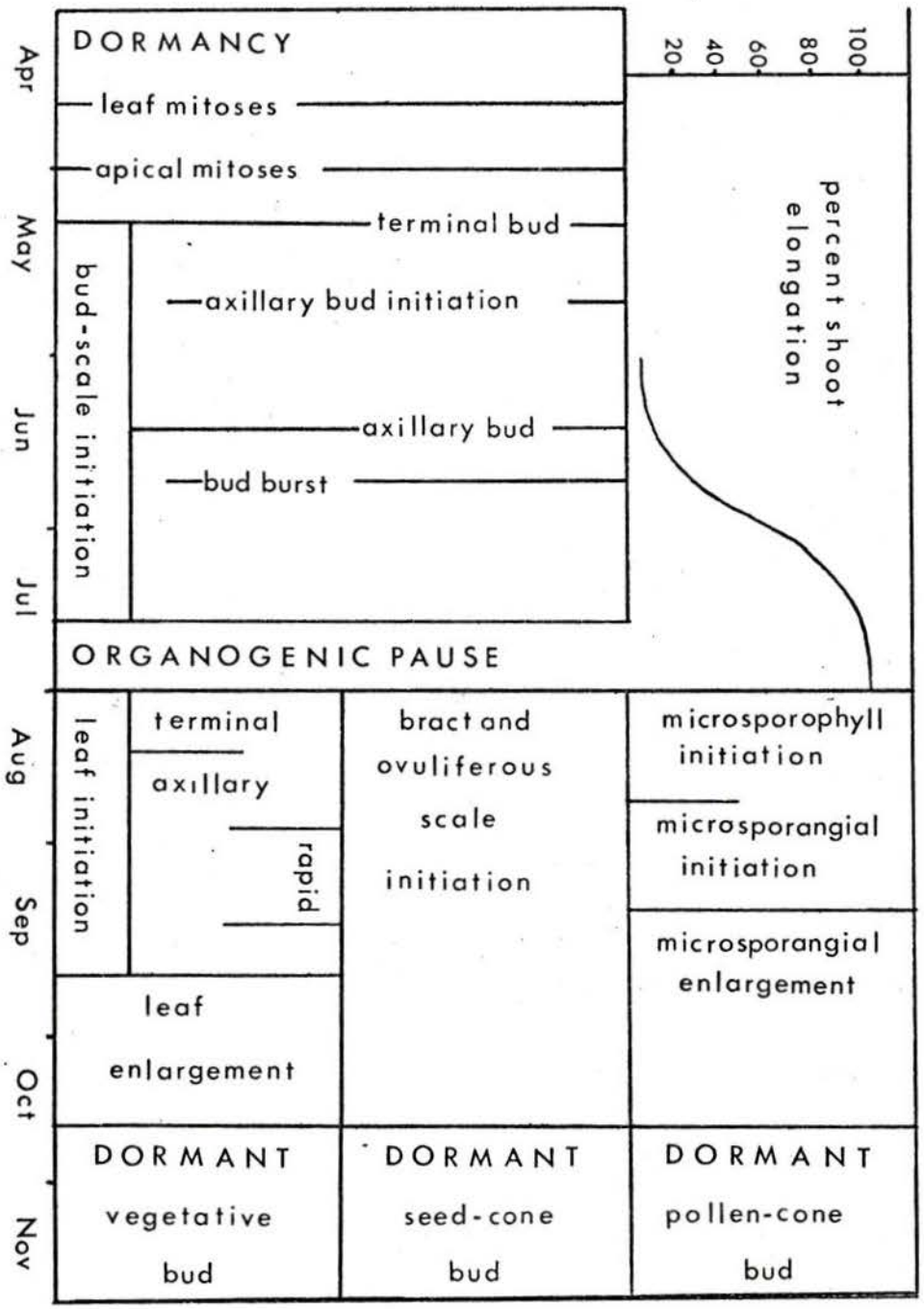

DORMANCY
leaf mitoses
apical mitoses
terminal bud
bud-scale initiation
axillary bud initiation
axillary bud
bud burst
20
40
60
80
100
percent shoot elongation
Apr
May
Jun
Jul
ORGANOGENIC PAUSE
leaf initiation
terminal
axillary
rapid
bract and ovuliferous scale initiation
microsporophyll initiation
microsporangial initiation
microsporangial enlargement
Aug
Sep
Oct
leaf enlargement
DORMANT vegetative bud
DORMANT seed-cone bud
DORMANT pollen-cone bud
Nov

Figure 14.

Reproductively mature Picea engelmannii collection tree located at an elevation of 1700 m. The tree was estimated to be 30 m tall and 108 years old.

Figure 15.

Seed-cones (megasporangiate strobilii) occupying both terminal and distal axillary positions on a lateral shoot selected from the upper nine whorls of the tree. Material was collected in late July.

2 x

Figure 16.

Pollen-cones intermittantly spaced along a lateral shoot.

1.5 x

Figure 17.

A proximal cluster of pollen-cones (microsporangiate strobilii) located at the base of a lateral shoot that elongated the previous year. Material was collected in late July.

1.5 x

Figure 18.

Bud scale buttress initiation confined to the peripheral zone. Note the anticlinal division of the protoderm (arrows) and the conspicuous appearance of the underlying peripheral zone cells. protoderm, pr; peripheral zone, pz.

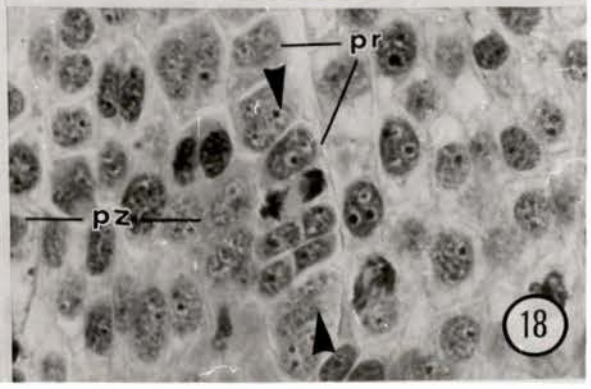
800 x

14

15

16

17



18

Figure 19.

Bud scale buttress initiation showing a number of protodermal cell which have recently undergone anticlinal division and are begining periclinal division. Underlying peripheral zone cells show randomly oriented mitotic divisions (arrows). protoderm, pr; peripheral zone, pz; bud scales, bs.

400 x

Figure 20.

Early development of bud scale buttresses by increased mitotic division of the underlying peripheral zone (arrows). bud scales, bs.

800 x

Figure 21.

Typical bud scales enclosing the undifferentiated apex. apex, ap; secondary bud scales, sbs; primary bud-scales, pbs.

55x

Figure 22.

The apical and sub-apical initials of a secondary bud scale representing the distal growing portion of the bud scale. The apex is approaching the period of anatomical differentiation, note the large conspicuous central mother cells (arrows). apical initials, ai; subapical initials, sai; bud scales bs.

300 x

Figure 23.

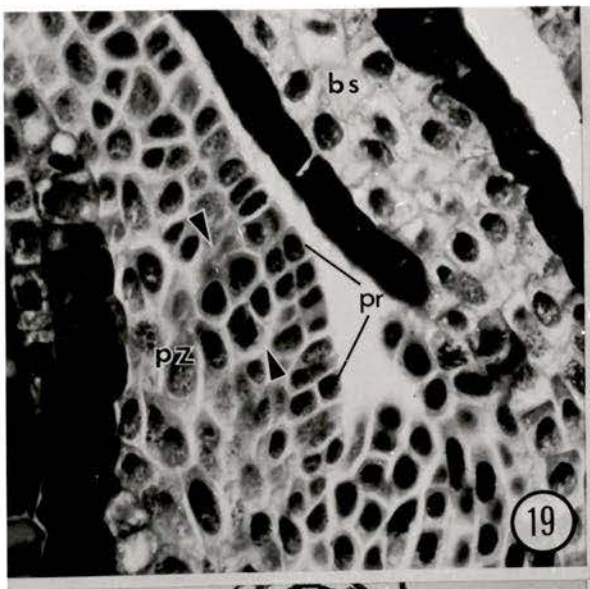
A cross-section showing the marginal meristems (arrows) of a scondary bud scale responsible for lateral bud scale growth. This micrograph was taken from the distal region of the bud scale.

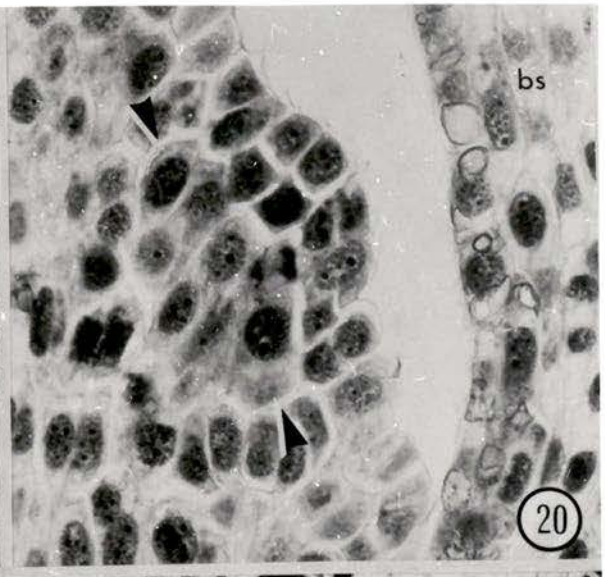
160 x

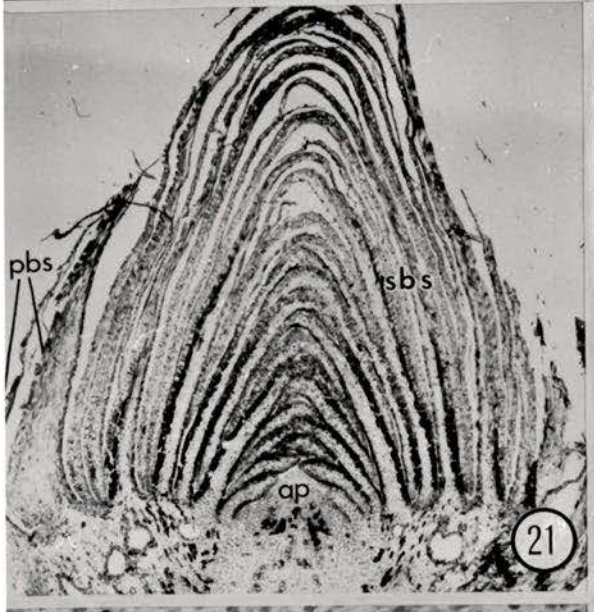
Figure 24.

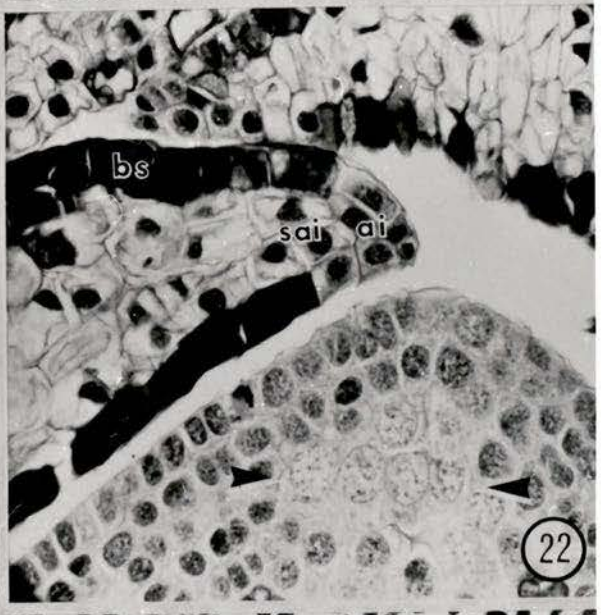
A cross-sectional view of the secondary bud scale basal region (approximately 18 um above the receptacle) showing the apparent loss of the meristematic margins and activity leading to a basal constriction of the bud scale (see Figure 25).

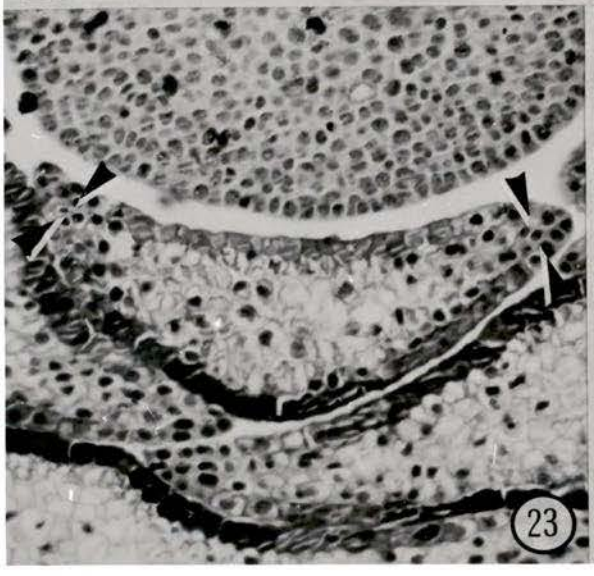
120 x


bs
pr
pz
19


bs
20


pbs
sbs
ap
21


bs
sai
ai
22


23

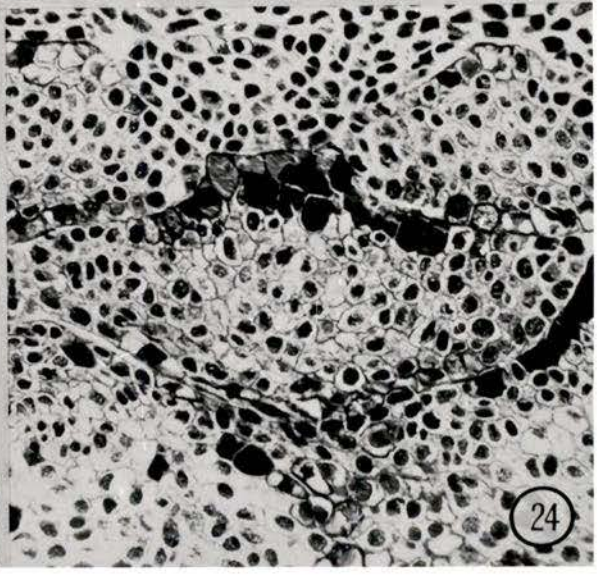

24

Figure 25.

The abaxial (outer) surface of an inner secondary bud scale illustrating the typical simple structure. Note: Figures 23 and 24 represent cross-sections at levels AA and BB respectively.

9 x

Figure 26.

Cross-section of a primary bud scale near the tip showing 'unusual' supportive and procambial tissue compare to Figure 23. supportive tissue, st; procambial strand, ps.

26 x

Figure 27.

Cross-section of a primary bud scale near the base exhibiting an indistinct, crushed procambim and a general loss of supportive tissue.

40 x

Figure 28.

The abaxial (outer) surface of a primary bud scale demonstrating the structural complexities derived by the supportive tissue, hairy margin and deltoid shape. Note: Figures 26 and 27 represent cross-sections at levels CC and DD respectively.

12 x

Figure 29.

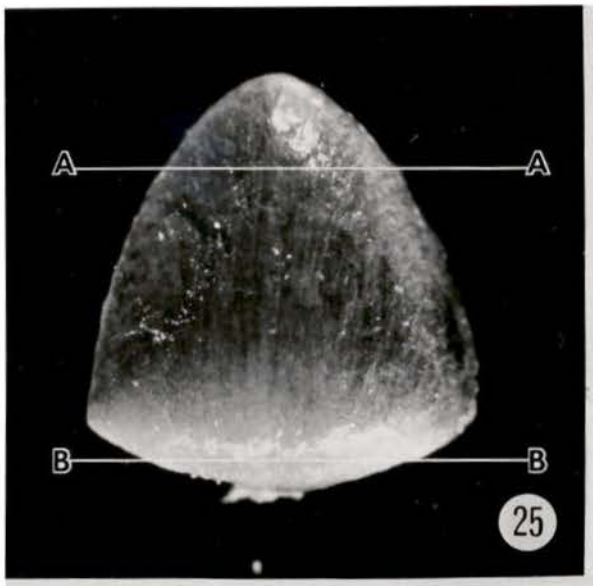
The terminal bud of a first order branch emphasizing the outer primary bud scales. Two such whorls surrounded the inner secondary bud scales. primary bud scales, pbs; secondary bud-scales, sbs.

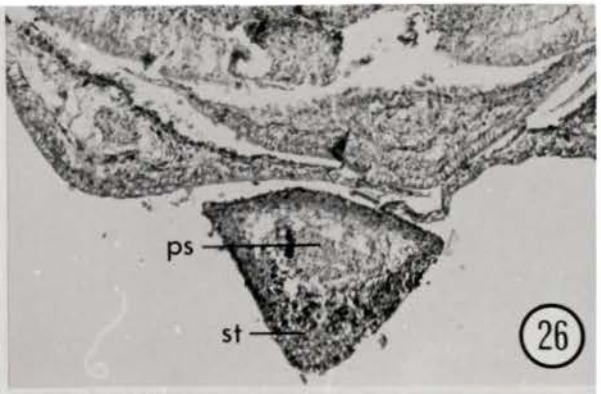
11 x

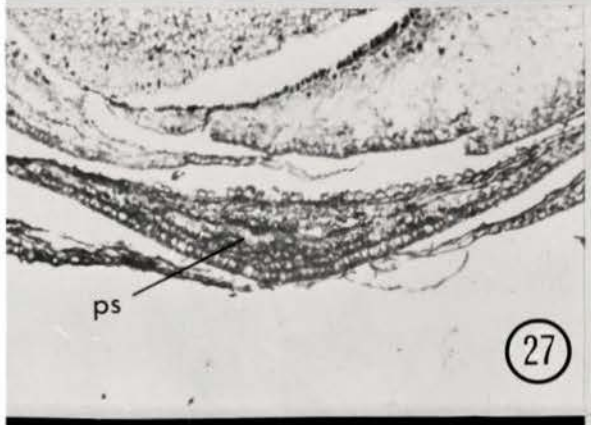
Figure 30.

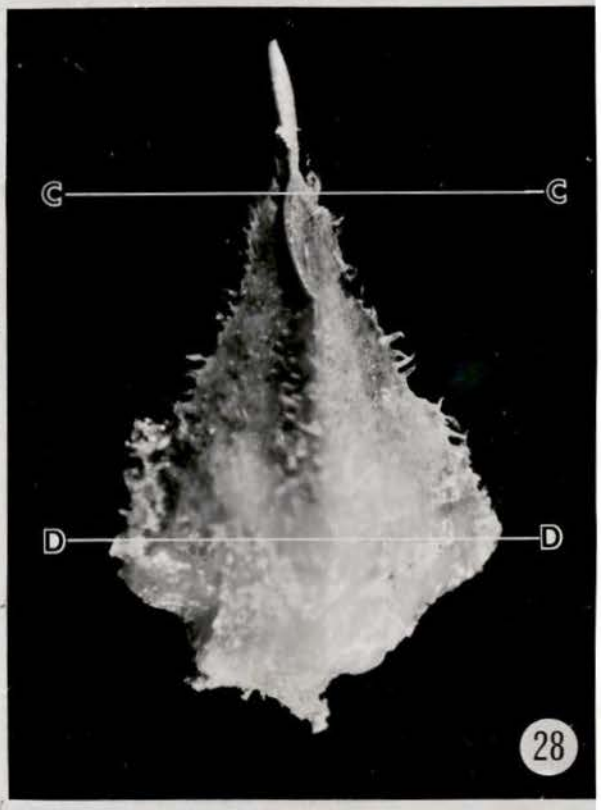
The adaxial (inner) surface of a primary bud scale cross-section exposed to the environment showing a build-up of protoplasmic ergastic substances.

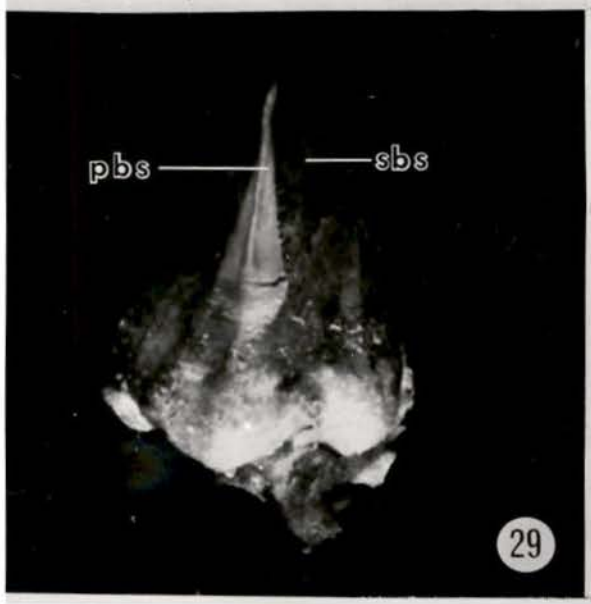
190 x


A
A
B
B
25


ps
st
26


ps
27


C
C
D
D
28


pbs
sbs
29

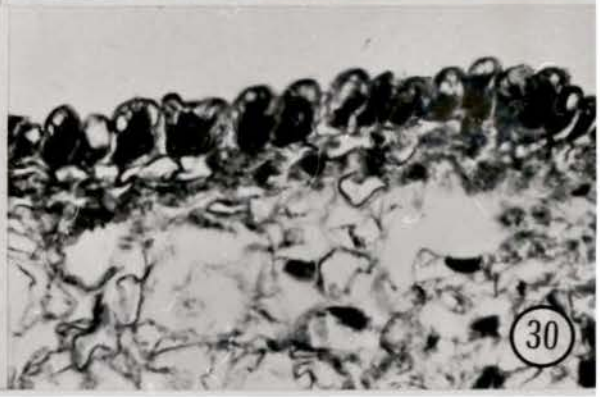

30

Figure 31.

The abaxial suface of a primary bud scale cross-section illustrating the epidermis layer with a thick cuticle. epidermis, ep; cuticle, c.

190 x

Figure 32.

A shoot tip cross-section throught the receptacle showing various sized and numberous resin ducts as well as procambial strand cross-sections (arrows). resin duct, rd; pith, pi.

32 x

Figure 33.

Longisection of a procambial strand (arrows) originating at the base of a secondary bud scale and merging with the underlying shoot vasculature. procambial strand, ps; shoot vasculature, sv; secondary bud scales, sbs.

50 x

Figure 34.

Procambial strand (arrows) cross-section at a level just below a secondary bud scale (see Figure 33 AA). Note the lack of differentiation.

380 x

Figure 35.

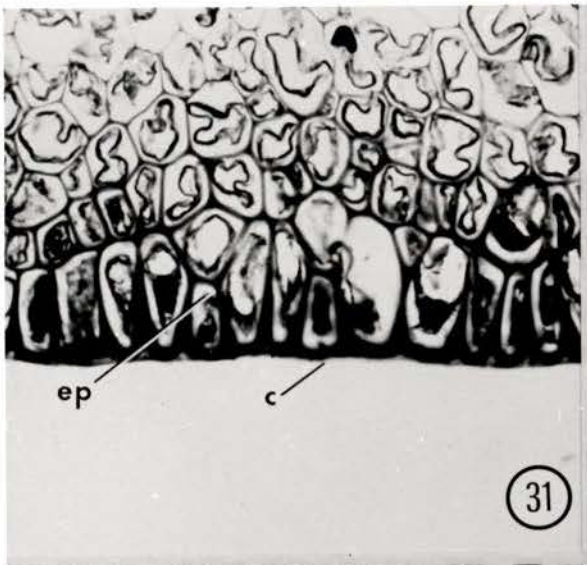
Procambial strand cross-section deep within the receptacle just above the point of vasculature mergence (see Figure 33 BB). Note: prospective xylem (arrows) is showing the development of a thickened secondary wall while retaining some cytoplasmic detail.

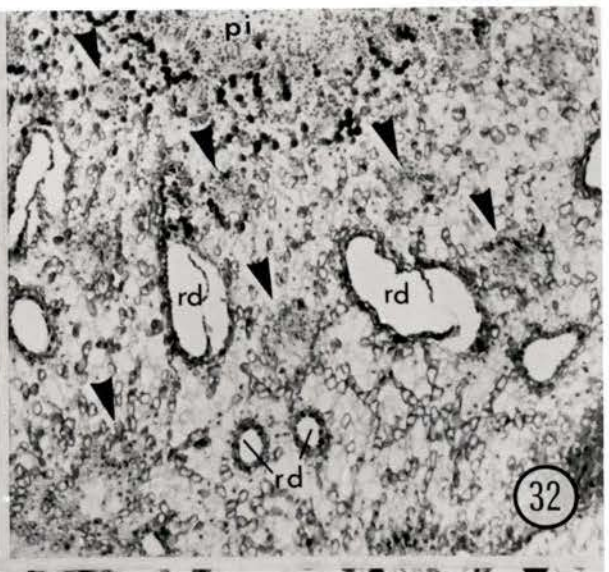
380 x

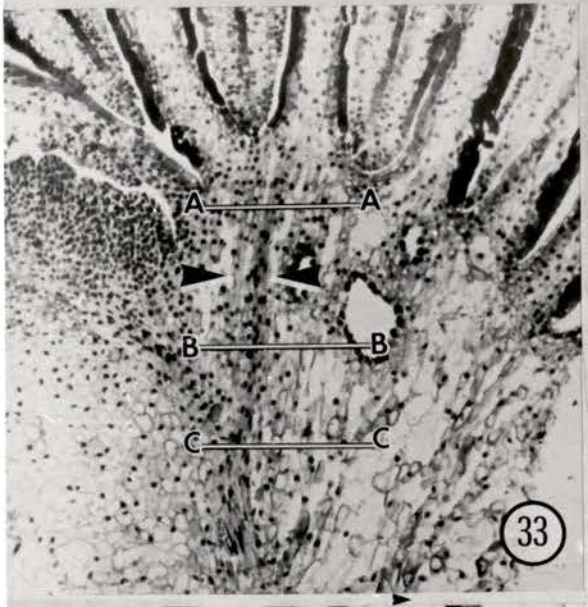
Figure 36.

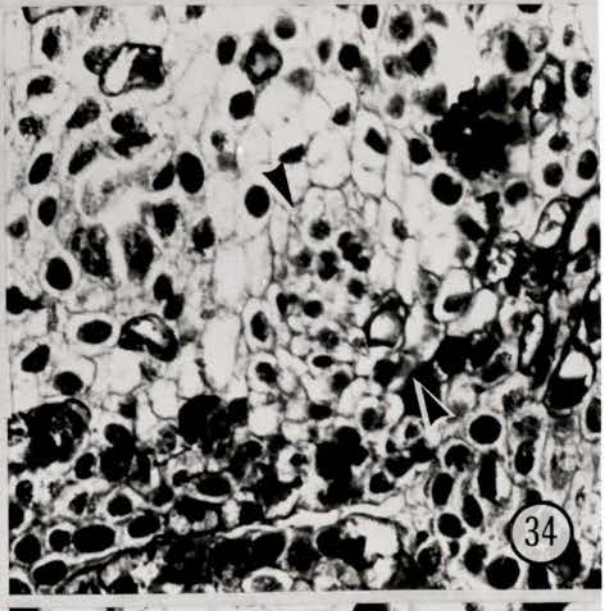
Procambial strand cross-section at a level of merger between bud and shoot vasculature (see Figure 33 CC). Note the xylem (arrows) has lost all cytoplasmic detail.

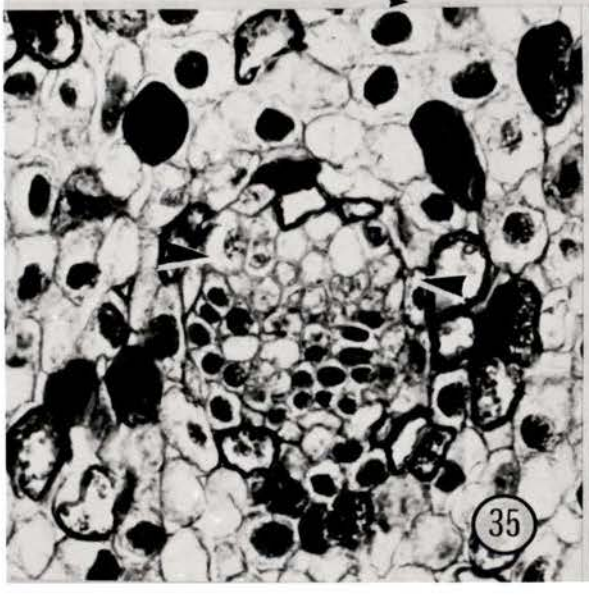
300 x


ep
c
31


pl
rd
rd
rd
32


A
A
B
B
C
C
33


34


35

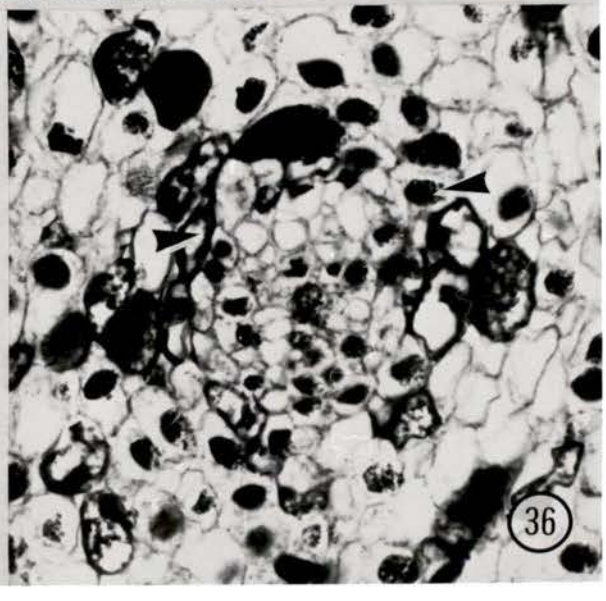

36

Figure 37.

The low, sharply conical appearance of an early terminal vegetative bud showing the initiation of leaf primordia (arrows). Note the complete infiltration of the pith region with ergastic substances and the appearance of the apex with it's characteristic height-width ratio. ergastic substance accumulation, esa.

128 x

Figure 38.

A tall, potential male apex from a whorl of basal lateral buds. This apex illustrates at least two and perhaps four regions of microsporophyll initiation (arrows) along the upper apical flanks leaving a stalk-like structure below. Note the limited and filed appearance of ergastic substance accumulation within the pith. Also note the high height-width ratio. ergastic substance accumulation, esa.

100 x

Figure 39.

A tall, potential female apex from a terminal bud showing the initiation of a bract (arrows) and the filed accumulation of ergastic substances. Again, note the high height-width ratio.

128 x

Figure 40.

The median longitudinal section of a typical mamillary apex. The central mother cells and apical initials have been displaced to the summit of the apex by peripheral zone and rib meristem activity. central mother cells, cmc; apical initials, ai; peripheral zone, pz; rib meristem, rm.

160 x

Figure 41.

SEM (scanning electron micrograph) of a mamillary apex at the same stage as in Figure 40 showing leaf primordium initiation and the bulgeing apical initial region. leaf primordium, lp; apical initials, ai.

240 x

Figure 42.

Leaf primordium initiation in the peripheral zone of a vegetative apex. This area is delimited by a slight protrusion and subtle changes in orientation of mitoses and cytoplasmic staining properties (arrows).

330 x

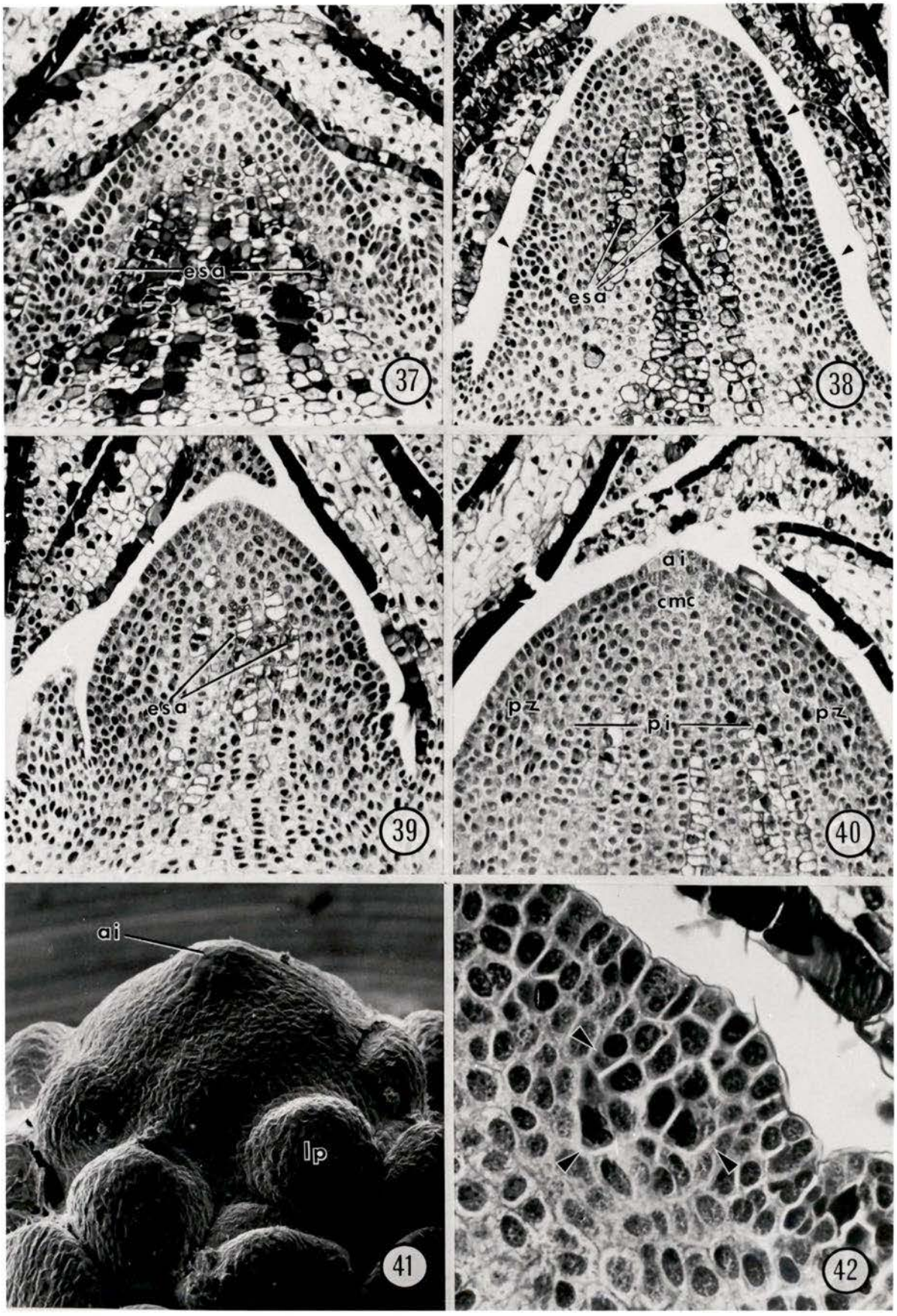

esa
37
esa
38
esa
39
ai
cmc
pz
pi
pz
40
ai
lp
41
42

Figure 43.

Median longitudinal section of an apex during leaf primordium initiation. Note the region of peripheral zone cell proliferation and periclinal division of the protoderm giving rise to a leaf buttress (arrows). protoderm, pr; peripheral zone, pz.

400 x

Figure 44.

A median longitudinal section of a leaf buttress demonstrating the lack of zonal organization or any differentiated tissue. Note the homogenous appearance of the cells within the buttress. leaf buttress, lb.

300 x

Figure 45.

A median longitudinal section of a leaf primordium showing the establishment of apical initials, subapical initials and the development of a central procambial strand (arrows). apical ai; subapical initials, initials, sai.

190 x

Figure 46. Scanning electron micrograph of a vegetative bud illustrating it's descriptive phyllotactic parastichies. Clockwise parastichies (8) are drawn in white while counterclockwise parastichies (13) are drawn in black.

80 x

Figure 47.

A median longitudinal section of a leaf primordium exhibiting the banding and the central location of the procambial strand (arrows). leaf primordium, lp.

180 x

Figure 48.

A cross-section of a leaf primordium showing the centrifugal division of the developing procambium. Differentiation of internal structure has not yet begun. procambial strand, ps.

300 x

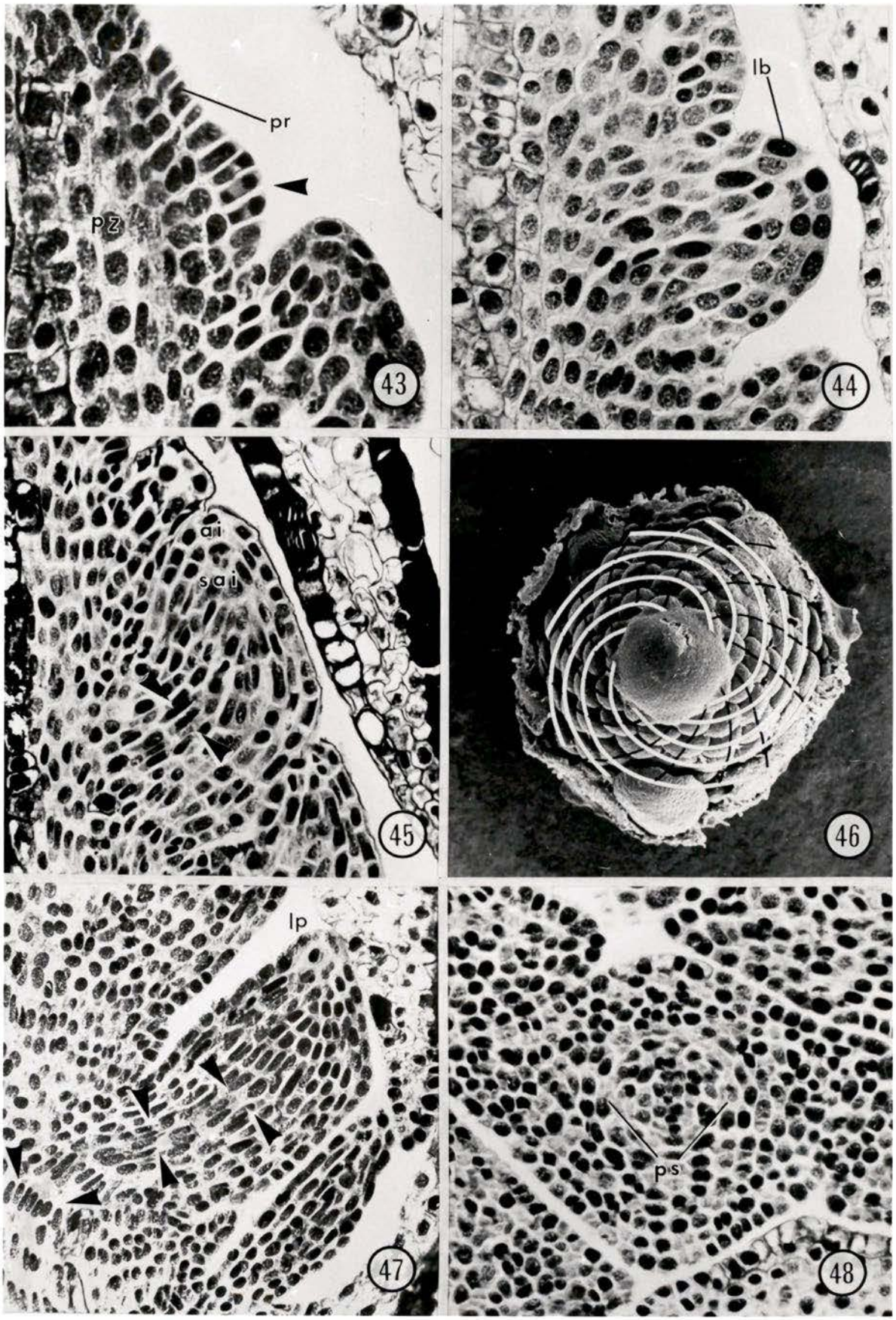

pr
pz
43
lb
44
ai
sai
45
46
lp
47
ps
48

Figure 49.

SEM of a terminal vegetative bud with a mamillary apex undergoing leaf primordium initiation. Material was collected in late August. leaf primordia, lp; mamillary apex, ma.

70 x

Figure 50.

A median longitudinal section of a terminal vegetative bud at the same stage of development as Figure 49. leaf primordium, lp, central mother cells, cmc; apical initials, ai; peripheral zone, pz; rib meristem, rm.

65 x

Figure 51.

SEM of a pollen-cone bud having almost completed microsporophyll and microsporangial initiation. Note: the microsporangia are not visible. Material was collected in late August (1980). microsporophyll, mi.

65 x

Figure 52.

Median longitudinal section of a basal axillary pollen-cone bud at a stage developmentally behind Figure 51. microsporophyll, mi; microsporangium, mic.

35 x

Figure 53.

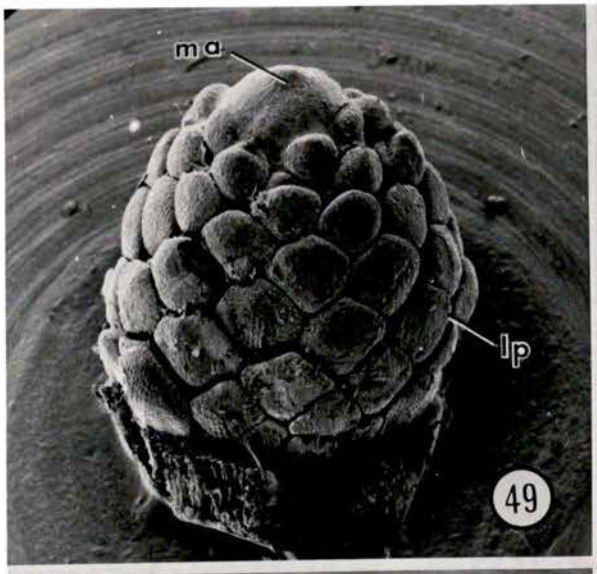
SEM of a seed-cone undergoing bract and ovuliferous scale initiation. Only the bracts are visible, the ovuliferous scales are located behind these bracts. Material was collected in late August (1980). bracts, br; apex, ap.

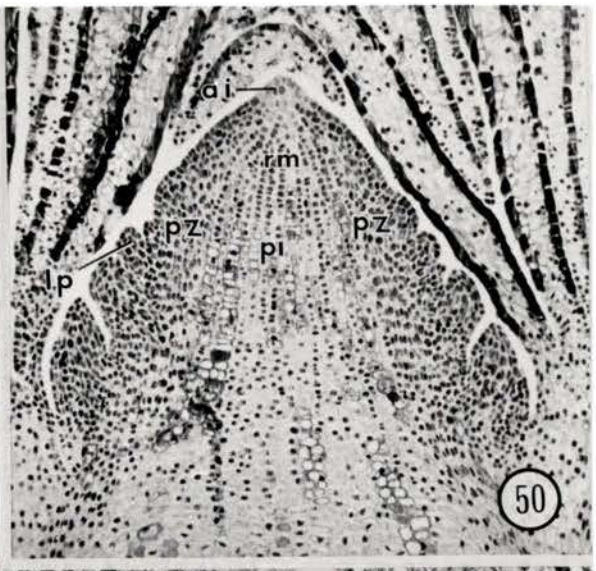
50 x

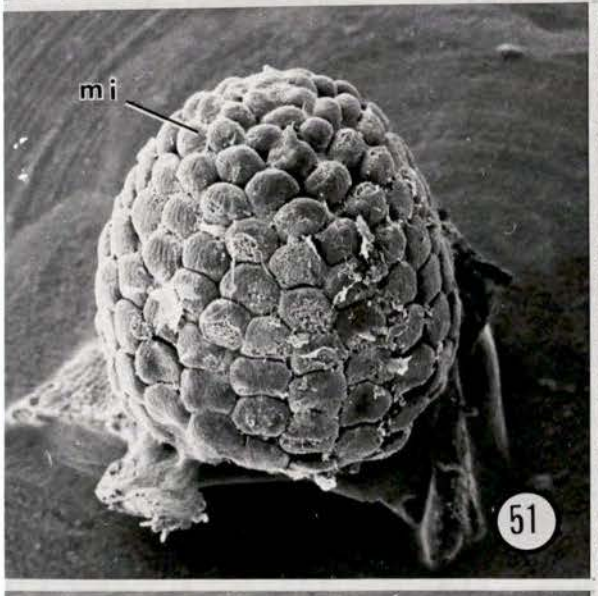
Figure 54.

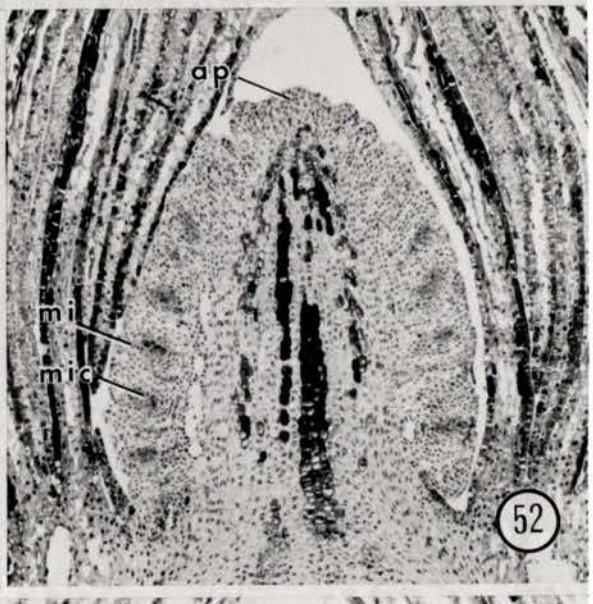
Median longitudinal section of a terminal seed-cone apex at a stage comparable to Figure 53. bract, br; ovuliferous scale, os; apex, ap.

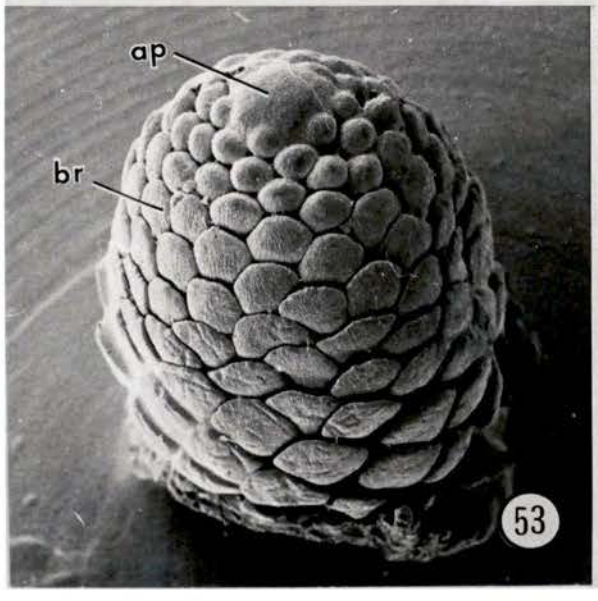
60 x


ma
lp
49


ai
rm
pz
pi
pz
lp
50


mi
51


ap
mi
mic
52


ap
br
53

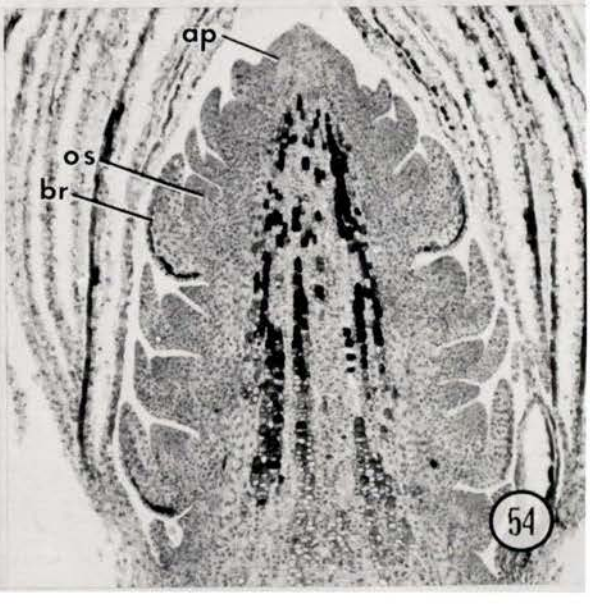

ap
os
br
54

Figure 55.

A fully developed vegetative bud with ergastic substances at dormancy. apex, ap; leaf primordium, lp; pith, pi; crown, cr; crown cavity, cc; bud scales, bs.

80 x

Figure 56.

Cross-section of a mature four sided leaf collected in early summer. The epidermis with an overlying cuticle and subtending hypodermis surrounds the mesophyll and large intercellular spaces which frequently open to the environment via stoma. epidermis, ep; hypodermis, hy; mesophyll, me; stoma, st.

30 x

Figure 57.

Higher magnification of Figure 56 showing the leaf vasculature with an endodermis, complete with casparian strip (arrows). Xylem and phloem are located centrally and transfusion tissue is found between this central core and the endodermis. endodermis, en; xylem, xy; phloem, ph; transfusion tissue, tt.

130 x

Figure 58.

Median longitudinal section of a pollen-cone bud just prior to pollination. Note: the apex has been completely utilized. microsporophyll, mi; microsporangia mic.

80 x

Figure 59.

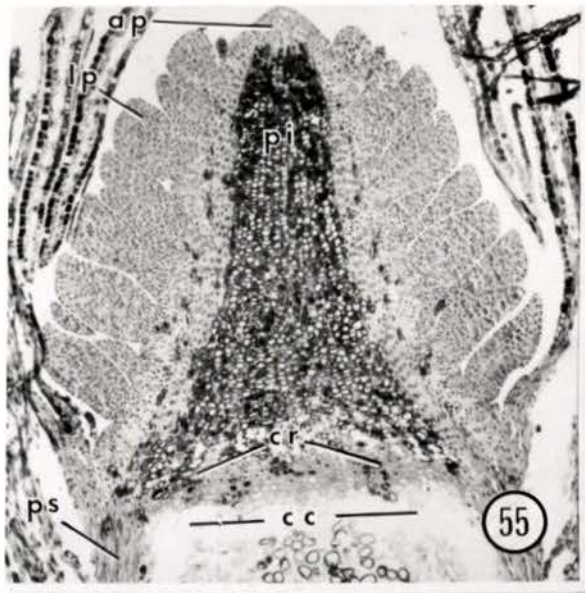
Microsporophyll primordia of a developing pollen-cone originating from peripheral zone activity. At the base of these primordia the development of resin ducts is obvious. microsporophyll, mi; resin duct, rd.

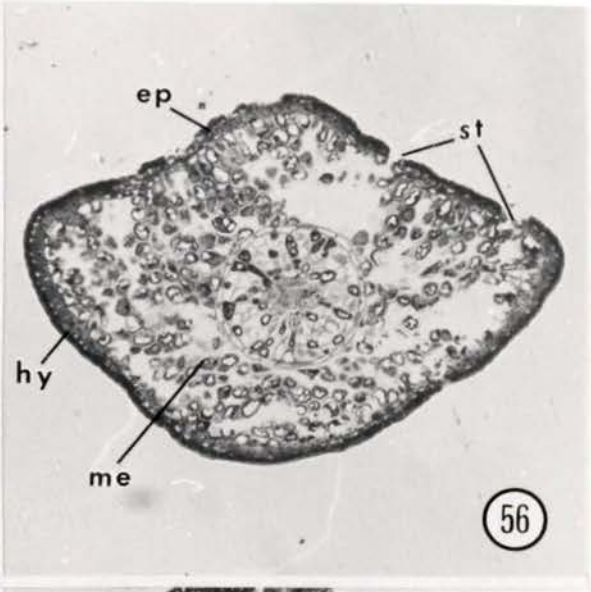
180 x

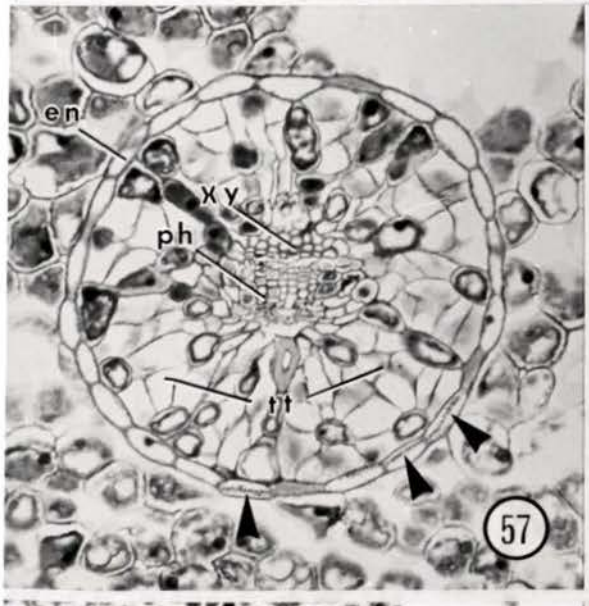
Figure 60.

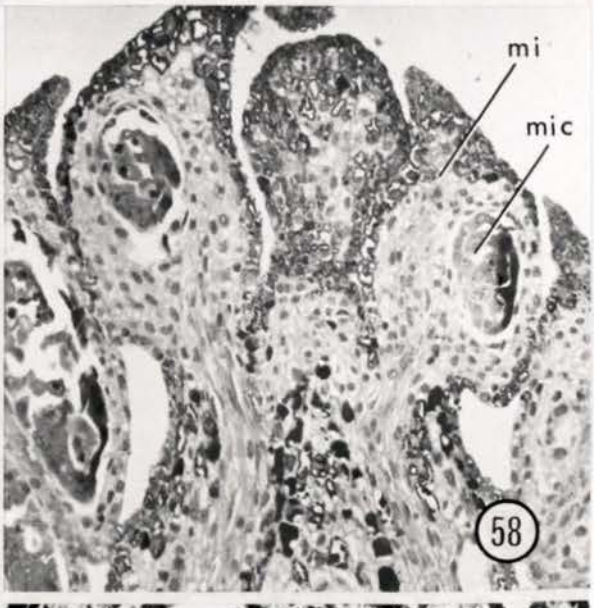
Median longitudinal section of a pollen-cone bud by mid-August just before microsporangial development becomes obvious. The apical initials and central mother cells are present but very indistinct. apical initials, ai; central mother cells cmc; microsporophyll, mi; bud scales, bs; ergastic substance accumulation, esa.

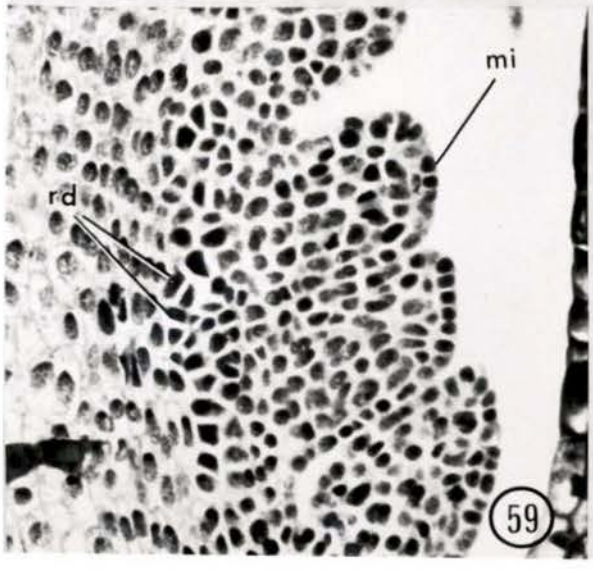
100 x


ap
lp
pi
cr
ps
cc
55


ep
st
hy
me
56


en
xy
ph
tt
57


mi
mic
58


mi
rd
59

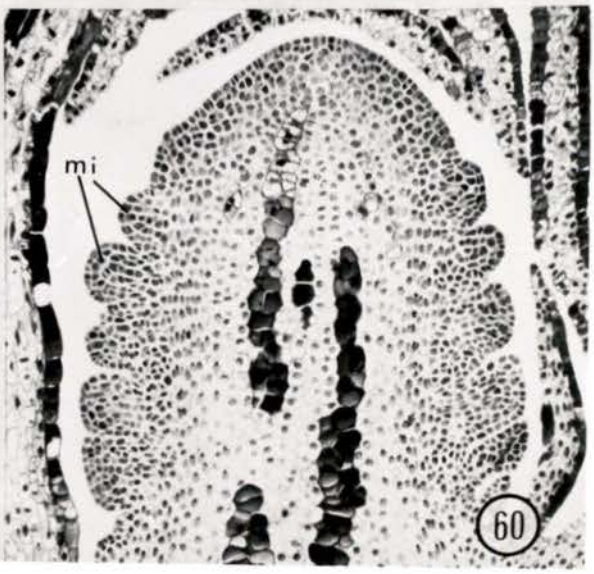

mi
60

Figure 61.

Early development of a pollen-cone peripheral zone resin duct (arrows). resin duct, rd.
400 x

Figure 62.

Late development of a pollen-cone resin duct showing the differentiation of the secretory cells. resin duct, rd; secretory cells, sc; epidermis, ep.
400 x

Figure 63.

Further enlargement of the resin ducts surrounding the single procambial strand of the microsporophyll. The enlargement of the resin duct appears related to the enlargement/elongation of the surrounding tissue of the cone axis. resin duct, rd; procambial strand, ps; microsporophyll, mi; pollen mother cells, pmc.
300 x

Figure 64.

The initiation of the microsporangium in the abaxial region of the microsporophyll. The cytoplasm of the presumptive microsporangial cells stain darkly, giving the first indication of initiation. microsporangium, mic; microsporophyll, mi; bud scales, bs.
190 x

Figure 65.

Early development of a microsporangium showing the differentiation of the sporogenous pollen mother cells. microsporangium, mic; pollen mother cells, pmc.
200 x

Figure 66.

Pollen-cone (microsporangiate strobilus) at dormancy At this stage the pollen mother cells show only a slight cytoplasmic stain variation when compared to earlier pollen mother cells (Figure 65). crown cavity, cc; crown region, cr; pollen mother cells, pmc; microsporophyll, mi; microsporangium, mic; resin duct, rd.
70 x

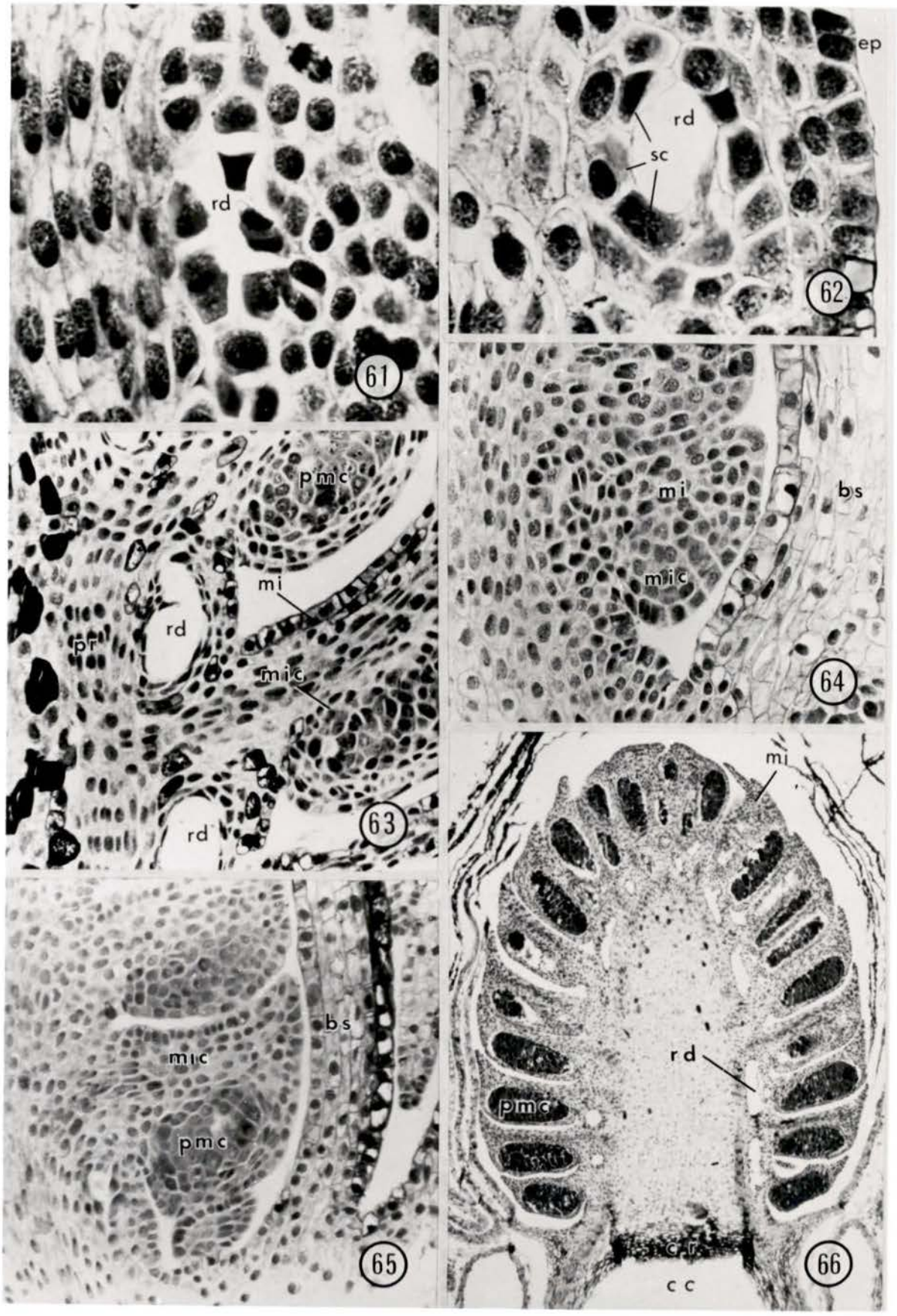

rd
61
ep
rd
sc
62
pmc
mi
rd
pr
mic
rd
63
mi
bs
mic
64
mi
bs
mic
pmc
65
mi
rd
pmc
cr
cc
66

Figure 67.

A longitudinal section of the microsporangial tapetal cells beneath the central procambial strand of the microsporophyll. Note the elongation of the tapetal cells. tapetal cells, tc; procambial stand, ps.

150 x

Figure 68.

A longitudinal section through the microsporangium showing isodiametric, normal sized, pollen mother cells reflecting an absence of elongation. microsporophyll, mi; pollen mother cells, pmc.

100 x

Figure 69.

A longitudinal section of the outer tapetal cells illustrating a regularity in cell size, reflecting an absence of elongation. tapetal cells, tc

150 x

Figure 70.

Median longitudinal section of a developing seed-cone apex exhibiting bract initiation along the apical flanks. bracts, br; bud scales, bs; apex, ap.

80 x

Figure 71.

Longisection of the most terminal bracts. The axil of the upper bract exhibits a group of conspicuous cells which will give rise to the ovuliferous scale. Also note the central procambial strand (arrows). Below, an ovuliferous scale is seen developing in a bract axil. bracts, br; ovuliferous scale, os.

130 x

Figure 72.

The initiation of an ovuliferous scale in the axil of a bract (as seen in Figure 71). The ovuliferous scale is conspicuous due to the nature of the nuclei and cytoplasm. ovuliferous scale, os; bract, br.

500 x

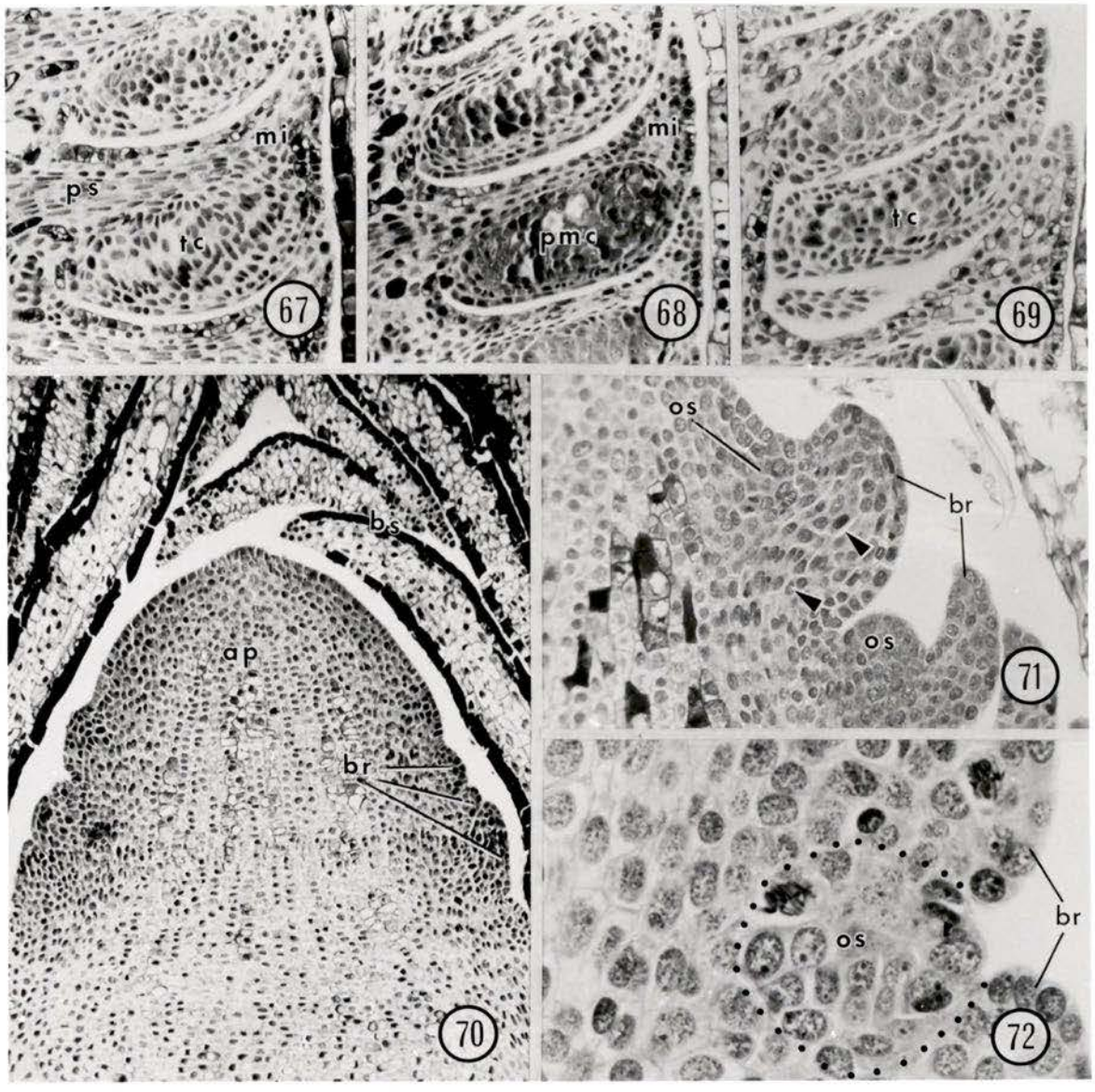

mi
ps
tc
67
mi
pmc
68
tc
69
bs
ap
br
70
os
br
os
71
os
br
72

Figure 73.

A median longitudinal section of an early ovuliferous scale in an axil of a bract. Mitotic orientation is random yielding a small buttress. ovuliferous scale, os; bract, br.

125 x

Figure 74.

The median most longitudinal section of an enlarging ovuliferous scale exhibiting what appears as a zonation pattern similar to that of leaf primordia (see Figure 45).

150 x

Figure 75.

A longitudinal section through the ovuliferous scale and megaspore mother cell showing the loss of the ovuliferous scale zonal pattern. megaspore mother cell, mmc; ovuliferous scale, os.

200 x

Figure 76.

Cross-section through an ovuliferous scale and bract showing size relationships and point of attachment (arrows). Also note the longitudinally oriented resin ducts. ovuliferous scale, os; bract, br; resin ducts, rd; procambial strand, ps; megaspore mother cell, mmc.

65 x

Figure 77.

A longitudinal section of an ovuliferous scale (with a slight swelling around the megaspore mother cell) subtended by a bract. Micrograph represents the same developmental stage as Figure 76. ovuliferous scale, os; bract, br; resin duct, rd; ovule primordium, op; megaspore mother cell, mmc.

100 x

Figure 78.

Longisection through the center of a developing ovule primordium showing a megaspore mother cell at the center. megaspore mother cell, mmc.

410 x

Figure 79.

Median longitudinal section of a seed-cone apex at the time of pollination. Note the apex can still be identified but the zonation has disappeared. apex, ap; ovuliferous scale, os; bract, br.

80 x

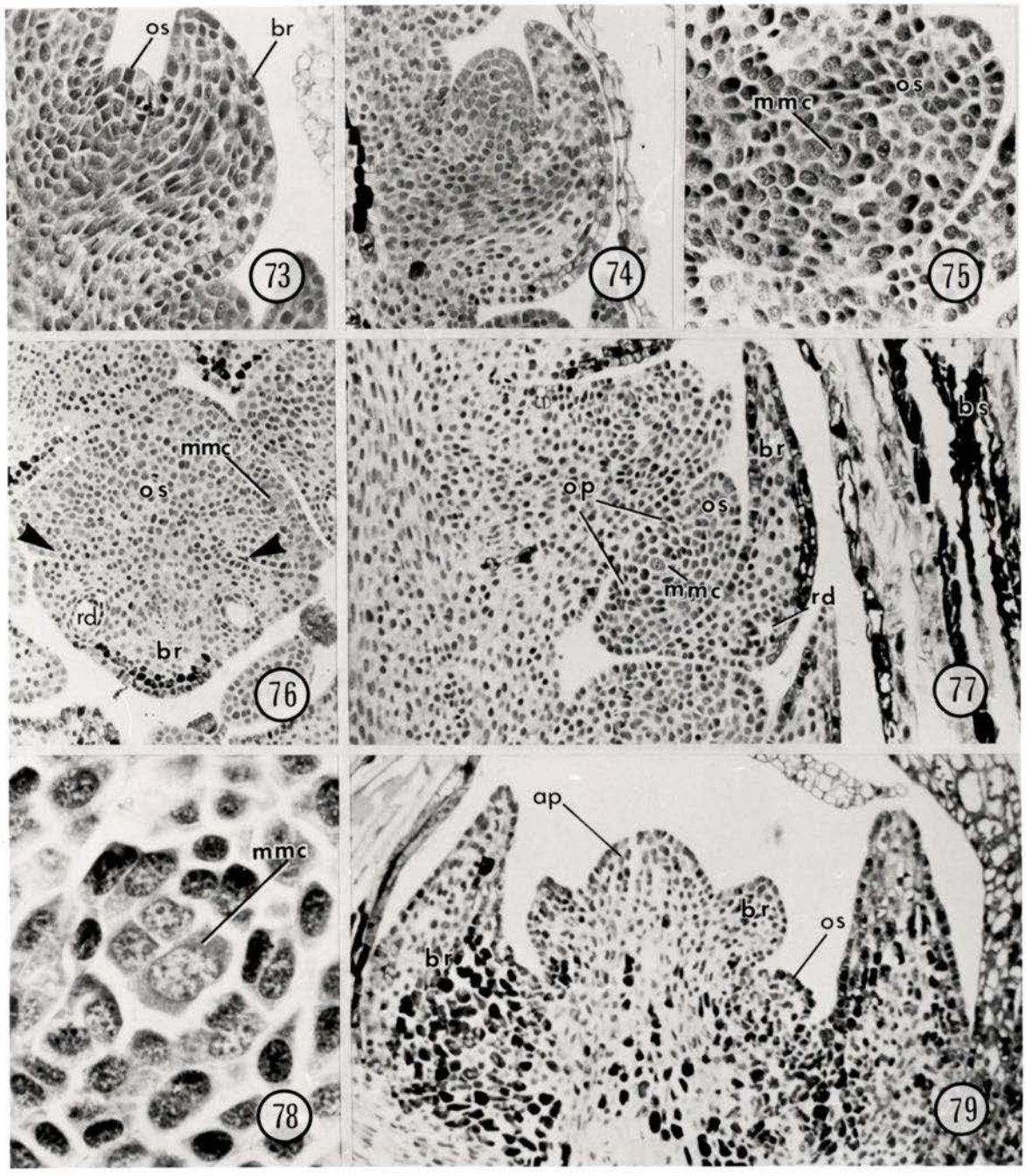

os
br
73
74
mmc
os
75
mmc
os
rd
br
76
op
os
mmc
br
rd
bs
77
mmc
78
ap
br
os
br
79

Figure 80.

A median longitudinal section of a seed-cone at dormancy. bract, br; ovuliferous scale, os; apex, ap; pith, pi; crown, cr; crown cavity, cc.

60 x

Figure 81.

A light micrograph of a potential vegetative apex during bud scale initiation. The median section normally shows the presence of starch granules (stippled area) between the level of the newly initiatied leaf primordia and the receptacle. bud scales, bs.

150 x

Figure 82.

A phase contrast dark background micrograph of a developing seed-cone, starch granules (as in Fig. 81) appear as the light region below the last initiated bracts. bracts, br; ovulidferous scale, os.

70 x

Figure 83.

Cross-section of the crown region separating the bud pith from the underlying shoot pith. Note the thickened cell walls, these walls are not lignified. crown region, cr; receptacle, re; resin duct, rd.

24 x

Figure 84.

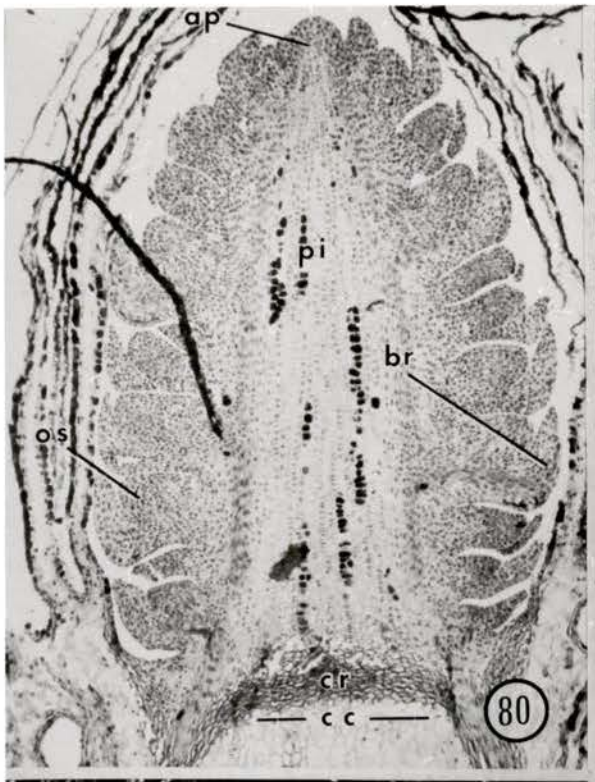
Higher magnification of the crown cells of Figure 83, showing the characteristic thick walled cells and darkly staining cytoplasm.

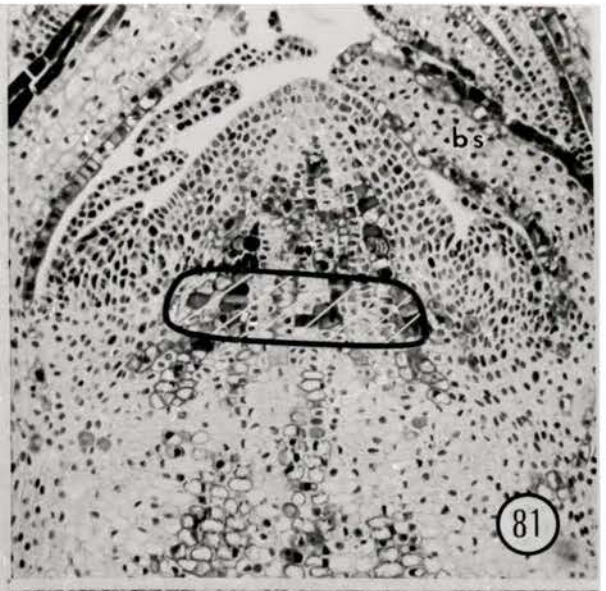
160 x

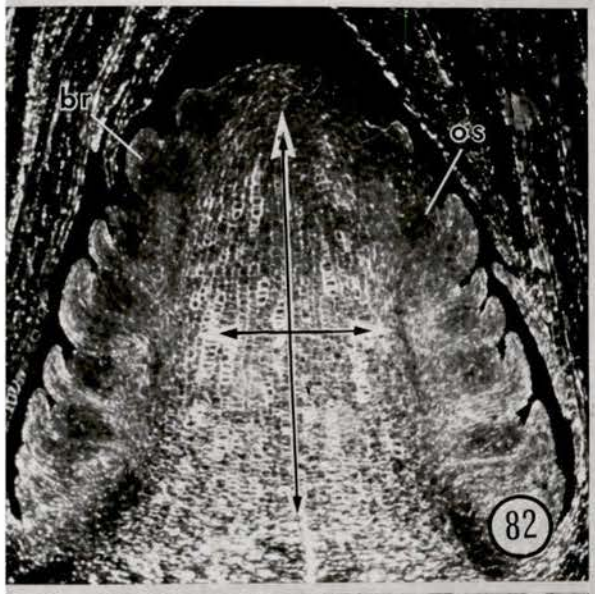
Figure 85.

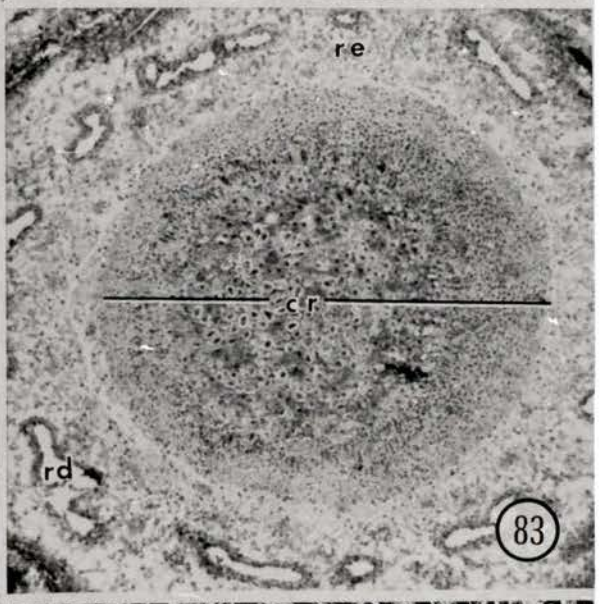
Median longitudinal section of an apex showing larval damage of the insect, Argyrestia sp. (Family, Yponomeutidae). Essentially the apex has been destroyed and the insect has burrowed into the pith of the subtending shoot towards the differentiating vascular tissue (arrows). bud scales, bs; pith, pi.

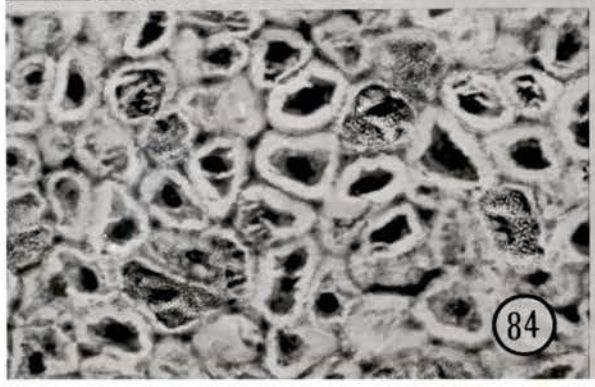
40 x


ap
pi
br
os
cr
cc
80


bs
81


br
os
r
82


re
cr
rd
83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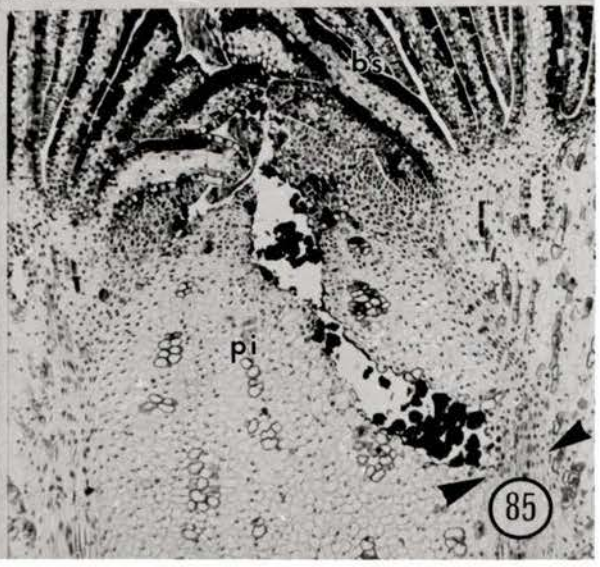

bs
pi
85

Figure 86. Median bud longitudinal section showing the spontaneous abortion and collapse of a proximal axillary apex. collapsed apex, ca; pith, pi. 50 x

Figure 87. A longitudinal section showing the formation of a region analogous to a secondary meristem (arrows). Note, this meristematic region servers procambial continuity between the upper degenerating bud and the underlying shoot. 60 x

Figure 88. A higher magnification of the secondary meristematic region of Figure 87.

Figure 89. The median longitudinal section of a latent lateral apex after dormancy. Note that the apical zonation pattern is indistinct compared to Figure 90 and the lack of lateral primordia. bud scales, bs; crown cavity, cc; rudimentary primordium, rp. 50 x

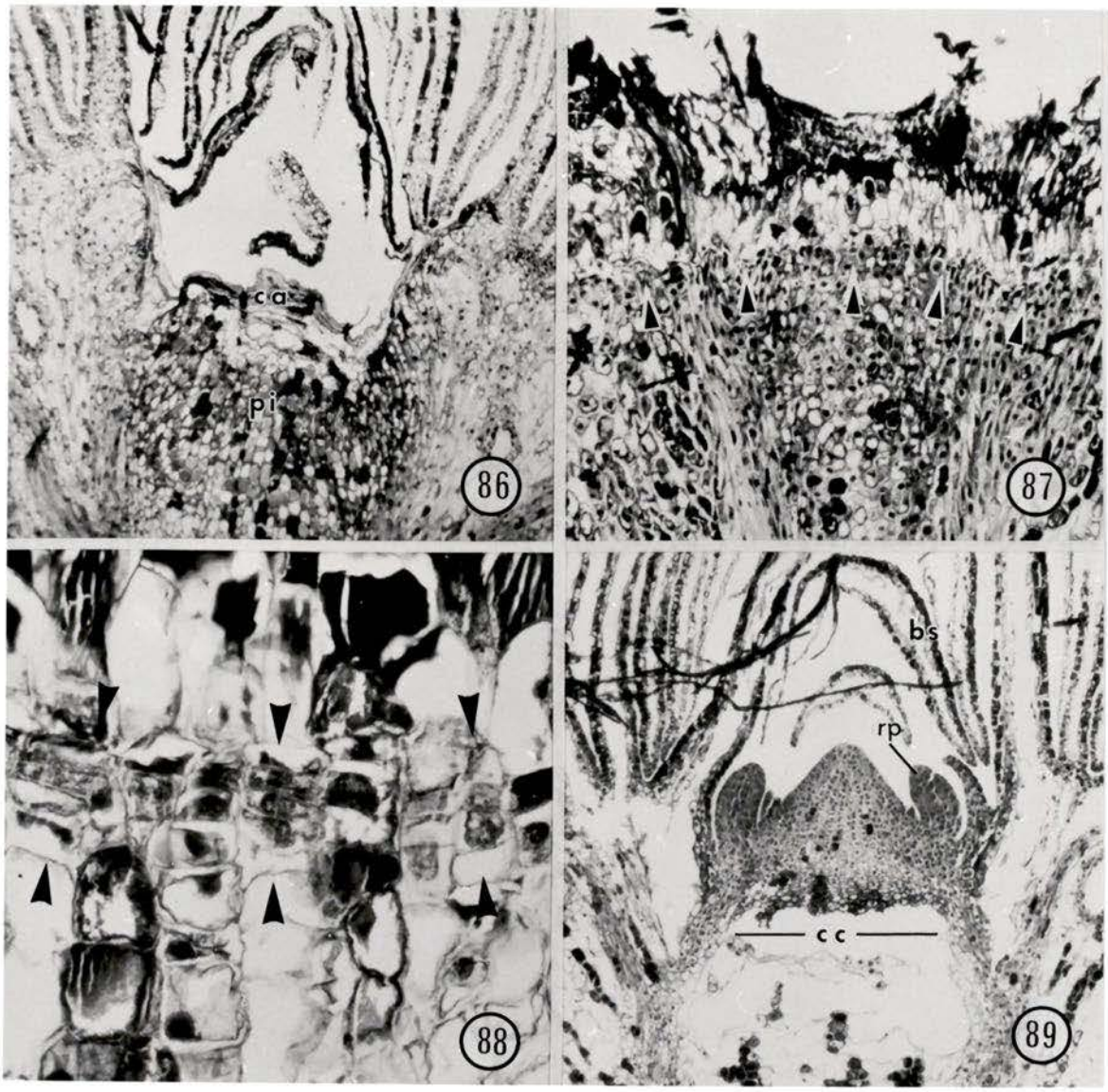

ca
pi
86
87
88
bs
rp
cc
89

Figure 90.

Median longitudinal section of a terminal vegetative apex at the end of dormancy. Staining differences are slight, still the zonal pattern may be delimited on the basis of cell size. Also note the conspicuous absence of ergastic substances. apical initials, ai; central mother cells, cmc; rib meristem, rm; peripheral zone, pz.

140 x

Figure 91.

Median longitudinal section of a terminal vegetative apex during early bud scale initiation. Note the mergence of the apical initials and the central mother cells. apical initials, ai; central mother cells, cmc; rib meristem, rm; peripheral zone, pz.

160 x

Figure 92.

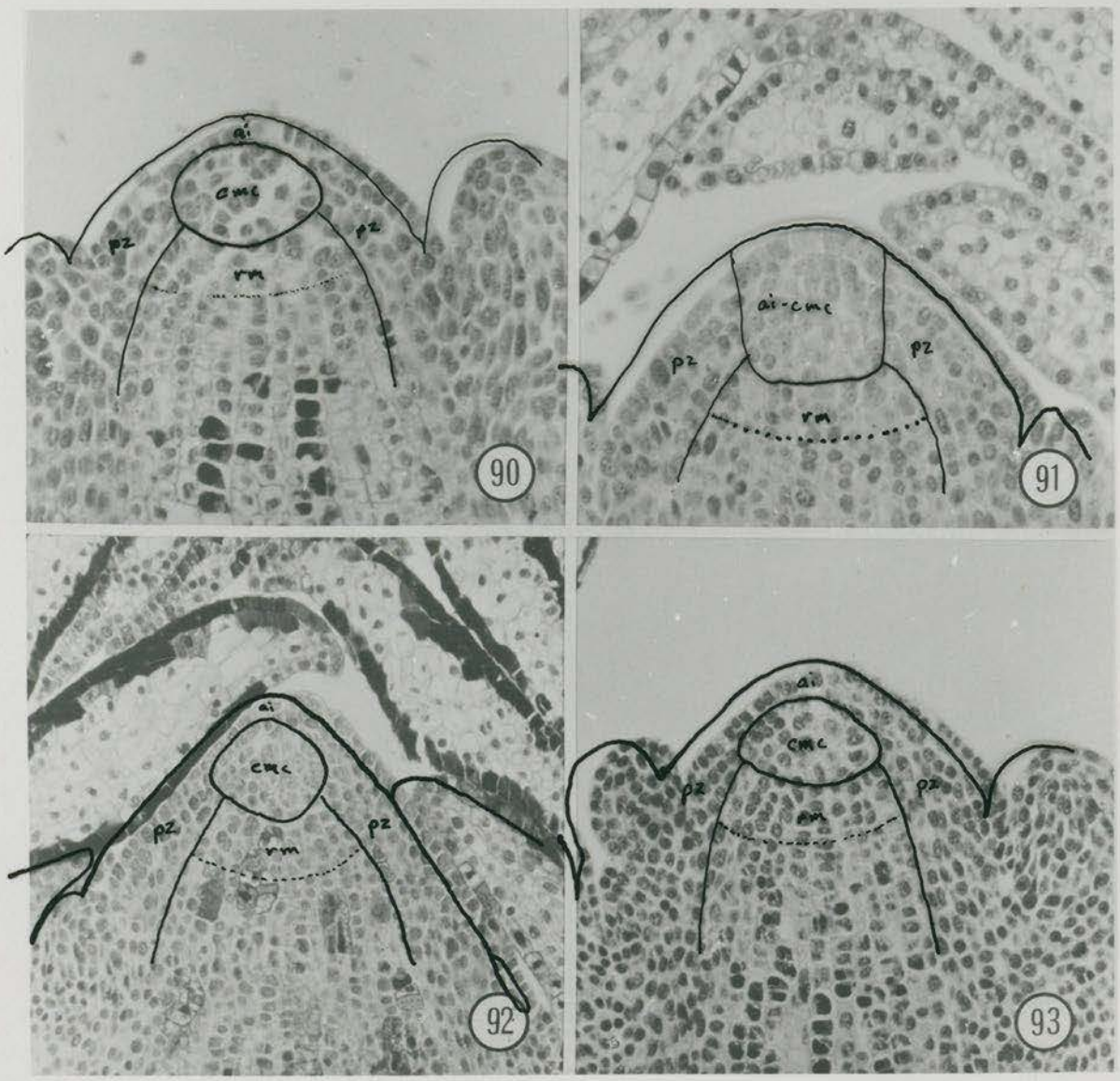
Median longitudinal section of a vegetative apex at the end of bud scale initiation exhibiting four distinct zones. apical initials, ai; central mother cells, cmc; rib meristem, rm; peripheral zone, pz.

160 x

Figure 93.

Median longitudinal section of a vegetative apex in a near dormant state. Zonal patterns are present in terms of cell size and arrangement but zonation is terms of differential stain uptake is indistinct. Compare to Figure 90 and note the lack of any permanent changes during dormancy.

140 x


ai
cmc
pz
pz
rm
90
ai-cmc
pz
pz
rm
91
ai
cmc
pz
pz
rm
92
ai
cmc
pz
pz
rm
93

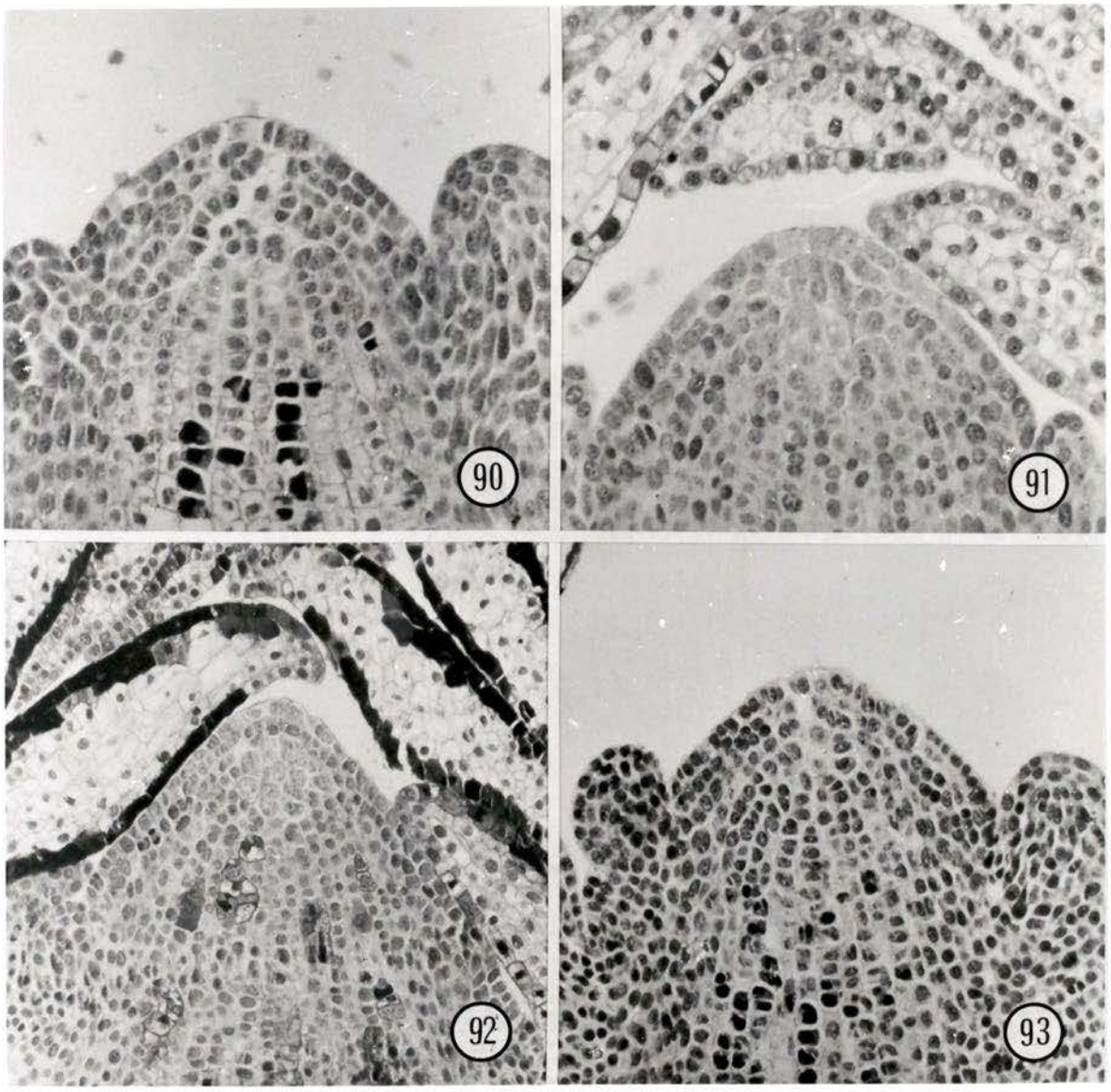

90
91
92
93

Figure 94.

A median longitudinal section of a Picea glauca terminal vegetative bud during late bud scale initiation illustrating zonation patterns. apical initials, ai; central mother cells, cms; peripheral zone, pz; rib meristem, rm; pith, pi; protoderm, pr.

360 x

Figure 95.

A cross-section of a plastic embedded vegetative P. glauca apex. Light micrograph illustrates the apical initial cells at level AA (Fig. 94). apical initials, ai.

920 x

Figure 96.

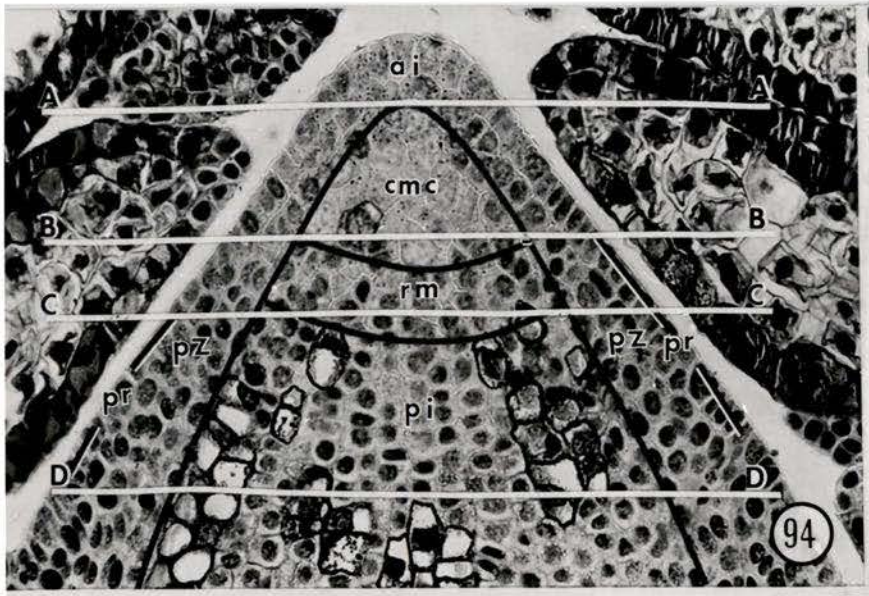
A light micrograph cross-section of P. glauca at level BB (Fig. 94). This micrograph represents the basal region of the central mother cells thus incuding the peripheral zone cells. central mother cells, cmc; peripheral zone, pz; prototderm, pr.

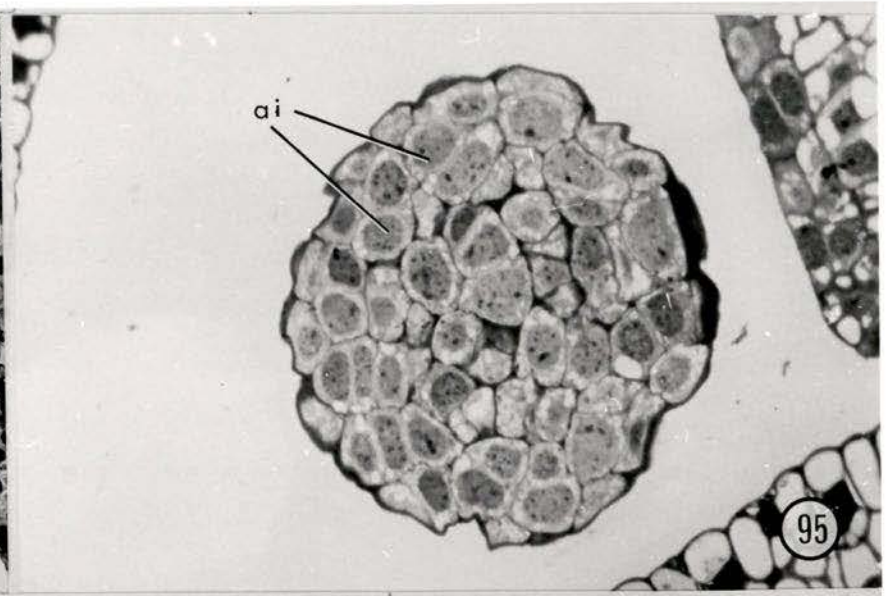
360 x

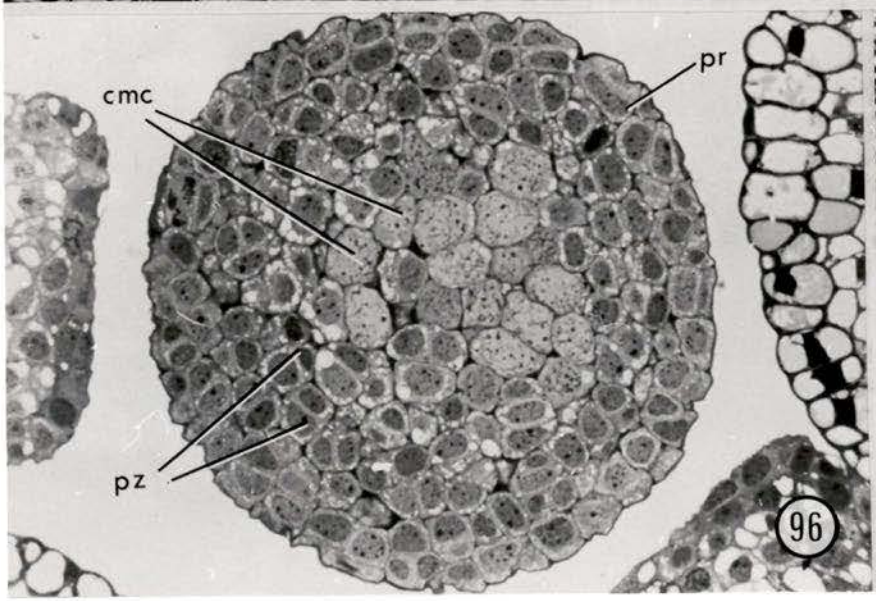
Figure 97.

Cross-section at level DD (Fig. 94) of a Picea glauca vegetative apex through the pith showing ergastic substance accumulation (darkly staining central cells). Material was fixed and embedded for transmission electron microscopy. pith, pi; peripheral zone, pz.

360 x


ai
A
A
cmc
B
B
rm
C
C
pz
pz
pr
pr
pi
D
D
94


ai
95


cmc
pr
pz
96

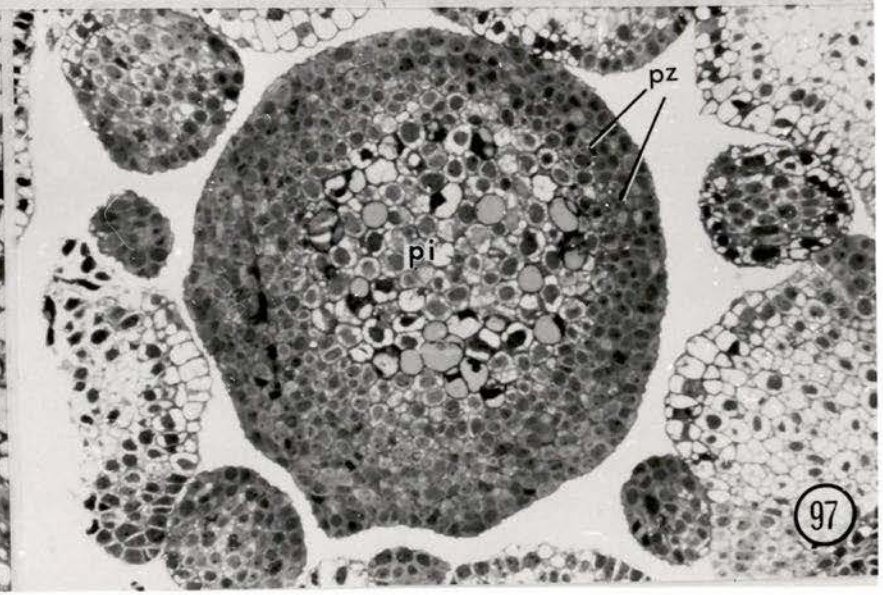

pz
pi
97

Figure 98.

A TEM micrograph of a Picea glauca vegetative apex. Cross-section shows pith and peripheral zone, at this period it clearly demonstrates that the ergastic substance accumulation is confined to the protoplasm. pith cells, pi; peripheral zone cells, pz; nucleus, N; vacuole, V; nucleoli, Nu; cell wall, Cw.

5600 x

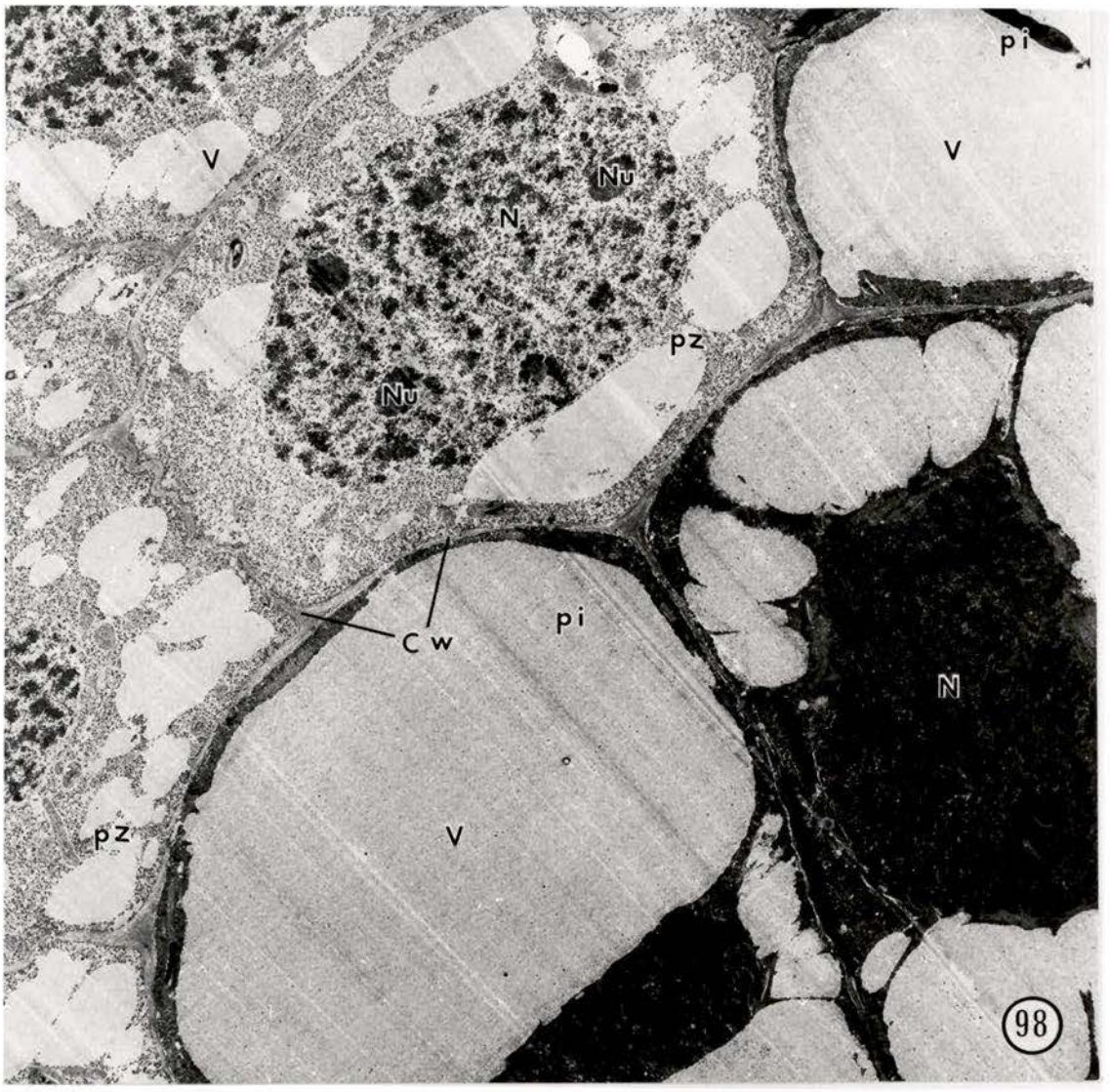

pi
V
Nu
N
pz
Nu
Cw
pi
N
V
pz
98

Figure 99.

A TEM micrograph of P. glauca terminal vegetative apex. Micrograph represents a cross-section of rib meristem cells located just beneath the central mother cell. Note the dense cytoplasm and condensed chromatin of the nuclei containing prominent nucleoli. nucleus, N; vacuoles, V; cell wall, Cw; chloroplasts, Ch.
6900 x

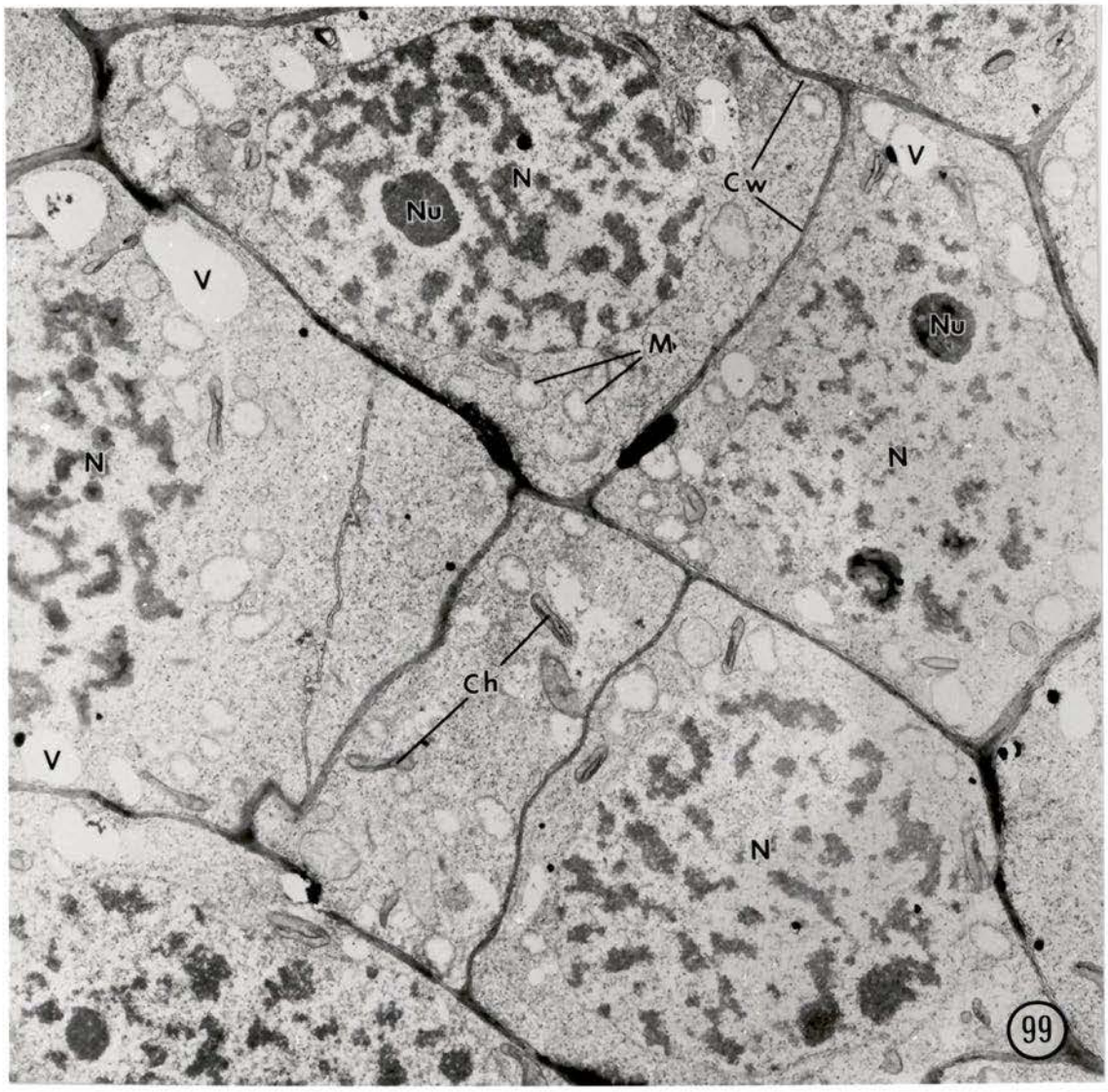

N
Nu
Cw
V
V
Nu
M
N
N
Ch
V
N
99

Figure 100.

A P. glauca micrograph of a central mother cell (mid-region) cross-section showing a large diffuse nucleus without any obvious nucleoli or vacuoles. Note the numerous mitochondria located throughout the cytoplasm. nucleus, N; cell wall, Cw; mitochondria, M; chloroplasts, Ch; vacuoles, V.

84100 x

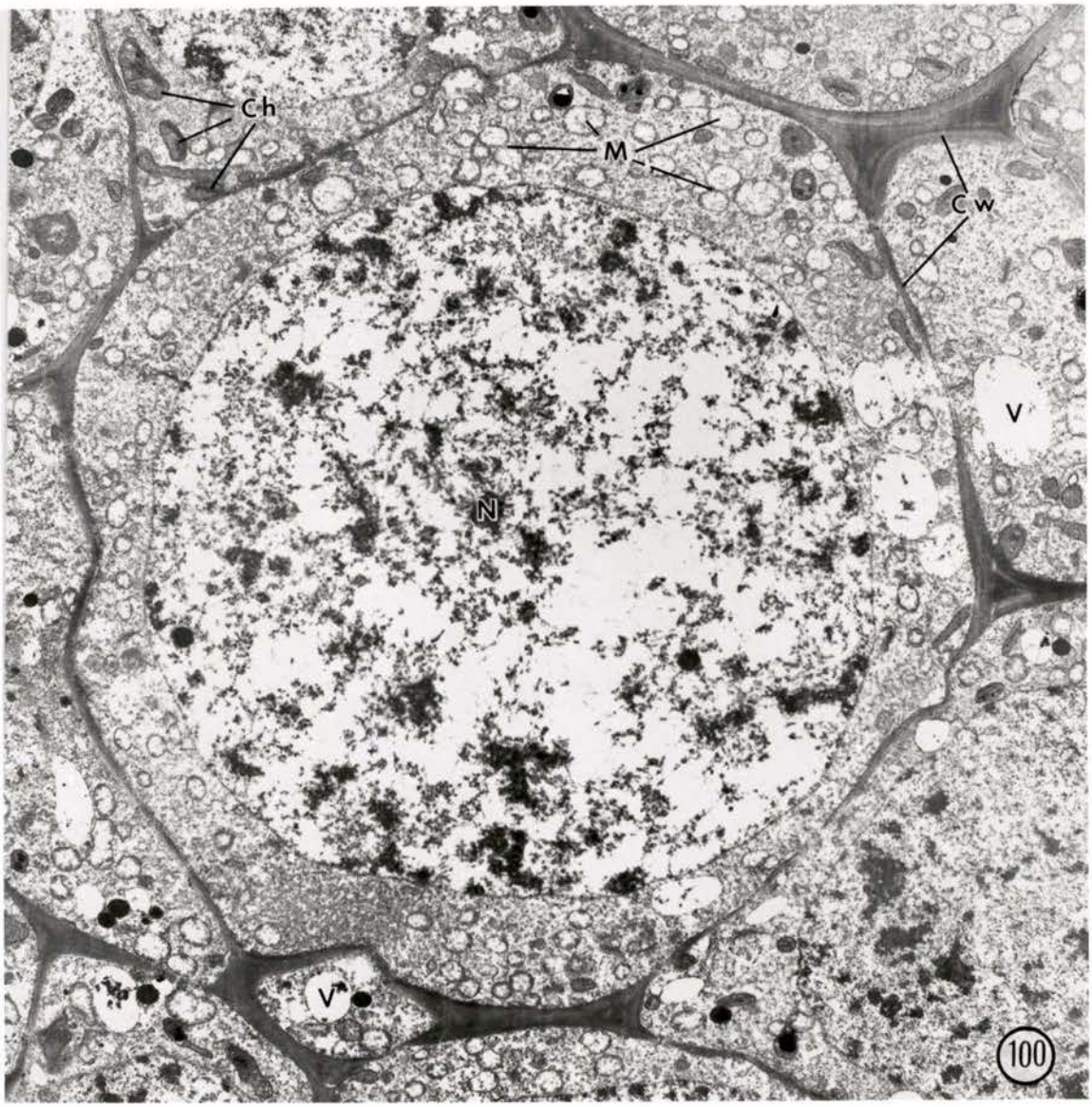

Ch
M
Cw
V
N
V
100

Figure 101.

An electron micrograph of peripheral zone cells in P. glauca taken at cell mid-point showing numerous small vacuoles and a conspicuous nucleus. Note: the prominent nucleoli and high organelle density. nucleus, N; nucleoli, Nu; cell wall, Cw; vacuoles, V; mitochondria, M; chloroplasts, Ch.

8400 x

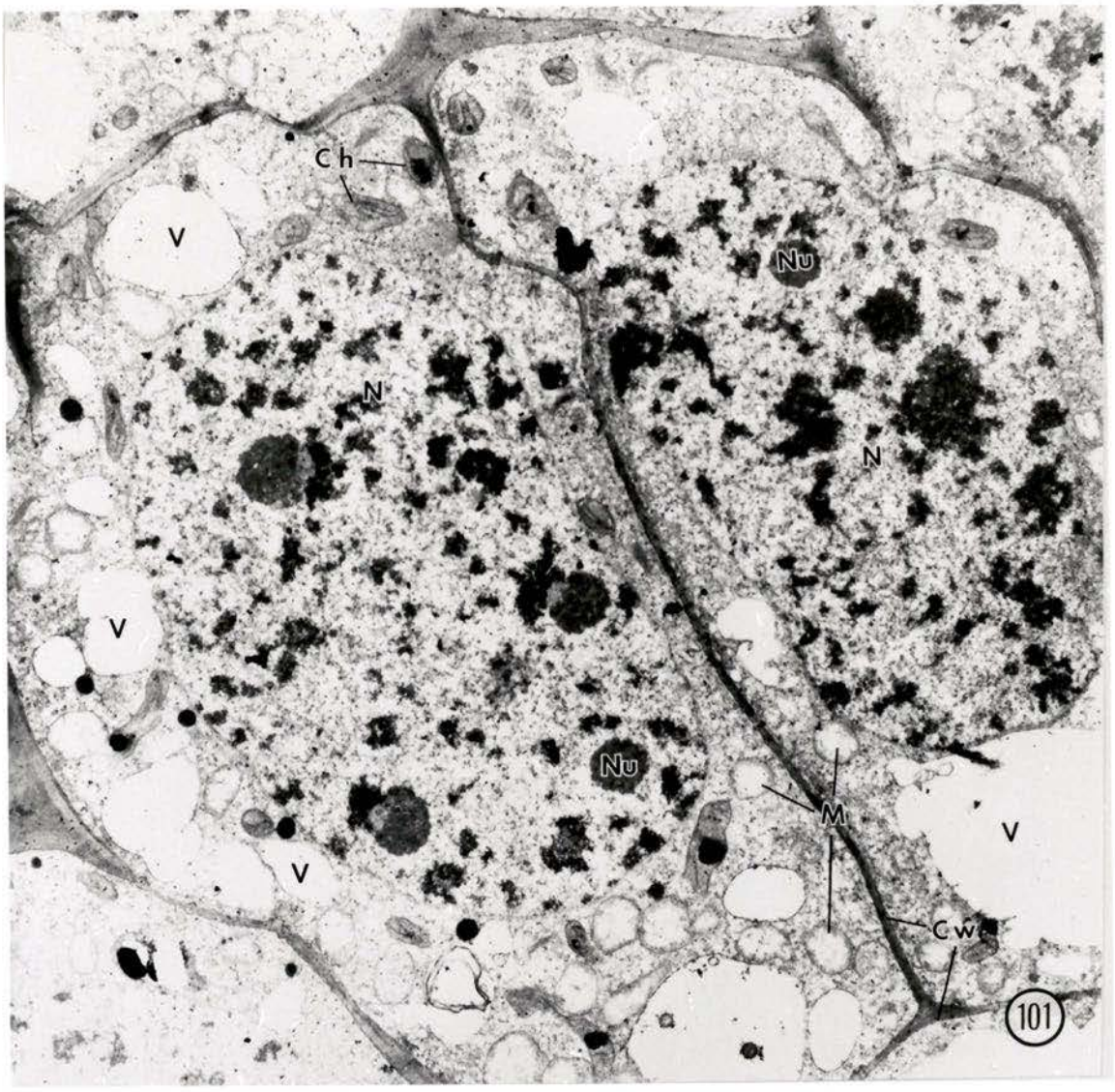

Ch
V
Nu
N
N
V
Nu
M
V
V
Cw
101

Figure 102.

An electron micrograph of an apical initial cell at mid-point. Note the large diffuse nucleus, high density of mitochondria and apparent lack of chloroplasts, nucleoli and vacuoles. nucleus, N; mitochondria, M; cell wall, Cw; dictyosome, D.

14000 x

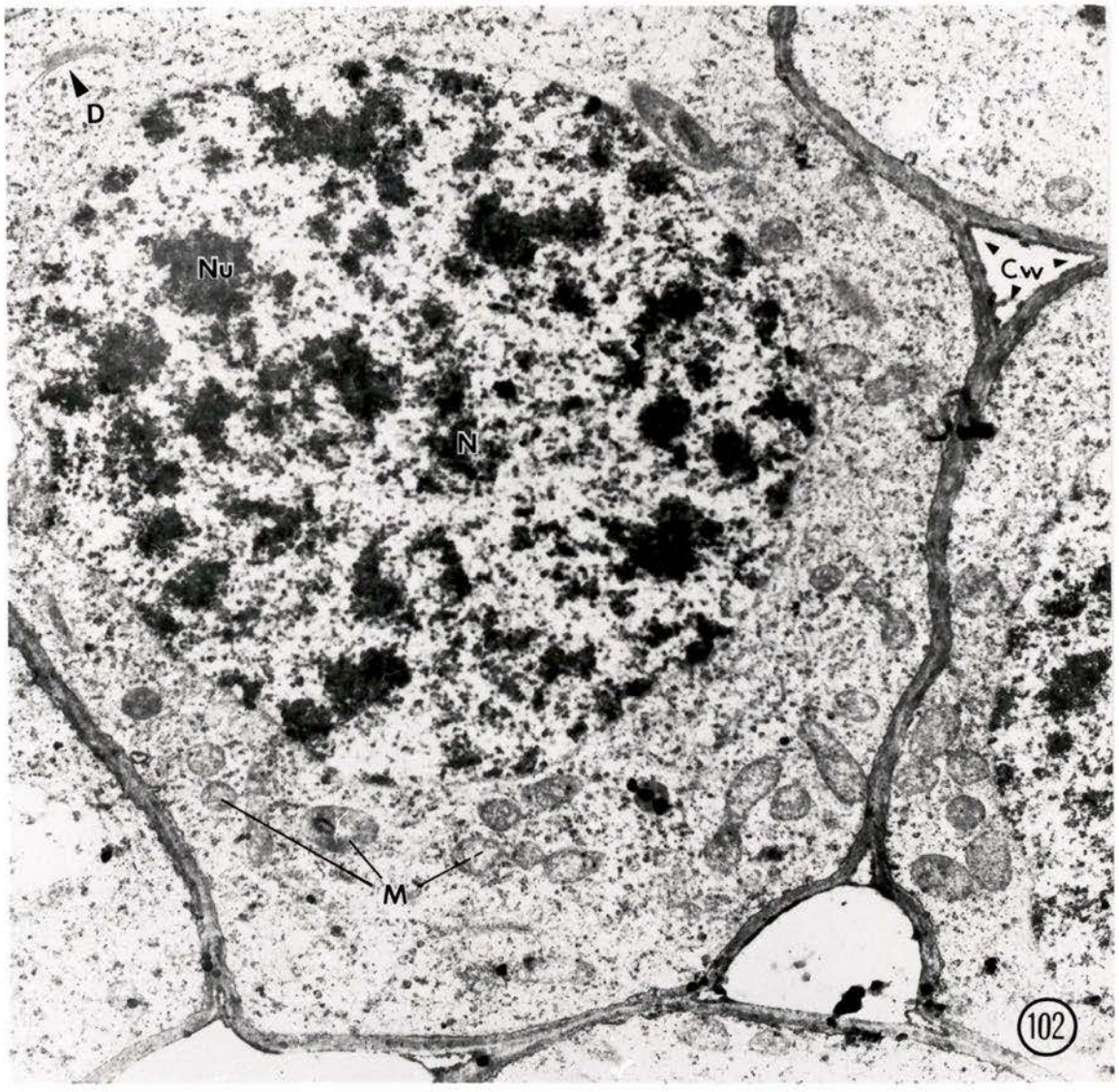
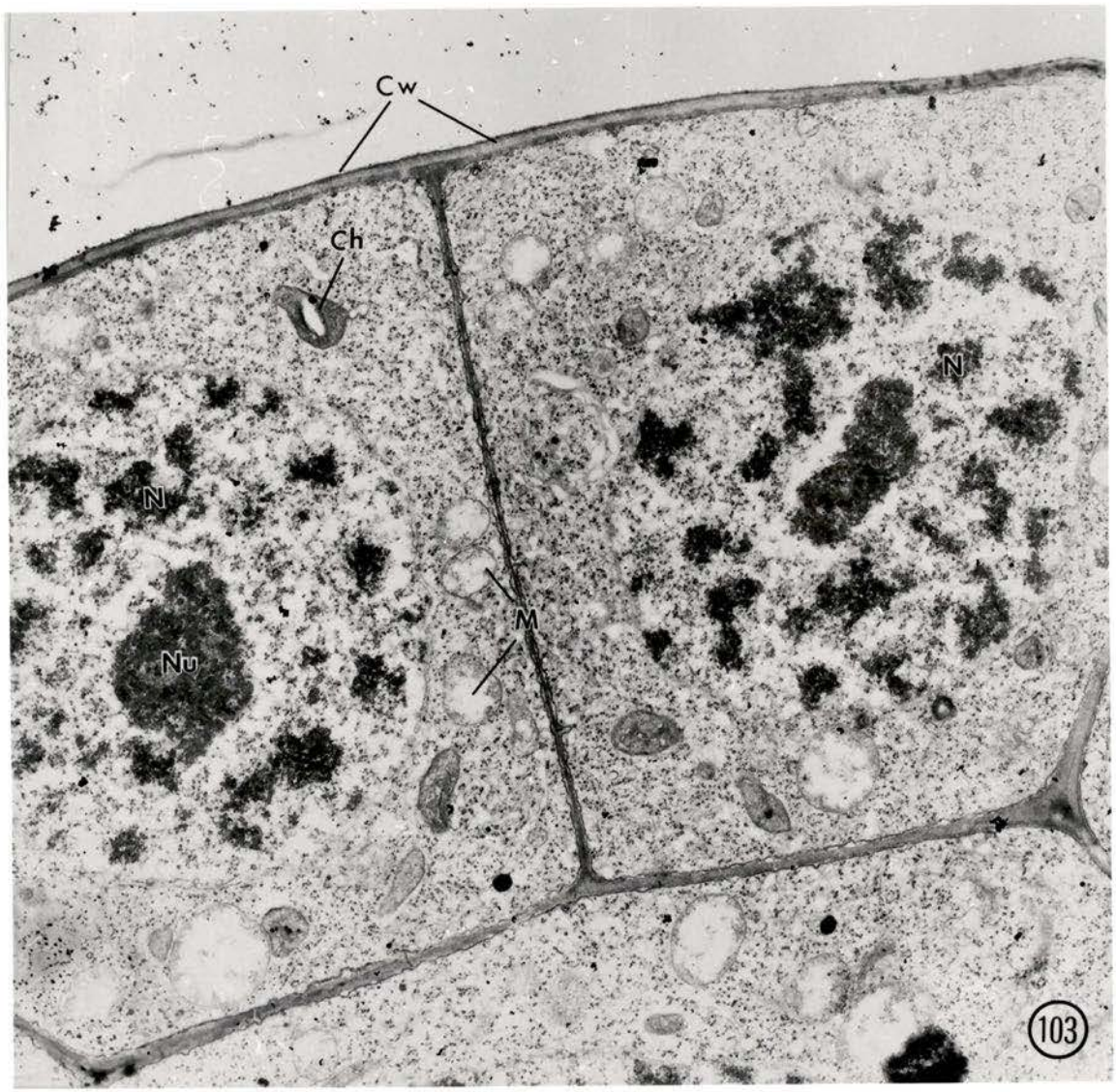

D
Nu
N
Cw
M
102

Figure 103.

A transmission electron micrograph of two protodermal cells at level CC (Figure 94). Note the lack of cell vacuoles and that the chromatin is rather condensed with characteristic nucleoli. nucleus, N; nucleoli, Nu; cell wall, Cw; chloroplasts, Ch; mitochondria, M.

12600 x


Cw
Ch
N
N
M
Nu
103

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VEGETATIVE BUD DEVELOPMENT, CONE DIFFERENTIATION AND DEVELOPMENT IN PICEA ENGELMANNII PARRY

Author [redacted]
(Signature)

DEREK L.S. HARRISON
Name (in block letters)

14th April, 1982
(Date)