PHENOLOGY AND ONTOGENY OF THE REPRODUCTIVE AND PRIMARY VEGETATIVE STRUCTURES OF *ABIES AMABILIS* AND *ABIES PROCERA*

by

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Approved by_____

Department_____

Date_____

May 17, 1966

Dear Jerry—

Here is a final copy of my thesis to use for our article. I didn't put in any of the photomicrographs because you indicated that you weren't going to include any anatomical data. If there are any figures you would particularly like to see, let me know and I'll print some up.

Let me thank you again for all the help and information you gave me.

Good luck on the manuscript —

Regards,
Gary

UNIVERSITY OF WASHINGTON

ABSTRACT

PHENOLOGY AND ONTOGENY OF THE REPRODUCTIVE AND PRIMARY VEGETATIVE STRUCTURES OF *ABIES AMABILIS* AND *ABIES PROCERA*

by

GARY ALAN RITCHIE

Chairman of Supervisory Committee:

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This paper presents a study which was conducted into the phenology and ontogeny of the shoot tips and reproductive organs of noble fir and Pacific silver fir, two important species of the Canadian Zone forests of the Western Washington and Oregon Cascades. Material for the study was collected from sexually mature and immature trees growing at an elevation of 4, 800 feet from late March to late October of 1965. Microscopic as well as macroscopic observations were made and the report is illustrated with numerous photomicrographs. Also a phenological calendar for both species was constructed.

It was found that each species underwent similar developmental

patterns throughout the season with significant differences in timing arising only with pollen and seed release. The vegetative buds passed through three growth phases during the year. The Resting Phase occurred from mid-October to early May and during this phase the buds were dormant. They were comprised of a mantle of bud scales protecting a "telescoped" shoot. The shoot contained a spirally arranged set of primordial needles. Vascularization was rudimentary and no secondary walls were present. The entire structure was subtended by a collenchyma-like crown. The apex was inactive.

Growth Phase I began in early May. During this phase, bud burst and shoot elongation occurred. Internally, the apex became active and produced a new set of bud scales. Expansion occurred in the pith meristem as cells divided and enlarged. The apex gradually changed in shape from a low, rounded dome to a higher and more pointed configuration. A new crown appeared simultaneously with the termination of this phase in late July.

Growth Phase II began in early August. No outward changes took place in the shoot and all development was internal. The apex assumed a high, pointed shape and rapidly initiated the primordia of next year's needles. The process of needle initiation and development continued through September and ceased in mid-October when the bud became dormant.

Microsporangiate strobili were initiated at the axils of the

needles in mid to late May. They followed a sequence of development similar to that of the vegetative buds. Bud scales were initiated first on about June 15. By early July the first sporophyll primordia were produced and their development continued through the summer. Sporangia became apparent by August and by October growth ceased. The sex cells were then in the primitive archesporial stage and overwintered in this form.

In early April, meiotic divisions began to occur in the sporangia and microspore mother cells were produced. Microspores were fully matured by the first week of June when pollen release occurred. Noble fir began to shed pollen about two weeks later than silver fir, but each species shed for about four weeks.

The initiation of megasporangiate strobili was not observed but may have occurred at approximately the same time as the initiation of the microsporangiate strobili. Their development during the first year followed similar courses. By July 1 all bud scales had been initiated and a few bract primordia were present on the female buds. The first cone scales were initiated by September 1. By mid-October the female buds also became dormant, with the cone scales in a primitive undifferentiated state.

Internal development of the female cones during the second year was not investigated. However, external measurements were made. On the basis of these measurements it is apparent that the female cones

undergo a very definite growth sequence. This sequence was divided into eight stages: bud swelling, bud burst, first elongation stage, receptivity, resting stage, second elongation stage, maturation stage and cone shattering. Silver fir cones disintegrated beginning September 15, and were completely broken up by October 7. Noble fir cones did not begin to shatter until October 7.

The development of the crown was observed. This structure, although existing as a poorly defined zone of cells during Growth Phase I, did not mature until the end of this phase. A strong correlation seemed present between the time of crown maturation and the cessation of activity in the pith meristem suggesting a possible relationship between its development and auxin movement. The crown was also found beneath reproductive buds.

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INTRODUCTION

In the high elevations of the rugged Cascade Mountain Range of Washington and Oregon long bleak winters dominate the climate, the snow-free period sometimes lasting only two or three months. The flora of this country must be able to survive the prolonged cold and heavy winter snows and undergo a complete cycle of growth in a few short summer months. The true firs (Abies) of the extensive Canadian Zone virgin forests of this area are no exception.[1] They must break winter dormancy with the melting of the snow and begin apical and radial growth. They must produce male strobili and mature them in time to pollinate the developing female cones. These, in turn, must be urged through a period of development from loosely differentiated dormant buds to mature cones releasing their fully developed seeds. At the same time buds for the next season's vegetative and reproductive growth must be produced. All of these functions plus the untold thousands of supporting and accompanying physiological processes must be accomplished in one very short growing season.

The rhythm of growth of an organism in relation to seasonal

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[1] The Canadian Zone as proposed by C. H. Merriam (1898) occupies elevations of between 3,000 and 4,000 feet in the Western Washington Cascades.

cycles (phenology) can give important insight into its ecological, physiological and genetic status. Phenological patterns are manifest in practically every phase of growth of most advanced living organisms from the ontogeny of plant roots to photosynthetic activities of cells. Some of these patterns are obvious and easily measured, others subtle and extremely difficult to quantify.

Two readily observed processes which occur during the growing season are the increase in length of the growing shoots (primary vegetative growth) and the development and maturation of the reproductive organs (called strobili in gymnosperms). Because the growth and development of all these structures are dependent on similar factors (genetics, climate, site) a reasonably similar pattern of development should exist between individuals of a species which are exposed to similar genetic, climatic and site situations. If such a pattern does exist what are its characteristics, how do each of the different aspects of growth relate to one another, how do they differ among individuals and species, and what is their significance in terms of the ecological role of the species?

The purpose of this study is to find and recognize, from scrutiny of various collected field and laboratory data, phenological patterns in two important high elevation Cascade Mountain tree species, Pacific silver fir (Abies amabilis) and noble fir (A. procera). Observations are not limited to gross phenological characteristics but include an

examination of the anatomy of the shoot tip throughout its development, the initiation and development of the vegetative and reproductive organs. Emphasis is placed on the concept and mechanism of the apical meristems of these organs as the understanding of their structure and function is prerequisite to an appreciation of tree growth.

REVIEW OF LITERATURE

Descriptions of the Study Species

Noble fir (Abies procera Rehd.)

To spare the reader a lengthy botanical or taxonomic description, which may be found in any good dendrology text (Little, 1953; Sargent, 1926; Sudworth, 1908), let us merely state that noble fir is a member of the pine family (Pinaceae) and of the genus Abies, to which all of the true firs belong. Of the four species of true fir which occur in the Northwest, noble fir reaches the greatest size and is probably the longest lived. The largest individual alive today is 87 inches in diameter at breast height and 260 feet tall (American Forestry Association, 1955). A study carried out in Oregon (Hanzlik, 1925) revealed that the average noble fir in a 400 year old stand was four feet in diameter and 200 feet tall. The typical stand-grown individual lifts a clean, symmetrical bole 100 feet or more to the first branches and forms a round, dome-like crown which is rather distinctive and occupies a dominant position in the canopy (Collingwood and Brush, 1964; Staebler, 1958). When growing in the open the characteristic bluish foliage reaches almost to the ground and assumes a drooping attitude as it approaches the lower crown. A young individual possesses a sharp,

sparse, spire-like crown which tends to become rounded at the top as maturity approaches. Fruits are borne annually with large crops being produced at three or four year intervals. Female cones arise atop the previous year's shoots and are restricted to the very upper part of the crown in a typical stand-grown tree. Male cones are purple, pendulent from the needle axils and occur throughout the middle crown branches. On open-grown trees they may occur on the lowermost whorls. Male strobili tend to be produced in greatest abundance on the open or sunny side of the crown.

The species is an impressive one and has been aesthetically described by various authors as "...one of the most magnificently tall and symmetrically formed trees of its kind (Sudworth, 1908)," or "...an aristocrat among firs...a magnificent specimen, lifting its crown on a clean and symmetrical trunk a hundred and fifty to two hundred feet in the sky (Collingwood and Brush, 1964)."

The geographic range of noble fir (see Figure 1) is incompletely known and many spurious statements have been forwarded concerning it. Franklin (1964) has summarized what is presently known and tempered it with extensive personal observations to arrive at the statement that the species occurs to a latitude of $47^{\circ}45'$ N in the Washington Cascades, in the southern Washington and northern Oregon Coast Range and probably not on the Olympic Peninsula in contrast to earlier reports by Sargent, Merriam, Piper and Sudworth. Gratowsky (1958) places

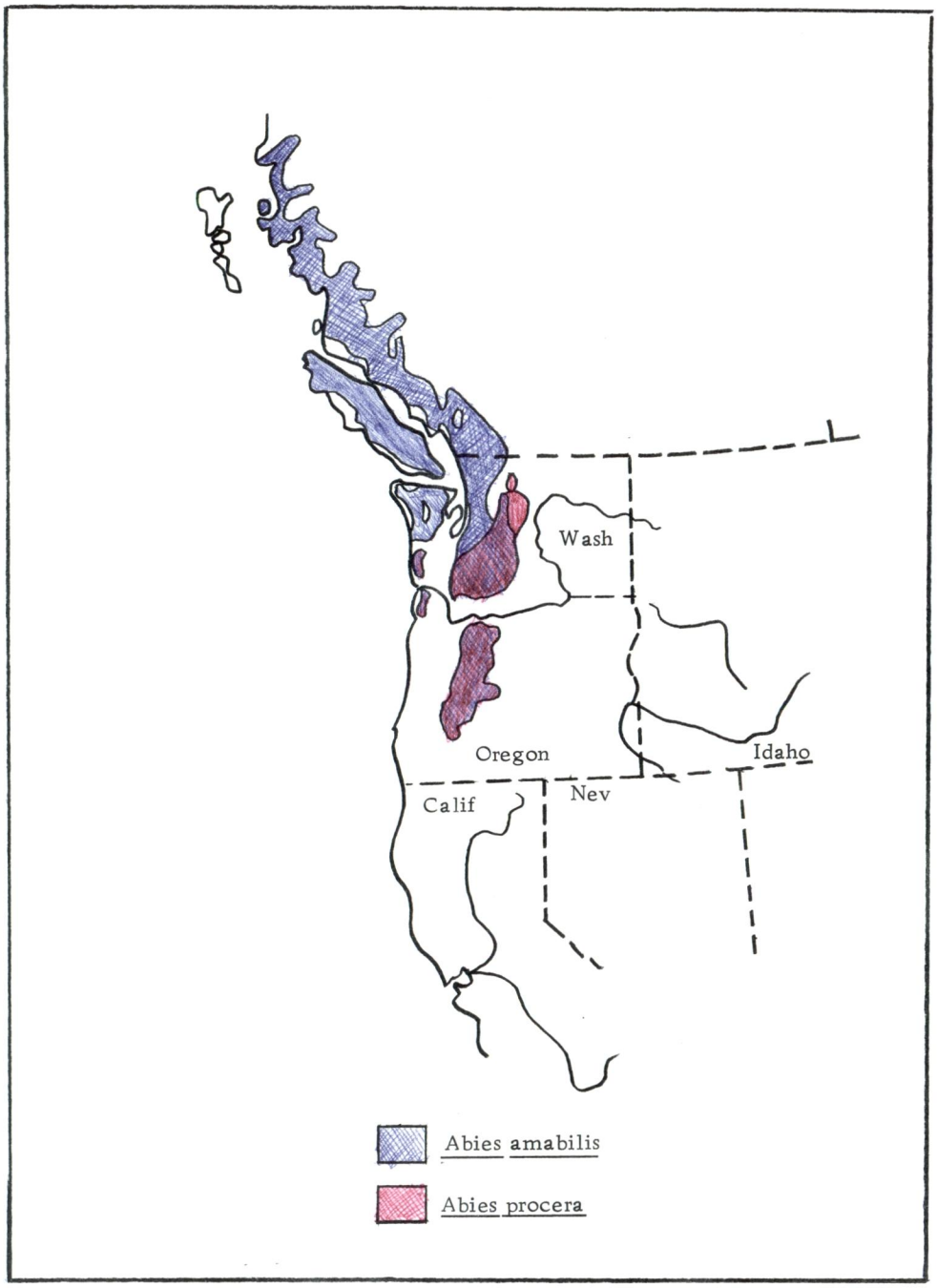


Figure 1: The geographical ranges of Abies amabilis and Abies procera (Staebler, 1958; Dimock, 1958).

noble fir's southern boundary at 43° 30' N in southern Oregon in which area it intergrades into the species Abies magnifica var. shastensis. The genetic and taxonomic details are poorly understood.

Noble fir is a mountain species occurring almost exclusively in the Canadian Zone of Merriam (1898). The climate lies mostly within the super-humid zone of Thornthwaite (1941). This area is typified by a heavy precipitation (70 to 100+ inches per year) most of which falls in the winter as snow, and mild summer-winter temperature gradients averaging 24° to 35° F in January and 50° to 62° F in July (Staebler, 1958). In northern Washington the species is found chiefly below 4,500 feet in elevation and almost always above 3,000 feet (Hanzlik, 1928). Betts (1945) places its altitudinal range at from 3,000 to 5,000 feet with extremes of 1,000 to 6,000 feet. In the Coast Range it occurs also in the Humid Transition Zone of Merriam (1898).

Soil conditions have a minimum effect on the development of noble fir (Betts, 1945; Redmond, 1950; Staebler, 1958; Sudworth, 1908). Although the species thrives on thin rocky well drained soil types its best growth is achieved, as might be expected, on deep fertile sites. As Sudworth (1908) and Staebler (1958) pointed out, soil moisture is more critical than soil quality to the survival of the species. This may in part be attributed to the fact that the seedling root systems are morphologically not adapted to low moisture conditions and thus cannot tolerate even slight drought. The one to three year old seedlings have

no prominent taproot, instead there is a slow growing main root which gives rise to several sparsely branched laterals (Wilcox, 1954).

According to Franklin (1964) noble fir is found regularly throughout its range in small pure stands despite disputing comments by Sudworth (1908), Hanzlik (1925), 1928) and others. But it more typically occurs in mixture with other species of the Canadian Zone. Among these are Douglas-fir, Pacific silver fir, western white pine, mountain hemlock and western hemlock in the lower Canadian Zone and Pacific silver fir, Alaskan yellow cedar, mountain hemlock and subalpine fir in the Upper Canadian. Southward it mingles with white fir and sugar pine (Harlow and Harrar, 1958). Baker (1949) classes noble fir as intolerant.

Pacific silver fir (Abies amabilis (dougl.) Forbes)

Pacific silver fir, also a member of the Pinaceae and the genus Abies, is the most abundant and most important (Franklin, 1964) species both economically and ecologically in the Canadian Zone forests of the Pacific Northwest, accounting for about one half of the timber volume of this area. On a tree-for-tree basis, however, Pacific silver fir cannot compare with noble fir in size or quality; however, since the advent of the Northwest paper pulp industry, it has become valuable as a prime pulp species. In the Olympic and Cascade Mountains of Washington where this species reaches its peak of development, mature

individuals can attain heights of 140 to 160 feet with diameters of two to four feet. The largest tree alive today is 186 feet in height and six feet ten inches in diameter; it is located in the Olympic National Park in Washington (American Forestry Association, 1955). Ages of up to 300 years are not uncommon with over-maturity usually being attained at 250 years (Harlow and Harrar, 1958) and death occurring at about 300 years (Hanzlik, 1938). This species is able to endure long periods of severe suppression and it is not unusual to find a specimen two feet in diameter which is 200 or more years in age.

In appearance, silver fir is quite readily distinguished from the other true firs occurring with it, due to a distinctive crown shape and color. The foliage assumes a more lustrous darkened tone than that of noble fir, its closest true fir associate, and the crown is generally much denser and more pointed, even in later life, than that of either grand or noble fir.

The following is summarized from Sudworth (1908), Little (1938), Sargent (1926) and others. The fruits, flowers and cones are similar to those described for noble fir. Male flowers are borne in the central section of the crown and below the twigs of the current summer at the needle axils. Female flowers are found exclusively on the uppermost branches of the crown and are borne upright on shoots of the previous year's growth. At maturity the female flowers assume a bright scarlet color and hang pendant on stalks beneath the branch. After pollen has

been released the male strobili turn brown and eventually dry up and fall to the ground. The female cones, upon opening in the spring, show an almost purple color and as they mature this hue is not lost. Upon reaching maturity these structures disintegrate and release their seed. The seed, which is produced in quantity at approximately three year intervals, falls to the ground and remains beneath the snow pack until spring when it germinates. Successful establishment may occur on moist, heavy duff or a mineral soil and the seedlings are quite tolerant. Similar to noble fir, the seed crops are adversely affected by severe weather conditions during pollination, various seed chalcids and rodents (Dimock, 1958). Germination is transient and unpredictable, and is typically very low. The young seedling is capable of growth both in shade or in the open if sufficiently supplied with soil moisture (Hanzlik, 1928). Growth in dense shade is extremely slow but the seedlings have tremendous tolerance toward this type of environment and can survive for many years in a severely suppressed state, and show remarkable response to release (Schmidt, 1957).

The mature trees occur in semi-pure or pure stands throughout their range which is described by Dimock (1958) as occupying extensive areas in the Cascade, Olympic and Coast ranges from 55° N latitude in southern Alaska to 43° N latitude in southern Oregon, and as far inland as the eastern slopes of the Cascades (see Figure 1).

Silver fir is not edaphically demanding and, providing that it

is supplied with abundant moisture, can persist on a wide variety of soils. It is a major occupant of both the Canadian and Hudsonian Life Zones where it associates with the same species listed as associates of noble fir.

Ecologically, silver fir is one of the most tolerant conifers occurring in its range and it is believed by most authors that, ignoring fire or catastrophe, this species would be a major component of the climax type throughout its range. Hanzlik (1928) suggests that it is more tolerant than hemlocks and cedars.

Apical Meristems

The Concept of an Apical Meristem

Plants obviously differ from animals in a multitude of ways; some of these differences are seemingly quite clear cut, while others are not. Since the mid 1830's, the concept of the cell as the fundamental building-block of plant and animal tissue alike has been accepted by biologists as unalterable fact, but it was not until a generation later that the cell origin by division idea began to take shape and replace the old myth of spontaneous generation. In animals, growth occurs in all parts of the body but sometimes at different rates (Clowes, 1961), whereas in plants, where growth is partially governed by the inherent rigidity of cell walls not evident in animals, cell division or growth is

largely confined to certain small isolated areas of the plant organism. These areas are called meristems (from the Greek word meristos meaning divisible). As Esau (1953) explains, these tissues are carry-overs from the embryonic stage of the plant as it existed within the seed and they remain young and divisible throughout the life of the plant. This feature allows plants to undergo growth throughout their entire life while animals reach a point in their ontogeny where this capacity for production and enlargement of new tissue is no longer apparent.

This characteristic of plants can be visualized with the simple concept of their being composed of two different types of cells; mature cells which have become differentiated and specialized within the organism and have lost any ability of undergoing further division, and juvenile cells occurring in meristematic regions and being totipotent, that is having the capability of dividing and their derivatives becoming virtually any type of cell. Phylogenetically this type of structure probably results from the evolution of complex forms from relatively simpler forms; with this change, a progressive separation of the two types of cells occurs and following this a greater distinction between the mature and juvenile tissues. Whereas in primitive forms all tissues tend to be rather similar and display a certain degree of totipotence, an evolutionary maturing has brought a higher degree of specialization of each type and concurrently a further separation.

All growth of plant organisms can be more or less ideally

classified into one of two different categories: primary growth and secondary growth. Primary growth can be visualized as that growth which occurs at the apex of the growing points of the plant both on the underground and above-ground organs. This growth is manifest through structures termed apical meristems. These, in effect, produce the "skeleton" of the plant. The covering over of the skeleton by supplementary tissues is called secondary growth and can only occur following primary growth. The production of secondary tissue occurs surrounding the cylinder of cells formed by the primary meristems and comes about via the sheath of vascular cambium which envelops the entire plant. As secondary growth is thought of as being seasonal and occurring each year after the first year of a plant's life, it is generally restricted to perennials.

This study is concerned with the primary meristematic tissues which occur on the above-ground portion of the plant and with the reproductive structures which have their origin on these apical meristems. More specifically, we shall deal with the apical meristems of two closely related species of higher plants, noble fir and Pacific silver fir. But in order to gain an appreciation of the variability of meristem regions and a glimpse into their development and histology, a review is presented of much of the pertinent English (and some foreign) literature on gymnosperm apices in general and more specifically the apices in the family Pinaceae of which both study species are members.

Historical

Meristems vary from species to species, often to considerable degrees. But some generalities can be assumed if it is borne in mind that these generalities do not apply in all cases and are listed only as a matter of convenience and not of biological accuracy.

For all practical purposes an apical meristem occurs at the tip of a growing shoot and is often enclosed within the bud. In transverse section it appears as a small rounded dome-like structure with various cellular arrangements within it. The first workers who viewed this curious organ through their crude optical instruments offered various interpretations as to its arrangement and cytohistological zonation. In 1845, Nägeli, a German working with apical tissues of certain mosses and algal forms, observed that in each apical region a large, single, apical cell was present and this cell seemed to give rise to all other cells in the meristem and thus all cells in the plant. So was born the Apical Cell Theory of meristem arrangement. It was not illogical to assume that this type of arrangement was also present in other forms of plants and another German botanist, Hofmeister (1852), recognized it in various species of higher plants. The theory gained popularity and its followers became numerous and included some of the most noted botanical workers and theorists of the time.

It was soon evident that the apical cell theory proved valid when

dealing with embryonic stages of plant development; however many workers failed to adhere to the theory as applied to mature individuals. Later and more sophistocated investigations bore out their contentions.

In 1868 Hanstein (reported by Esau, 1953; Romburger, 1963) who had as support extensive studies on root and shoot tips of angiosperms, proposed and published a theory of apical organization which he called the Histogen Theory. The mandates of this concept read:

1. a mass of meristem in the shoot apex and not a single initial cell gives origin to the main plant body, and
2. this mass is made up of three parts called histogens which may be differentiated on the basis of their origin and development.

The histogens are arranged in this manner; a core of irregularly arranged cells is surrounded with many mantle-like layers of cells. Each layer and the core are produced by single rows of initial cells. Romburger (1963) has interpreted Hanstein as having named the apical initiating cells the histogens, while Esau (1953) interprets him as referring to the zones of cells produced by these initials as the histogens. Clowe's (1961) interpretation parallels Romburger's and will be accepted in this treatment of the subject. These rows of apical initiating cells called histogens were named the dermatogen, which is the surface layer giving rise to the epidermis, the underlying periblem producing the cortical tissue, and the plerome producing the pith and

procambial areas. The theory, in effect, assigns to each histogen a predestiny. This concept of predestiny of these tissues met with considerable criticism from various of the contemporary workers. Romburger (1963) recalls discussions in the literature of the pros and cons of the concept as given by Schmidt (1924), Koch (1891) and Korody (1937). As a matter of interest, the histogen concept was also applied to root meristems and, according to Clowes (1961) "Serious work on this subject (the behavior of root meristems) was delayed by the acceptance of the histogen theory of Hanstein."

Still another theory of apical arrangement which had been forwarded by many authors is the tunica-corpus theory of Schmidt. He disagreed with Hanstein on the point of the number of layers of tissues in the apex, contending that there were not three but two. One of these was a regularly arranged, multi-layered mantle to which he assigned the name tunica, and the second was an underlying, loosely arranged aggregate of cells called the corpus or body. This concept has found considerable merit in work with angiospermous shoot apices but has proven to be of little value in the interpretation of gymnosperm material or angiosperm root tips.

In commenting on the significance of the various theories, Romburger points out "...it should be remembered that there may be some truth in each theory even when applied to higher plants. Some pines have single apical cells during embryonic stages (Johansen, 1950).

Numerous root apices and a few shoot apices...are apparently well interpreted by the histogen theory."

The Shoot Apex in Gymnosperms

Treatments of this phase of developmental plant anatomy are not uncommon in the botanical literature. Noteworthy examples are considerations by Esau (1953, 1961); Clowes (1961); Romburger (1963); Foster (1939, 1941); Crafts (1943); Johnson (1951); Korody (1937); and Lewis and Dowding (1924). This can, in part, be explained by the evolutionary position of the gymnosperms between the primitive vascular cryptogams and the angiosperms, providing botanists and morphologists with a developmental link connecting these two forms. Evolutionary progress in the plant kingdom seems to have involved a simplification of the apical regions resulting in fewer and less complicated histological zones (Esau 1953).

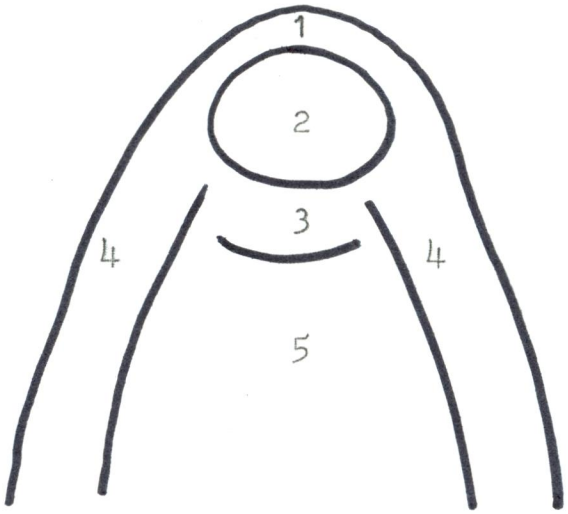
The presence of a tunica-corpus arrangement in an apical meristem is determined by the plane of division of the upper row of cells. If all divisions occur anticlinally and none periclinally, a tunica-corpus arrangement exists. If any periclinal divisions occur in the outer layer, then it does not exist. In most gymnosperms (there are a few exceptions) periclines do occur in the outer layer so the tunica-corpus theory does not explain the zone pattern. Furthermore, research has failed to bear out the theory of the apical cell. Although a large

central cell often does occur in the apex it does not cut off in regular planes and maintain itself (Johnson 1951). Similarly the histogen theory has proved inadequate (Johnson 1951; Romburger 1963). These earlier theories were written around the idea of deciding on the origin and predestiny of a particular cell or tissue, and it was not until interest turned in the direction of physiology and developmental morphology that the entire cell complex of the meristem was dealt with as an entity (Romburger, 1963).

Foster (1938), in an investigation of the shoot apex of Ginkgo biloba, made the first significant contribution to the concept of a cyto-histological zonation within the shoot tip (Esau, 1953) and this provided the foundation upon which subsequent interpretations of apical meristems have been based. Following is a summarized presentation of the cyto-histological breakdown of the various apical zones which Foster described for Ginkgo. This information is included as a frame of reference as most authors prefer to consider this the basic or ancestral type of zonation pattern and most other gymnosperms and coniferous meristems form a modification of this basic pattern (Esau, 1953; Foster, 1938).

Foster (1938) contends that this pattern is typical of gymnosperms and not found in the meristems of either angiosperms or cryptogams, which suggests their intermediate evolutionary position. The apical meristem of Ginkgo biloba is described in terms of five well defined zones: the apical initial group, the central mother cells, the

transition zone, the peripheral zone and the rib meristem (see Figure 2).



1. Apical initial group.
2. Central mother cells.
3. Transition zone.
4. Peripheral zone.
5. Rib meristem.

Figure 2: Zonation of the apical meristems of Ginkgo biloba.

Apical initial group: The apical initial cells occupy the summit of the growing surface and are the ultimate origin of all cells in the shoot. They may undergo either anticlinal division adding to the surface of the growing tip, or periclinal division donating cells to the central mother cell group. These cells are larger than the other surface cells with large nuclei and vaculoes. They stain only lightly with safranin. There is no evidence of a single apical cell.

Central mother cells: Occupying a cup shaped area directly beneath the apical initial zone are the so-called central mother cells which are derived from the apical initials. These cells may be recognized by their large size (the largest of any cells in the apex), well

vacuolated cytoplasm and large lightly staining nuclei. They also display an irregular arrangement compared to the adjacent zones due to the irregular growth pattern which they undergo. Another somewhat striking feature of these cells is the collenchyma-like wall thickenings. Typical of the central mother cells is a relative infrequency of mitotic activity, growth being accomplished primarily by expansion.

Transition zone: Bordering the central mother cell zone on the basal end is a rather poorly defined region of cells referred to as the transition zone. It is here that the mitotic activity is renewed and the peripheral and rib meristem tissues are derived. This zone is not typical, however, of many gymnosperm apices and is often ommitted in literature pertaining to the subject.

Peripheral zone: Anticlinal divisions in the apical initial zone produce a layer of cells which surround the apex in a cylindrical manner. Inner layers of this zone are cut off the transition zone and may be several cells in thickness. These two layers of cells comprise the peripheral zone. Periclinal divisions occur throughout this zone and can give rise to further layers of the zone or to primordia which are initiated in the tip.

Rib meristem: Enveloped within the peripheral layers and capped by the transition layer of the central mother cell zone is the rib

meristem. This type of primary meristem is responsible for length growth of young internodes, roots, and petioles (Schuepp, 1926). It originates at the base of the transition zone where periclinal divisions produce parallel rows of small cells. Width growth is achieved by occasional periclinal or oblique divisions.

Similar descriptions of typical zonation patterns in all of the various gymnosperms are beyond the scope of this paper. A more valuable contribution would be a brief review of the apical characteristics of the coniferales and in particular the Pinaceae. Since the advent of modern histological techniques and in the light of modern concepts of apical arrangements numerous reports have been made on apical meristems in conifers. In order to preserve space, these reports have been summarized in Table I.

Table I: A summarization of investigations on the shoot apex in conifers.

Author	Species	Observations
Cross (1939)	Taxodium distichum	Originally this species was thought of as having a tunica-corpus zonation, but Cross demonstrated that in fact a modified Ginkgoid pattern exists.
Cross (1941)	Cyptomeria japonica	This species has a zonation intermediate between tunica-corpus and Ginkgoid and is thought to be an evolutionary transition form.
Cross (1942)	Cunninghamia lanceolata	Zonation is much less specialized and more complicated than that of Taxodium and Cryptomeria and the genus is probably more primitive.

Table I: A summarization of investigations on the shoot apex in conifers (continued).

Griffith (1952)	Araucaria spp.	This is an advanced conifer showing a modified tunica-corpus arrangement. The corpus is divided into regions and is thus probably more primitive than angiosperms.
Crafts (1943a 1943b); Cross (1943); Sterling (1945); Popham (1951)	Sequoia gigantea, Sequoia sempervirens	The zonation is similar to other members of the Taxodiaceae and is also described as Ginkgoid. A structure called the "crown" is reported; a discussion of this structure will be presented in a later section.
Kemp (1943)	Torreya californica	A zonation pattern fitting the Ginkgoid description was also found in Torreya. This paper has particular significance in that the author recognized and described changes in the nature of the apical meristems with changes in the seasons. He recognized what he called the resting period, the period of bud expansion, and the period of new terminal bud formation. This emphasized the importance of relating work done on apical meristems to the season in which they are collected. Most earlier papers failed to do this.
Sterling (1946); Allen (1947); Popham (1951)	Pseudotsuga menziesii	This species domonstrates Ginkgoid zonation and undergoes phenological changes similar to those described for Torreya and Abies concolor (to be presented in detail later).
Sacher (1954)	Pinus lambertiana, Pinus ponderosa	This genus also has Ginkgoid zonation and shoot tip periodicity. It, however, undergoes a slightly different growth sequence in that terminal bud scales are initiated at the end of the growing season rather than the beginning.

The most relevant paper to date is one by Parke (1959) on the growth periodicity and shoot tip of Abies concolor. It also bears mentioning that Korody (1937), a German worker, presented a definitive paper on the shoot apex of this species and two others; this paper has been liberally quoted and referred to throughout the literature on apical meristems. The aforementioned work by Parke will be treated here in some detail due to its close bearing to the present research, and it will serve to culminate this section on apical meristem literature.

Three growth phases were recognized and named by Korody (1937) in the shoot tip of Abies concolor, the terminology used by Park is identical to that of Korody in describing these phases. The following data were obtained from studies of six to ten year old trees growing in the Sierra Nevada mountains in California at an elevationof 4, 000 feet. Phase one is called the Rest Phase. The Rest Phase lasts from September to early April. During this period the fully formed, "telescoped" shoot with 50 to 70 needle primorida and 20 to 30 primordial bud scales remains in a state of suspended growth within the terminal bud. The shoot is delimited from the cup-like structure bearing the cataphylls and tissues of the mature shoot by a "crown". This structure has been reported in several genera including Pseudotsuga, Picea, Sequoia, Torreya and others. It is basically a plate of thick walled, isodiametric cells extending across the shoot beneath the bud. Its function is unknown (Romburger, 1963). Growth Phase I: from early April to mid-June

rib meristem activity causes a vertical expansion of the shoot. The bud scales are stretched by the expanding needles and are held together by a sticky resinous substance until they are broken from the base of the bud and fall to the ground. During the early phase of elogation the apex remains inactive and in the shape of a low, broad cone reminiscent of the Rest Phase. As elongation proceeds, the apex gradually becomes steeply conical. When the shoot has elongated to about two or three centimeters the apical meristem begins to form new scale primordium initiation continue until mid-June. Growth Phase II: this period lasts from mid-June to September. A marked tapering off in the rate of shoot elongation and a ceasing of cataphyll formation seems to occur simultaneously. Then the apex increases markedly in size, becomes more steeply conical, and soon begins to form new needle primordia. As these are initiated the new telescoped shoot being formed gradually increases in height due to rib meristem activity. During this period the crown region becomes clearly delimited across the base of the new shoot. Needle primordia continue to be formed until late September when the apex again assumes the shape of a low, flat, broad dome. The annual growth sequence is then completed and the buds enter the Rest Phase.

Four zones in the shoot tip are described and these zones change during the different growth phases (see Figure 3). The zones were first described by Korody (1937) and Foster (1941a) and Parke has used the

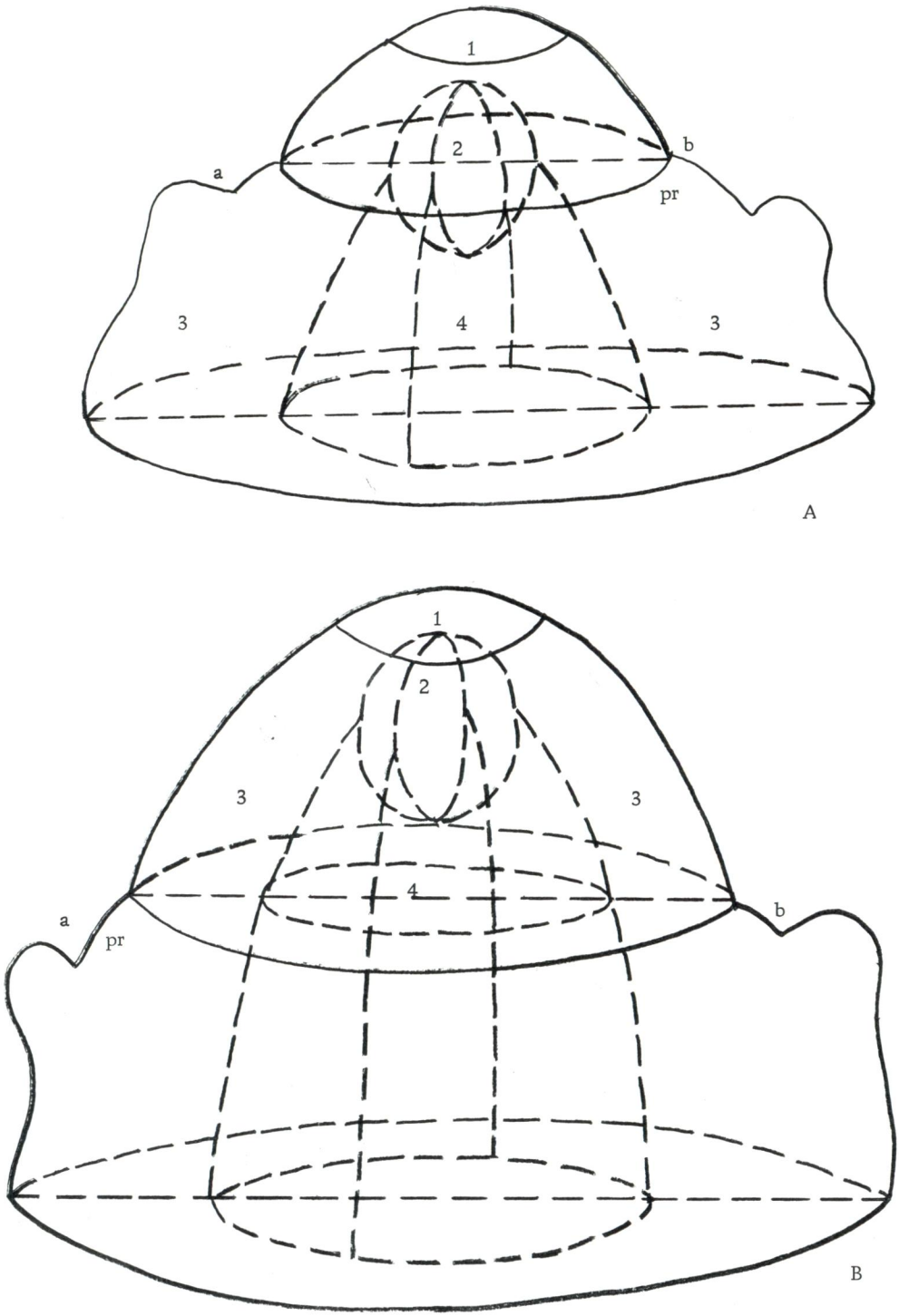


Figure 3: Zonation in the shoot tip of Abies concolor during resting (A) and growth (B) stages. Zones are: 1, apical initials; 2, central mother cells; 3, peripheral zone; 4, central of pith meristem. Plane ab delimits the shoot apex above the youngest primordium pr. The shoot apex differs structurally in the two shoot tips. (Parke, 1959).

Ginkgoid terminology of Foster in his treatment of these zones (see Figure 2), but has chosen to consider the transition zone as the lower section of the central mother cell zone. The seasonal periodicity in zonal patterns occurs as follows. During the Rest Phase the apex is a low, broad dome 65 μ high by 290 μ in diameter. The zones are all represented but are not clearly defined. In the early stages of Growth Phase I the structure of the shoot remains essentially unchanged from the Rest Phase. But soon, due to a resumption of growth in the pith meristem and the peripheral zone as well as slight activity in the central mother cell zone and the apical initials, the apex increases in height and to a lesser extent in diameter. Due to renewed activity in the various zones in the shoot tip, the zonal structure becomes more easily discernible. As the process of elongation and differentiation continues in the more basipetal regions of the shoot apex and in the needle primordia, the shoot apex continues to slowly increase in height and diameter. During scale formation the apex remains fairly constant but the basal diameter and thus the total volume decrease. Scale formation and shoot elongation continue concurrently until approximately the middle of June when scale formation ceases and shoot elongation tapers off markedly. During the initial stages of Growth Phase II the shoot apex increases conspicuously in both height and basal diameter. At this time zonation reaches maximum definition. When the apex has reached a height of about 170 μ and a basal diameter of about 360 μ it

again becomes active organogenetically. Cells in the peripheral zone give rise in rapid acropetal sequence to numerous spirally arranged needle primordia. This activity initiates formation of the new telescoped shoot. Overall growth or reconstitution of the shoot apex proceeds at a much slower rate than the formation of needle primordia and, as a consequence, the flanks of the shoot apex are gradually used up. That is, the height and subsequently the basal diameter of the apical cone are gradually decreased as new needle primordia are formed. This continues until September when the apex is reduced to a shallow dome. The shoot is formed and growth is completed.

Floral Ontogeny in Conifers

Many eighteenth century workers investigated floral initiation and development in conifers and their findings are summarized by Coulter and Chamberlain (1910) and Chamberlain (1935). The major objective of these earlier workers seems to have been to determine homologies between angiosperm and gymnosperm floral parts. The prevailing theory holds that the male strobilis is a simple structure whose sporophylls are borne directly upon the cone axis. Because there are no bracts the staminate cone is considered a flower, while the female strobilis is an inflorescence because the bract which occurs is homologous to the male sporophyll and the ovuliferous scales are modified shoots which bear the ovules (Chamberlain 1935). Literature dealing

with this subject is extensive and has been reviewed by Doak (1935), Thompson (1940) and Florin (1954).

The apical meristem producing the floral structure has been discussed in terms of its origin, some workers (Gregoire, 1938) holding that it is morphologically different from the vegetative meristem, and others claiming that it is identical to a vegetative apex and its differentiation into a floral structure is merely due to a change in size and shape of the vegetative meristem. The latter view is the one supported by most modern workers (Clowes, 1961; Gifford and Wetmore, 1957).

The Microsporangiate Strobilus

All members of the Pinaceae are monoecious. The microsporangiate, or male, strobilus comprises a central axis with a series of spirally arranged sporophylls bearing two sporangia each and is typically eusporangiate. The initiation of this structure occurs in early spring or summer at the axil of a needle and by fall it is fully formed and inclosed within a sheath of cataphylls. At this time the sporangia are well developed and areas of sporogenous tissue are visible within. It is in this condition that the structure endures the winter dormant period. With the coming of spring, meiotic divisions forming haploid tetrads occur and the characteristic winged pollen grains are soon formed. Within each pollen cell a nucleus divides three times to form two prothallial cells, a generative cell, and a tube cell with the latter two

functioning in fertilization. Soon afterward the sporangium wall dehisces and the pollen grains are released thus completing the function of the microsporangiate strobilus.

Initiation of the strobili occurs in much the same manner as the initiation of any vegetative organ, that is by localized divisions occurring on the main stem at the needle axils causing a distinct swelling. The time of initiation in various species has been investigated by several workers. The pines have been studied in this light rather intensively. Strassburger (1872, reported by Mergen and Koerting, 1957) stated that the male primordia in Pinus pumilo were initiated in early August. In her classical work on the life history of Pinus, Ferguson (1904) indicates that the primordia of all species studied, with the exception of P. strobus, appeared in October or early November. This observation was fortified by Coulter and Chamberlain (1910) with P. laricio in the Chicago area. Doak (1935) reports that the deposition of male strobili of Pinus occurs in late July. For P. elliottii growing in the area of Lake City, Florida, Mergen and Koerting (1957) observed the initiation of a strobilus in late June.

Observations on other genera are less numerous. Some notable examples are, however, works on Pseudotsuga menziesii by Allen (1946), and Owens and Smith (1964), Taxus canadensis was observed by Dupler (1919, 1920) and described with emphasis on vascularization. Allen (1941) observed that with Douglas-fir, grand fir, and western hemlock

the ceasing of vegetative growth also ushered in the differentiation of the reproductive organs. Owens and Smith (1964) described the initiation and development of both vegetative and reproductive apices in Douglas-fir near Corvallis, Oregon. They found that each type of structure is initiated in a similar fashion during the second week of April. This date will vary due to the environmental conditions outlined by Mathews (1963). The microsporangiate strobilar apex is distinguishable by its smaller size up until the time of first microsporophyll initiation. By mid-July the initiation of foliar organs is marked by apical enlargement. The apices of each bud type (microsporangiate, megasporangiate and lateral vegetative) show similar growth periodicity and become reduced in size and zonal definition during foliar initiation. Microsporophyll initiation is similar to the initiation of other foliar organs and is described in detail by Allen (1946). The bud enters the dormant period with microsporophylls differentiated to within 60 μ of the shoot, the apex of which loses visible zonation patterns, shrinks in size and becomes a low, flattened dome. Within the sporangia, sporogenous tissue is visible. The duration of inactivity of this tissue during the winter varies with species and according to the climate. Mergen and Koerting (1957) report that for slash pine in Florida a definite resting period does occur and the strobili undergo this period in the microspore mother cell stage. They suggest also that in more southern latitudes, development may continue throughout the winter. They also point out

that some of the northern pines reach the same stage of development before winter dormancy sets in.

Microsporogenesis has been treated in the pines by Ferguson (1904) and in general by Chamberlain (1935). Mergen (1961) has investigated in some detail the development of the male strobilus and microsporogenesis in the genus Abies. He made the following observations on A. nobilis glauca growing on the Yale University campus. Buds collected on January 27 were covered with a white resinous material. Within the sporangia, primitive archesporial cells with pronounced nucleoli were visible throughout. These cells had differentiated to the microspore mother cell stage by April 6 within the then enlarged strobili. In strobili collected on April 19, he observed meiotic divisions in progress, this was followed by rapid expansion of the strobili and bud burst. By April 26, well developed microspores were present and these were being shed by May 18.

The Megasporangiate Strobilis

The megasporangiate, or female, strobilus also originates from a primordium initiated on the stem. Female strobili in all genera of the Pinaceae undergo a two-year developmental cycle except for Pinus which require three or even four years (little, 1938). In Abies they follow a developmental pattern not unlike that of the male strobilus. Initiation occurs in the spring or early summer. Bud scales are

produced by meristems resembling the vegetative and microsporangiate meristems, then bracts are produced within the bud scales. On each bract a primordial cone scale begins to develop and the overwintering stage occurs with these structures quite visible but not differentiated. Activity is resumed the following spring as swelling and bursting of the female buds occurs. The bracts preceed the scales in development and are the first structures to protrude from the distended winter bud scales. Pollen release occurs, followed by fertilization, seed development and finally seed release in the autumn.

In this study, microscopic examination was carried out on the female buds during the first year of development. During the second year, only growth measurements were made and anatomical studies were not pursued. Thus in this review of the literature only papers dealing with similar studies will be treated in any detail.

General morphological characteristics of these structures in gymnosperms are given by Chamberlain (1935) and Coulter and Chamberlain (1910). Hashizumi (1962) reported on the initiation and development of flower buds in Cryptomeria japonica. The ovuliferous structures of Taxus canadensis were studies by Dupler (1920). Buckholz (1938) reports that the female primordia of Sequoia gigantea are initiated sometime during the summer. Pollination takes place in late April or early May. By July the cones are half grown and succulent and do not attain full size until August. Seeds, however, are not mature until

August of the next year, thus three years are required for their production.

A number of studies have been performed on pines. Gifford and Mirov (1960) present in tabular form an outline of these studies, their geographical locations, the species which were studied and the dates of initiation of male and female strobili. Summarizing from this table: for more than ten different species studied in all parts of the United States and some parts of England initiation of female buds occurred in either late August or September. In one case they were not found to occur later than November. Doak (1935) studied seven species widely scattered throughout the United States and found that in all cases female primordia were initiated in mid-August. Gifford and Mirov (1960), in the paper from which this summary was extracted, report that in Placerville, California, female strobilus initiation occurred in the first two weeks of September on Pinus ponderosa. On September 18 the apices were still devoid of appendages, and the apex had a zonation similar to vegetative apices. Strobili continued development through the winter and by November 19 bract initiation had occurred acropetally. By May 1 ovuliferous scales were visible in the axils of the bracts.

While investigating the initiation and development of flower primordia in slash pine, Mergen and Koerting (1957) noticed a time lapse between the first evidence of male primordia and that of female primordia. The latter part of August saw the first initiation of the

female structures, a slight swelling in the axil of a developing cataphyll. Bud scales were initiated and by October 18 were about 12 layers thick around the developing strobilus. At this time, early vascularization was occurring in the midrib and the apex had become quite flattened. Bract primordia were initiated acropetally during late October. Collections made on January 6 showed evidence of ovuliferous scales and ovular initials were contained within them.

Initiation and development of the megasporangiate cone in Abies prior to dormancy has apparently been ignored as no accounts of this phenomenon are to be found in the recent literature. Fertilization and embryogeny in this genus are treated in older investigations by Hutchinson (1915, 1924). However, several accounts have been given of cone development in Pseudotsuga menziesii (Allen, 1943, 1963; Owens and Smith 1964, 1964). The first paper of Owens and Smith gives detailed accounts of anatomy and morphogenesis of male, female and vegetative buds in the first year of development and it is followed by a detailed presentation of the anatomy and development of the female cone in the year following dormancy. Because Douglas-fir requires two rather than three years to mature the female cone, as does Abies, and because of some striking similarities in development between the female strobili of these two genera, Owens and Smith's first paper will be summarized here in some detail.

From its initiation in early April and up until the time of bract and leaf initiation, the vegetative primordia and the megasporangiate

primordia are remarkably similar in appearance. Although the vegetative and female buds were initiated simultaneously, development of the vegetative bud preceeds development of the female bud by about two weeks. Cataphyll initiation proceeds in a similar fashion in each structure and cataphylls are borne on a receptacle-like structure on each sex strobili. Apical enlargement occurs near the end of cataphyll initiation. In mid-July, three and one-half months after initiation of the bud, bract initiation begins, and proceeds in the same manner as that of a leaf or microsporophyll. At this time the apex becomes enlarged and zonation is most pronounced. Bract elongation after early development proceeds as a result of divisions in intercalary meristems in the bract bases. Bract initiation continues for three and one-half months, and during this period various changes take place in the apex. When about one-half the final number of bracts have been initiated, the first scales are initiated acropetally and basipetally. This occurs about the first week in September. The scale arises from a truly axillary position on the bract. No vascular tissue is present in the scales before dormancy. Mitotic activity continues in both scales and bracts until dormancy in early November.

As mentioned earlier, in the present study we will not be considering a treatment of the anatomy of the female cone following dormancy but rather we will be concerned with external changes in size and appearance. In the genus Abies and more specifically A. ambilis

and A. procera no previous study of this type is presented in the literature, with the exception of a few very general accounts in certain botany and dendrology texts. However, Franklin (unpublished data 1965) has carried out some informal but systematic observations of this phenomenon in A. procera in the Washington and Oregon Cascade and Coast Ranges. He plotted cone length and breadth against time during the summers of 1961 and 1965 for 50 cones. For the two seasons the graphs closely followed a very distinct trend (see Figures 4 and 5). The growth cycle has been conveniently divided into seven distinct stages. These are summarized in Table II.

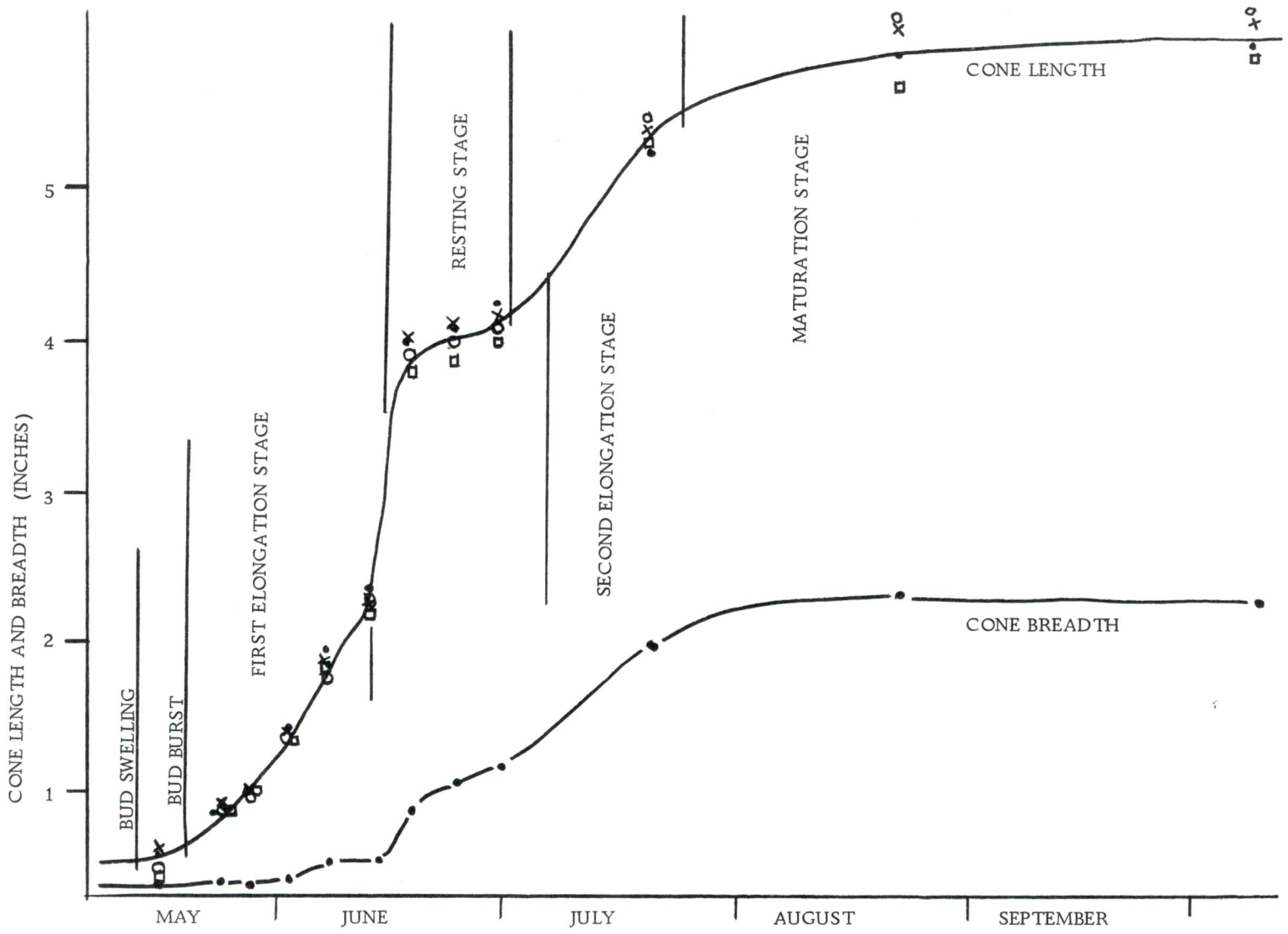


Figure 4: Growth of female cones in length and breadth on four trees at Mary's Peak, Oregon Coast Range, 1961. (Correspondence with J. F. Franklin).

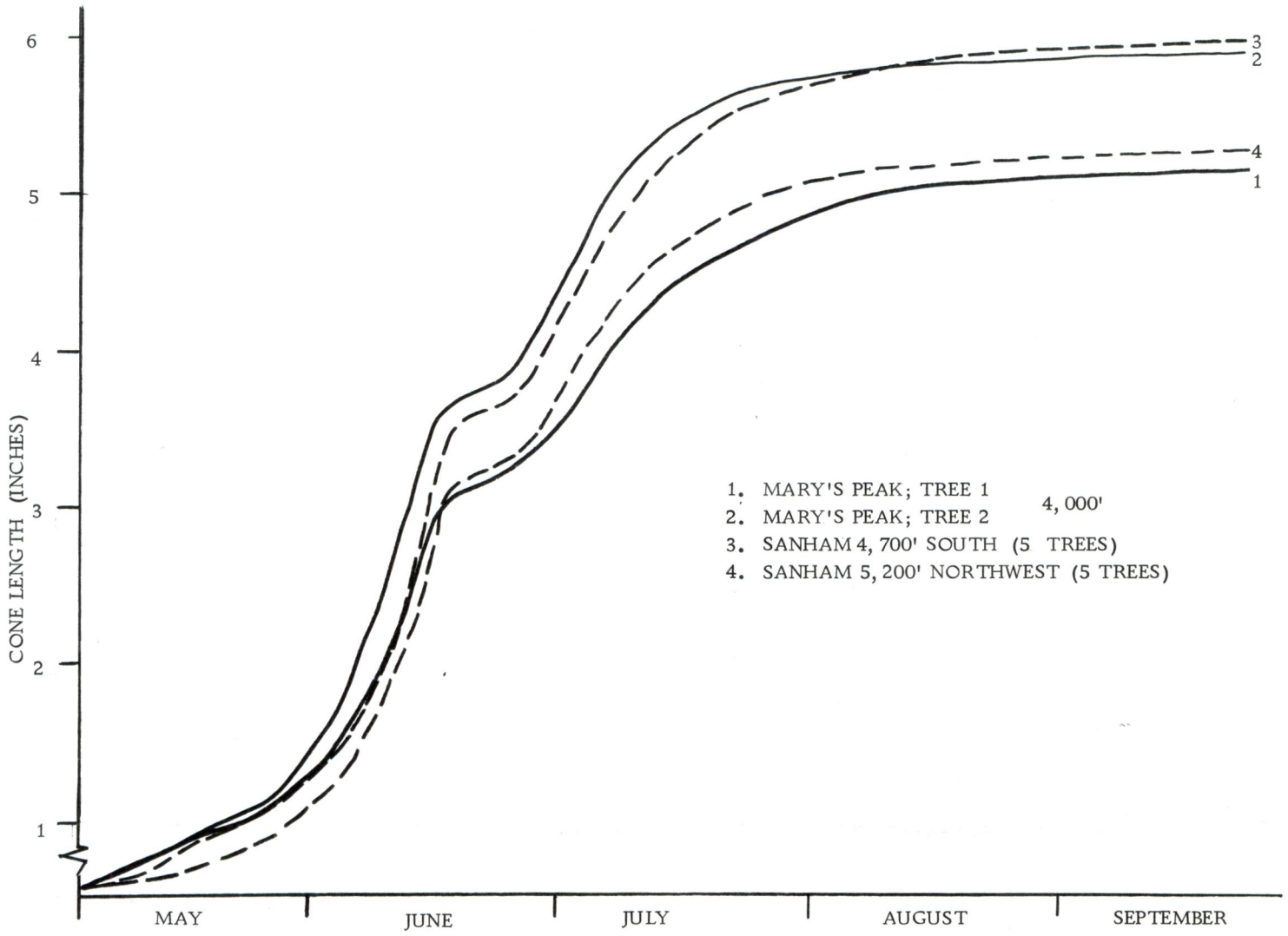


Figure 5: Growth of female cones on two noble fir trees at Mary's Peak and ten noble fir trees at Iron Mountain in 1965. (Correspondence with J. F. Franklin).

Table II. Stages in development of female Abies cones

Stage	Name	Description
I	Bud swelling	
II	Bud burst	Defined as the first projection of the cone bracts beyond the overwintering bud scales.
III	First elongation stage	The first phase of growth, initially slow but culminating in a period of very rapid growth just prior to Stage IV.
IIIa	Receptivity	A substage concurrent with a major portion of the first elongation stage. It begins when the bracts are fully freed from the bud scales and projecting from the axis at a steep angle causing an opening for the entrance of pollen. This stage ends when the cone scales grow up between the bracts and close this opening.
IV	Resting Stage	Period of very slow growth following the first elongation stage.
V	Second elongation stage	A second major growth phase during which the cone attains its final dimensions.
VI	Maturation	No changes occur in the external dimensions. This is the period of seed maturation.
VII	Cone shattering	Scales abciss from the cone axis and seeds are dispersed.

(This table is modified from a similar table compiled by Franklin, 1965; personal communication).

MATERIALS AND METHODS

Field Methods

Description of the Study Area

In order to perform this study it was necessary to select an area which met the following requirements:

a. The two study species must be present in sufficient numbers to provide collection and study material.

b. Trees in mature and immature (sexually) age classes must be present.

c. The mature trees must have evidence of a promising cone crop.

d. The area must be accessible during the winter.

An area meeting these requirements was located in Mount Rainier National Park along the Paradise Valley Road at an elevation of 4, 800 feet. See Figures 6 and 7. Historically, this area was part of a large tract of boreal forest which had been burned just before the turn of the century. The present stand is comprised of a mixture of noble, silver and sub-alpine firs, Douglas-fir, mountain hemlock, Alaskan yellow cedar, western white pine, and various associated subordinate species, primarily Vaccinium spp. and Alnus sinuata. Snags and stumps of Alaskan yellow cedar from the original stand litter the area. A

Figure 6: The study area located just off the Paradise Road in Mount Ranier Park on the south rim of the Nisqually Glacier Canyon. Note the intimate species mixture and predominance of Alaskan yellow cedar snags from the 1890 fire.

Figure 7 : Typical study trees. Notice semi-open grown conditions and the mixture of Abies amabilis and A. procera.

heterogenous patchwork of dense stands with intermittent openings and scattered individual trees typify the forest cover with 60 to 70 year old 60 to 80 foot tall dominant noble and silver firs occurring scattered throughout the area, being most dense on north-facing slopes. Old suppressed noble and silver fir trees occur sparsely at stand borders and more abundantly in openings in competition with the Vaccinium. Many of these trees are 2 to 4 inches in diameter and 50 or 60 years old. Physiographically, the area is rugged with intermittent andesite outcroppings forming sharp peaks and separating steep-sloped valleys. Soils are generally uniform, shallow pumice overlying bedrock of hypersthene-augite andesite and minor olivine andesite of Pleistocene or recent origin (Fiske et al., 1963).

The climate is distinctly seasonal and typified by short, warm, dry summers and long wet winters. The following data were obtained from the Paradise Ranger Station in Mount Rainier Park, a distance of about one mile west of the study area and at an elevation of 5, 500 feet.

PRECIPITATION (inches)				TEMPERATURE (F^{o})			
				Mean Monthly			
Total Annual	June July August	Annual snowfall	Average Annual	January	July	Average	Average
101.49	9.08	536.4	38.5	26.3	53.1	20.1	62.8

Sampling Procedure

Eight sexually mature noble and silver firs were chosen for the measurements and sample collections. See Appendix I for descriptions

of the individual study trees. For supplementary data on immature trees, 24 seedlings growing on sites ranging from dry, south-facing to wet, north-facing were located. During late March and early April the eight mature trees, which were dominant, open grown, and showed evidence of large potential cone crops, were climbed and inspected for occurrence of overwintering male and female flower buds. Eight of the female buds were numbered and permanently tagged on each tree. Ten dormant vegetative buds plus the terminal leader were similarly marked. The fertile crowns were arbitrarily divided into eight sections: upper and lower north, south, east and west. See Appendix II for a diagram of crown subdivision, location of reproductive structures, and pattern of marking.

Dormant vegetative buds on the immature trees were similarly marked later in the summer when the snow had melted. These structures on both the immature and mature trees were selected from the north and south sides of the crown and at varying heights. The growth data were tallied and averaged. This was done in order to obtain more representative estimates of growth because it was suspected that rates and total amounts of shoot growth may vary within different areas of the crown. This supposition was borne out by the study data.

At approximately weekly intervals throughout the growing season from early April to late October, measurements were made with a vernier caliper of the length and breadth of the female buds and cones

and the length of elongating shoots on all trees. At the same time the following structures were removed from the trees and fixed in FAA solution (Johansen, 1940).

a. male buds or strobili from each area of the crown of one tree of each species

b. female buds or cones from one tree of each species (the scarcity of these structures limited the number which could be taken), and

c. the new vegetative buds from several sections of the crown; as the buds elongated into shoots and produced reproductive and vegetative buds, these were also taken.

The male strobili, just prior to and during the period of pollen release, were immediately taken into the laboratory and inspected for the stage of development with reference to pollen release. This was accomplished by dropping each strobilis on a black surface and noting the amount of pollen spilled. They were then placed into one of three classes depending upon whether they had just begun to shed, were actively shedding or had completed shedding.

Laboratory methods

The material to be evaluated microscopically was dehydrated in a tertiary butyl alcohol series, embedded in 56°C Tissuemat, and sectioned to 10 to 12μ in thickness on a rotary microtome. Staining

was accomplished with safranin and fast green. See Appendix III for the staining schedule.

Photomicrographs were taken with a 35 mm. camera atop a Zeiss microscope and using Kodak Panatomic-X film; prints were made on Kodak Polycontrast Type F paper.

In order to study a particular structure at any one particular phase of its development, no less than two nor more than five of these structurs were sectioned. The statistical inadequacy of this procedure was necessitated by the amount of time required for slide preparation. This deficiency in sample numbers has been inherent in anatomical studies similar to this one in the past for the same reason (Owens, 1965, personal communication). So any interpretations made from the following data should be considered with this sampling deficiency in mind.

RESULTS

To preserve organization, yet at the same time perpetuate the feeling of a continuum of development of all structures in relationship to each other, the entire organism, and the environment, the results of this study will be presented in the following manner: the developmental phenology of each type of structure will first be treated separately, then with the aid of a comprehensive phenological calendar (see Figure 30) the developmental sequences of these structures will be compared.

The two species studied were found to be remarkably similar in developmental anatomy and phenology and for this reason will not be considered separately except in cases where marked differences do occur and these will, of course, be illucidated. The same policy will be followed in the presentation of photomicrographs; that is at any particular stage of development which is illustrated by a photomicrograph, specimens of both species will rarely be presented together unless significant differences are suspected. The photographs presented will be those considered to be representative and of good, reproduceable quality and may be of either species.

The Vegetative Bud

The following anatomical description will represent an examination of the buds of lateral shoot leaders of mature trees. During the winter months the shoot leaders of both species are embedded beneath a heavy sheath of inner and outer bud scales. The inner scales are considerably thinner and more succulent than the outer scales and lack a cutinized outer layer. Within this mantle of bud scales are the "telescoped" shoot and primordial needles, which will expand and differentiate into mature structures during the coming growing season (see Figure 8A). Within the shoot is a pith region comprising a loosely organized mass of parenchyma cells whose periphery is bounded by early provascular strands. The strands, showing no secondary wall thickenings, extend from the shoot into the needles where they can readily be distinguished from the surrounding pro-mesophylar regions. These regions are, in turn, bounded by a protodermal layer generally one cell in thickness and exhibiting a greater degree of development towards the needle tip than inwards. The shoot apex shows neither signs of activity nor zonation, and has the form of a low, slightly rounded dome (see Figure 8B). The entire structure is subtended by a sclerenchyma crown. Similar structures have been reported in the buds of A. concolor (Parke, 1959; Korody, 1937); Pseudotsuga and Larix (Lewis and Dowding, 1924; Sterling, 1946); Sequoia sempervirens

Figure 8: A, overwintering stage of the vegetative bud, noble fir, collected April 14, 1965; B, apical meristem of the same bud; C, the initiation of the first bud scale on May 13; D, the entire terminal bud at the beginning of Growth Phase I, note meristematic activity in rib meristem and needle primordia, note also obscurity of apical zonation, collected May 13, 1965, silver fir.

A X25
B X300
C X300
D X25

(Sterling, 1945a); Torreya californica (Kemp, 1946) and several other genera with exception of Pinus. Romburger (1963) summarizes some findings of the above investigators as follows:

> In effect [the crown] is a plate across the base of the bud consisting of perhaps 5 to 10 rows of somewhat isodiametric cells the walls of which have been thickened with deposits of cellulose, hemicellulose, and some pectinaceous materials. Lignin and lipids are reported almost absent. This plate isolates the tissue of the young shoot in the bud from the mature tissue beneath except where it is penetrated by vascular traces.

In the material investigated in this study, it appeared evident that the vascular traces did not actually penetrate the crown, but passed around it. The crown seems to mark the zone dividing vascular tissue which has undergone secondary development from that which has not. Above the crown, no significant development of the vascular traces has occurred (see Figures 9A and 10D). The base of the bud scales is consistently at or just above the crown region.

In the vegetative buds collected in mid-April there was no evidence of mitotic activity. The first signs of growth were noticed in buds collected on May 13 (Figure 8D). Mitotic figures were evident throughout the sections and were especially numerous in the needle primordia. Expansion and division of cells in the pith meristem were also occurring. Vascular strands at the needle bases and in the shoot were beginning to show secondary wall thickenings. From this it may be surmised that the first activity had begun in early May. In the apices of the buds collected on May 13 the first new bud scale primordia were

being initiated indicating that the apex had resumed activity, although at this stage apical zonation had not yet become obvious (Figure 8C).

External evidence of bud swelling did not become apparent until about the first week of June, at which time (see Figure 9A) three or four bud scales had already been initiated and vascularization in the shoot and needles had undergone considerable differentiation. Between June 7 and 15, buds began shedding their outer scales and by June 20 most of the buds of both species had burst. At the time of bud burst the needles had progressed to a state of advanced development with a well defined and slightly cutinized epidermis with stomatal openings, a loosely organized spongy mesophyll which demonstrated a high affinity for the stains used and an endodermis recognizable by a lack of cell content and Casparian strips. At this stage secondary thickening was readily visible throughout the protoxylem. Sieve tubes were developing sieve plates and losing their cytoplasmic content. Although mitosis was generally occurring throughout the needles, an obvious meristematic region was present in the base of each needle and these intercalary meristems were the site of most divisions (see Figure 9B). Divisions likewise occurred throughout the pith meristem but there an apparent zone just beneath the apex seemed to be maintaining a higher intensity of division. The apex is at this stage quite active and has produced a number of new bud scales. With the exception of a protoderm, little in the way of differentiation had occurred in the

Figure 9: A, noble fir vegetative bud during early Growth Phase I, note cell division and expansion occurring in rib meristem causing shoot elongation, collected May 30, 1965; B, vegetative bud of silver fir during mid Growth Phase I, note gradual change in shape of apex, well developed bud scales and basal needle meristems, collected June 17; C, silver fir vegetative bud in late Growth Phase I, collected July 7; D, apical meristem of the same bud showing the distinct Ginkgoid zonation which reaches its most defined state during this period.

A X25
B X25
C X25
D X300

scales. Still the classical Ginkgoid pattern of the active conifer apex did not become apparent until early July, about two weeks preceding the completion of bud scale initiation (Figure 9C). A closeup of the apical meristem at this stage is presented in Figure 9D. Readily evident is the central mother cell zone of large lightly stained cells with conspicuous grainy nuclei. They show no regular arrangement. Directly above them is the zone of apical initials, giving rise anticlinally to the protoderm and periclinally to the central mother cell (cmc) zone. The lower periphery of the cmc zone is mitotically active and giving rise to the pith meristem and lateral zones.

Between July 10 and 20 the cessation of scale initiation occurred and the apical meristem completed a change in shape and zonation. During this change the apex assumed the shape of a high, almost pointed dome, rising above the base of the last bud scale. The first needle primordia were initiated on the transformed meristem on about July 20 to 25 (see Figure 10A). At this point a distinct layer is present across the shoot at the base of the bud scales. This layer seems to be the precurser of the crown and its development is easily observed from this point in time. Its location is reminiscent of the meristematic area subtending the apex during the later stages of scale initiation (Figure 9B). Provascular strands can be seen appearing just above the crown region and extending upward into the base of the apex. Needle primordia fail to show any cellular organization at this point.

Shoot elongation ceases during the last days of July concommitant with the initiation of the first needle primordia, and a change in the shape of the apical meristem. Following this event, although no more external evidence of growth is visible, next year's shoot and a full complement of needles will be formed before winter within the new bud scales. Figures 10B and 10C show the progress of this development during the months of August and September. The apex has lost the distinctive zonation pattern evident during scale formation and is now wide and flattened. But zonation does most certainly exist for protodermal, provascular and pith areas are being initiated at the apex along with the needle primordia. Development of needle primordia occurs in rapid basipetal and acropetal sequence. Their development, however, occurs in a slow basipetal seauence so that while the most recent primordia show no cellular organization whatsoever the basal primordia on the same bud are already undergoing various degrees of development, notable preliminary vascular differentiation.

The final collection was made on October 24 at which time the bud no longer shows signs of mitosis, the apex has become inactive, and the structure can be considered dormant (see Figures 10D and 11A). The description given for buds collected in mid-April would suffice to describe also the buds collected in late October, as no subsequent development occurs during the winter.

Figure 10: A, silver fir vegetative bud during the early part of Growth Phase II, note the full complement of bud scales, needle primordia, and crown, collected July 27; B, silver fir vegetative bud during mid Growth Phase II, several needle primordia have been initiated and vascularization is occurring, note the change in shape and zonation of the apex, collected August 16, 1965; C, vegetative bud of silver fir during later stage of Growth Phase II, note that the apex is still active, collected September 1, 1965; D, vegetative bud of silver fir at beginning of the Rest Phase, note dormant apex and fully developed crown, collected October 24.

A X25
B X25
C X25
D X25

The phenology of seeling lateral and terminal buds, along with the phenology of the leaders of mature trees, was also recorded, but microscopic examinations were confined to branch leaders of mature trees. As is illustrated in Figure 30 the macroscopic phenology of these different types of vegetative buds does vary. It then follows that there will also be microscopic differences, but it may be safe to assume that the major observable events occur throughout the different types of buds in similar sequences with similar relative time intervals. The macroscopic patterns will be discussed in a later section.

The Microsporangiate strobilus

The male strobilus displays similarities and dissimilarities to the vegetative bud. They are similar in that both are formed by an apical meristem, have as analogous structures the shoot and needles of the vegetative bud and the axis and sporophylls of the strobilus. These structures are initiated and developed in a very similar fashion in each organ. Basic differences between the vegetative and male structures include their relative location on the shoot, the strobili being borne at the needle axils and the vegetative buds at the shoot tip. The vegetative bud is an indeterminate structure and the strobilus is a determinate structure; the vegetative bud requires one growing season to complete its development while the strobilus requires about one and one-half.

To study the initiation of a male (or female) strobilus one is faced with considerable and technical difficulties. The spiral arrangement of needles on a terminal bud shoot complicates the location and identification of primordial strobili because an obliquely sectioned needle base may have many of the characteristics of a sexual primordium, notably a rounded or dome-like configuration. However, in a serially cut terminal bud, a suspected primordium can be followed through a sequence of sections and its nature interpreted in this manner. This is probably the only reliable, although quite tedious, method of interpretation.

By resorting to this procedure, the first evidence of male primordia was observed on terminal buds collected in early June (Figure 11B). These primordia had undergone some enlargement and cell division and at this point the first bud scale primordia were being initiated suggesting that initiation of the bud primordium probably occurred in late May although no primordia were found on buds collected at that time. The apex of the bud primordium had taken on an appearance quite similar to the vegetative apex at a comparable stage in development. A rudimentary zonation was evident in some of the sections. Bud scale initiation and development continues for about three weeks as the apex begins to assume a high, rounded, dome-like appearance (Figure 11C). By the first of July most of the bud scales have been initiated and the apex has assumed a more flattened and

Figure 11: A, vegetative apical meristem of noble fir collected October 24, 1965; B, primordial male strobilus of silver fir just after initiation and showing first signs of cataphyll production, collected June 11, 1965; C, male strobilus primordium during the process of bud scale initiation, collected June 17, 1965; D, early stage in development of male strobilus of silver fir, bud scale initiation is complete and the first signs of sporophyll primordia are evident, collected on July 22, 1965.

A X250
B X400
C X300
D X100

broader configuration, apparently preparing for sporophyll production which begins between July 1 and July 6. In Figure 11D taken of a male strobilus collected on July 22 several sporophylls have developed. In addition, the lowermost pith region begins to accumulate ergastic material. The bud scales show a considerable degree of differentiation with provascular strands forming beneath the entire structure. Soon the pith cells accumulate heavy ergastic deposits (Figure 12A). The apex continues to flatten and broaden. Well defined vascular strands become evident in the bud scales and form the base of the strobilus. A crown similar in structure and location to the crown beneath the vegetative bud has formed. Like the needles on the vegetative bud, the primordia sporophylls show acropetal initiation and basipetal development. In the oldest and lowermost primordial sporangia (Figure 12B) the beginnings of an epidermal layer are apparent.

By mid-August (Figure 12C) definite cellular organization is evident in the older sporangia. An outer epidermis has formed and sporogenous initials are evident within the sporangium. These cells are characterized by their seemingly erratic planes of division producing an irregular organizational pattern. At the base of some sporangia provascular strands are appearing, the forebearers of a rudimentary vascular system. Early September (Figure 12D) sees a continuance of sporangial development along with the emergence of a distinction between cells destined to remain vegetative and those

Figure 12: A, male strobilus of noble fir collected on July 27, note heavy accumulations of ergastic material in pith and developmental stage of sporophylls; B, closeup of developing sporophyll, note primary differention of epidermal areas; C, sporangium collected from male strobilus of silver fir on August 16, 1965, differentiation is becoming more distinct and vascular strands are beginning to form at the base; D, sporangium from silver fir collected on August 31, the sporogenous zone in the central region is becoming apparent by the irregularity of planes of division.

A X25
B X350
C X350
D X400

destined to undergo reduction-division and form the microspores. The reproductive cells typically form the heart of the sporangium and have thin cell walls and large nuclei, while the vegetative cells have formed a regular, thickened outer sporangial layer and have developed hard cell walls.

The overwintering stage is achieved by early October (Figure 13A), at which time the outer bud scales have developed a heavy cutin layer and the crown has achieved a high degree of maturation. Vascular strands have emerged outside the pith and can be seen entering the sporangia. The pith has accumulated dense ergastic material. The sporangia (Figure 13B) now show a clearly defined epidermis, a resin duct with epithelial cells, a distinct three or four layered tapetum and a central mass of primitive archesporial cells. An abcission line is also forming in the sporangium as evidenced by reduced thickness of the epidermis and tapetum on the lower surface.

Activity again resumes within the dormant strobilus in early April of the following year, and by mid-April the microspore mother cells have formed and are entering the first prophase of meiosis (see Figures 13C, 13D). Meiosis requires approximately one month and by mid-May the microspores are in the haploid tetrad state. The balance of microsporogenesis takes place from late May, when wing formation occurs by separation of the inner and outer microspore wall layers (Figure 14A), to early June when the final nuclear divisions

Figure 13: A, overwintering stage of male strobilus of noble fir collected on October 3, 1965; B, sporangium of the same strobilus showing the wall layers, tapetum, resin duct, and primitive archesporial cells; C, male strobilus of noble fir collected on April 14, 1965; D, sporangium of the same strobilus showing microspore mother cells entering the first prophase of meiosis.

A A25
B X350
C X25
D X350

have produced the tube and generative nuclei (Figure 14B). Pollen release occurred during a four week period in the month of June. During this time the strobili were released from their cup-like receptacles at the needle bases and dangled freely from tiny stalks. They had changed in color from a bright red in A. amabilis or purple in A. procera to a tawny brown and became dry and brittle. Cells in the wall of the pollen sacs had developed secondary thickenings reminiscent of proxylary strands (Figure 14C) presumably as an opening mechanism. Secondary wall thickenings had developed in the protoxylem strands surrounding the pith; development of sieve cells did not occur although rudimentary phloem tissue was present adjacent to the xylary cells. This was especially evident in cross section.

The Megasporangiate Strobilus

The technical difficulties mentioned in conjunction with study of the male strobilus are multiplied when attempting to study the female strobilus in its early developmental phases. These phases occur within the swelling vegetative buds in early spring and there is no visible evidence of the absence or presence of sexual bud primordia. Male strobili are abundant and harbored in practically any terminal bud on the upper middle section of the crown. This is not the case with the females. There are relatively few buds harboring female strobili and there is no known method of detecting these. Furthermore, male

strobili generally occur with great abundance on any one shoot, so sections of the vegetative bud cut at almost any plane can be expected to reveal good median sections of at least a few male primordia. Again this is not true of the female. Generally one expects to find no more than one (rarely two or three) female primordia per shoot and thus, blindly sectioning a terminal bud in search of such a structure is, at best, a matter of luck. A final difficulty which was encountered was the predominance among samples of atypical, withered primordia destined to abort and thus valueless for interpretive studies. This high abortion rate may have physiological and practical significance and is discussed in a later section.

Resulting from the abovementioned difficulties, the first female structure obtained for study was not taken until June 27 (Figure 14D) just after the period of vegetative bud burst. Although this particular specimen is probably not typical, we can observe that at this date a complete complement of bud scales was present and the apex had flattened out and was beginning to produce bract primordia. Also a crown region is visible subtending the pith area which has begun ergastic accumulation. Bract initiation continues throughout the remainder of the summer until late August. By this time the oldest bracts (those initiated first and lowermost on the axis) show a considerable degree of development. Early epidermal and cortical tissues are distinguishable and vascular strands have progressed up the axis

Figure 14: A, the formation of wings on noble fir microspores collected May 30, 1965; B, mature microspores of silver fir during the period of pollen release collected June 18, 1965; C, mature sporangium of silver fir showing resin duct, mature microspores and wall details; D, female cone primordium of noble fir collected June 27, 1965, note that a full complement of bud scales has been initiated and the apex is probably now producing cone scale primordia although the specimen is damaged.

A X400
B X400
C X100
D X25

and well into the bracts. No secondary vascular development has occurred.

The next step in development is the initiation of the cone scale which occurs at the axil of the bract. Figure 15A depicts the first evidence of this event occurring on September 1. Subepidermal and perhaps epidermal divisions occur producing a swelling at the axil. This event occurs basipetally. By early to mid-October, female development is arrested with the scales somewhat swollen but not showing any degree of organization (see Figures 15B to 15D). At this point a definite species difference can be recognized. Noble fir female buds showed a heavy ergastic accumulation in the pith while silver fir had none. Also bract differences were apparent; noble fir bracts were long and pointed in relation to the short, stubby silver fir bracts. This difference will become more apparent when the cones reach maturity as the scales of the noble fir cones fail to overgrow the bracts as do the silver fir scales. During dormancy (mid-October to mid-April) presumably little or no further development of the female cone occurs.

Observations of female cone development during the second season of growth were limited to weekly measurements of length and width in centimeters because of the difficulties mentioned in the methods section. Also photographs were taken at selected intervals to illustrate morphological features. One graph was plotted for each individual tree (see Figures 17 to 24). At the beginning of the study eight female buds

Figure 15: A, immature cone bract of noble fir showing the slight swelling at the bract axil indicating the initiation of a cone scale, collected September 1; B, overwintering state of a female noble fir cone collected October 3, 1965, note the heavy ergastic material in the pith, the large pointed bracts and the primordial scales at the bract axils; C, closeup of scale from the same cone illustrating the overwintering stage and lack of any cellular organization; D, silver fir female cone in overwintering stage collected on October 24, 1965, note lack of ergastic material and short, rounded bracts in contrast to the noble fir cone.

A X300
B X25
C X300
D X25

Figure 17: Seasonal growth of female cones and vegetative shoots and pollen release for noble fir No. 1.

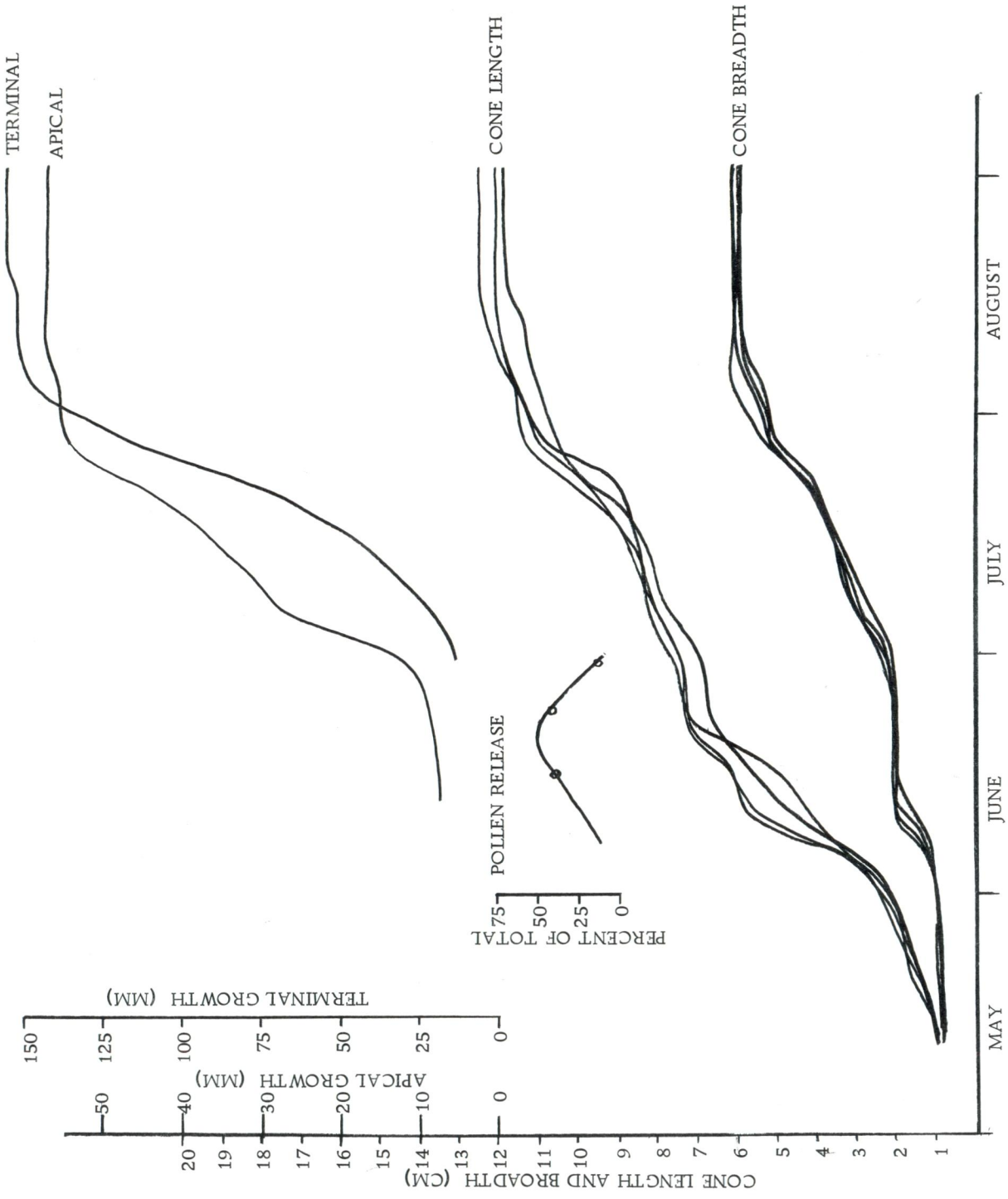


Figure 18: Seasonal growth of female cones and vegetative shoots and pollen release of noble fir No. 2.

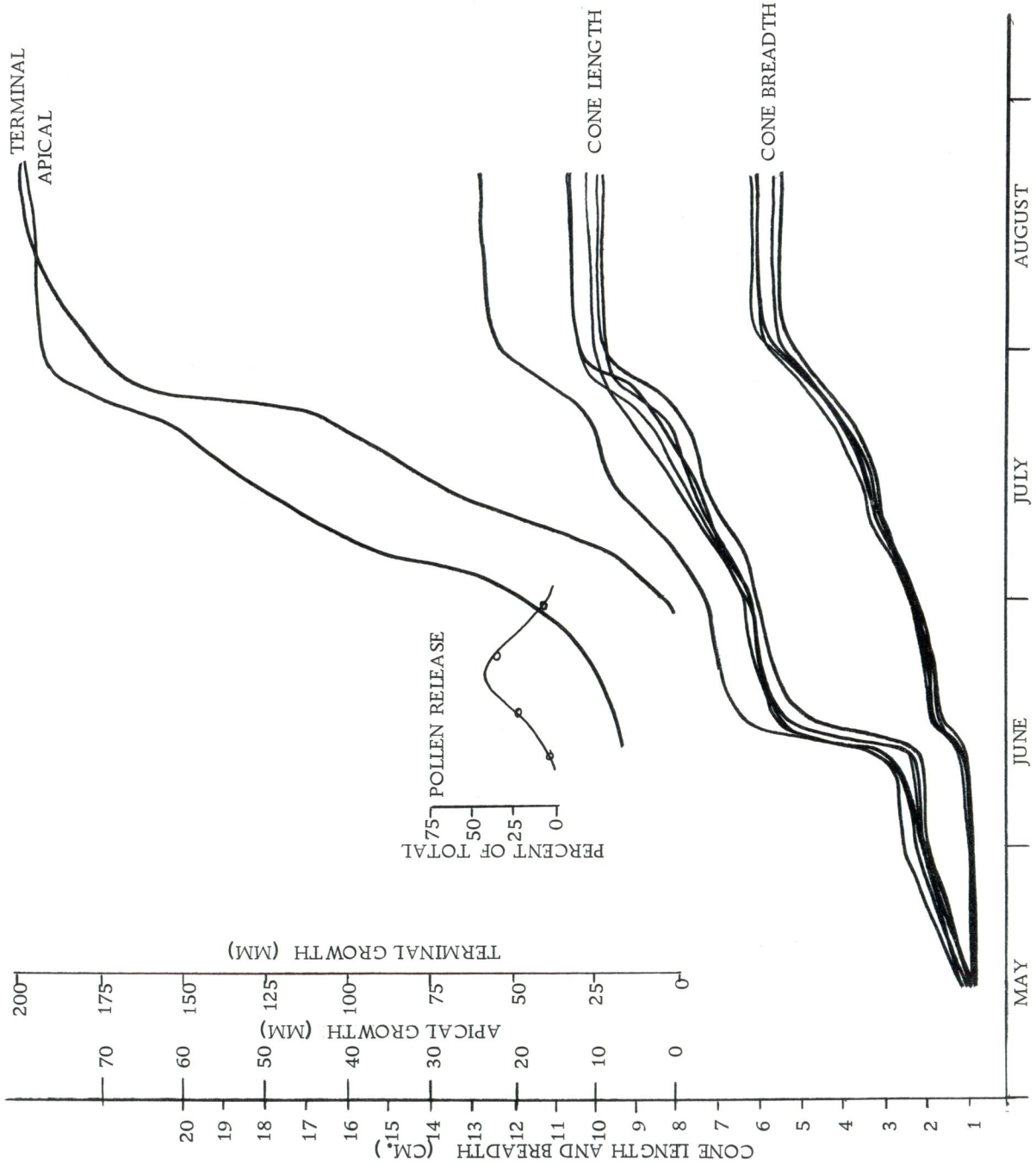


Figure 19: Seasonal growth of female cones and vegetative shoots and pollen release of noble fir No. 3.

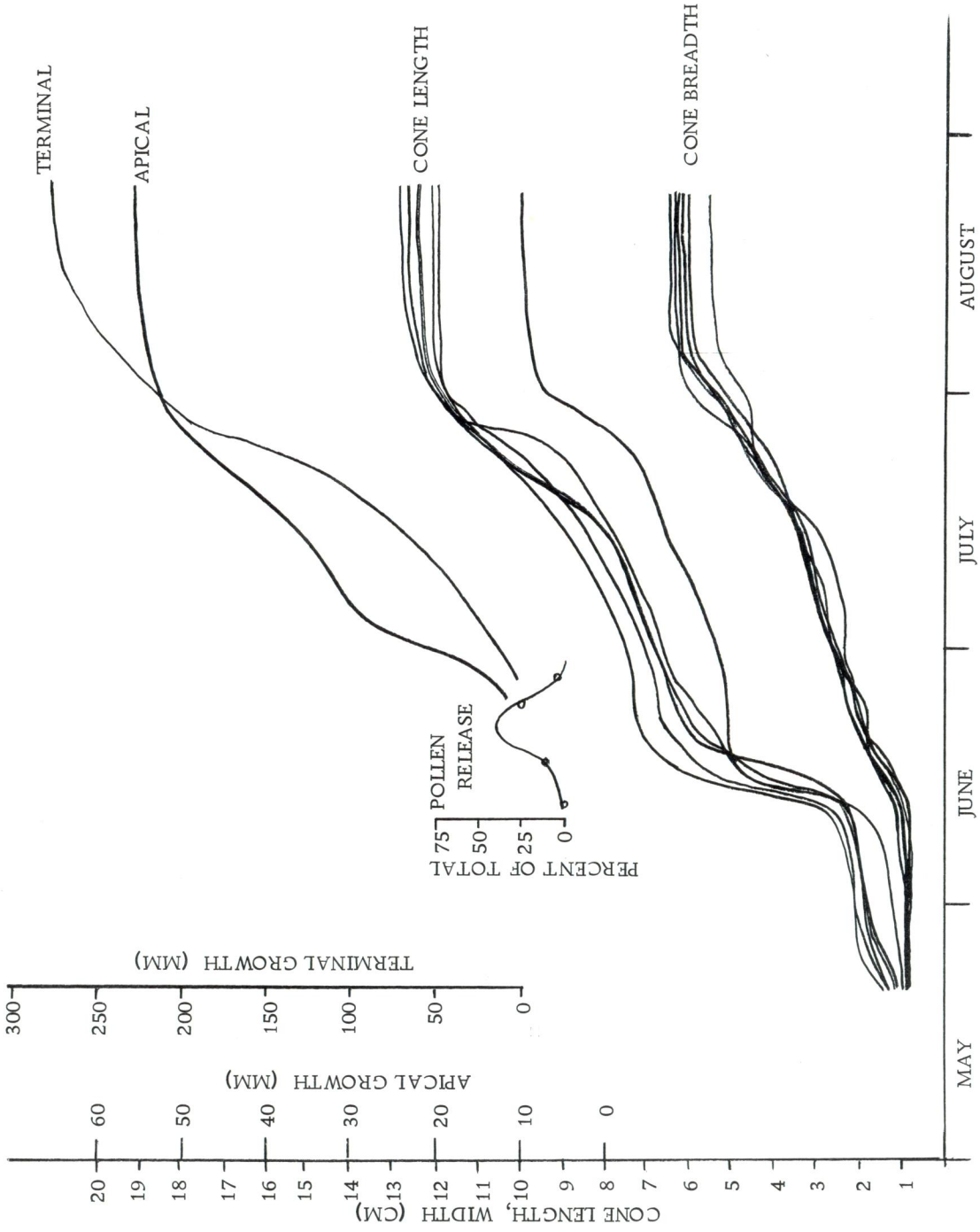


Figure 20: Seasonal growth of female cones and vegetative shoots and pollen release of noble fir No. 4.

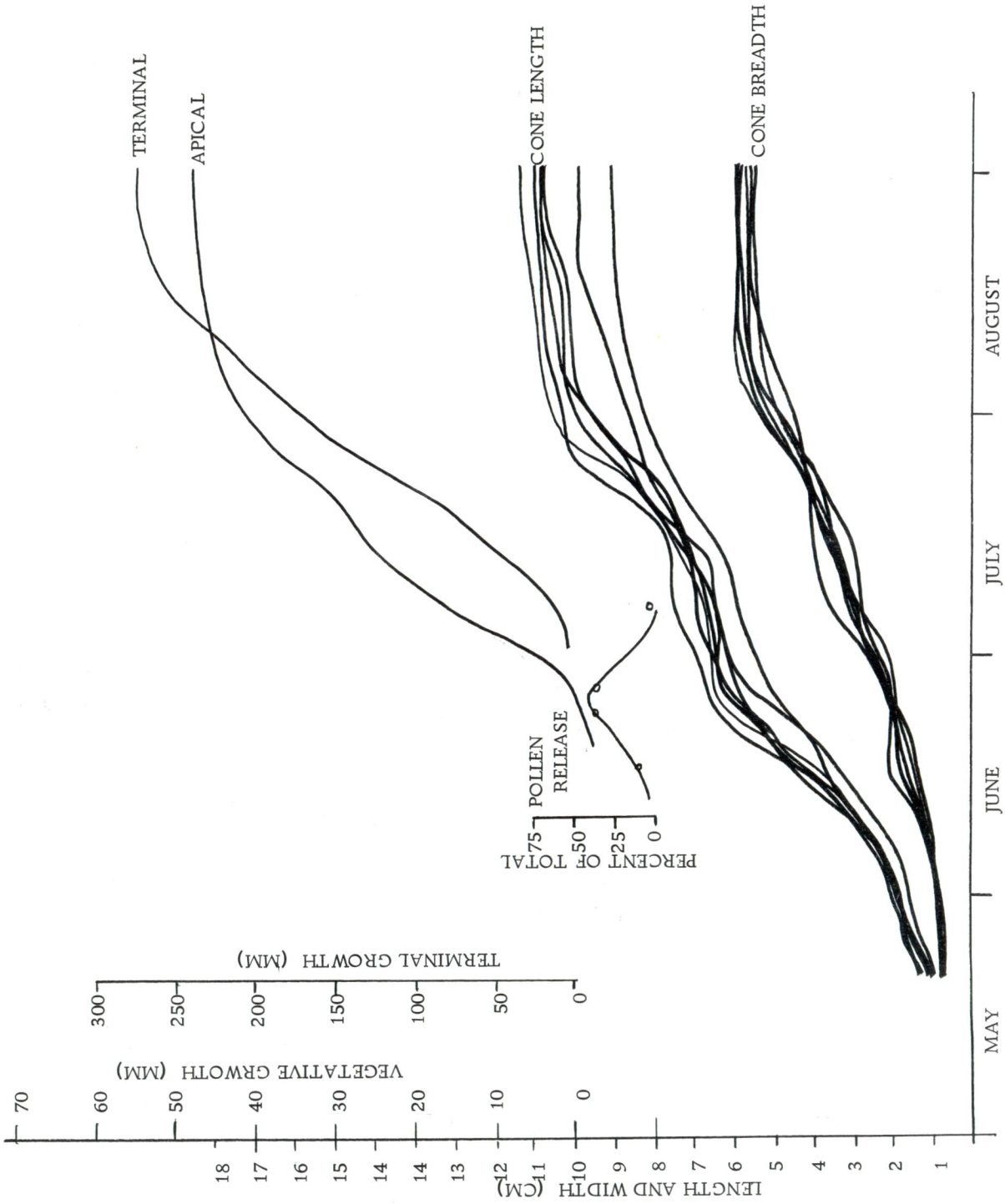


Figure 21: Seasonal growth of female cones and vegetative shoots and pollen release of silver fir No. 1.

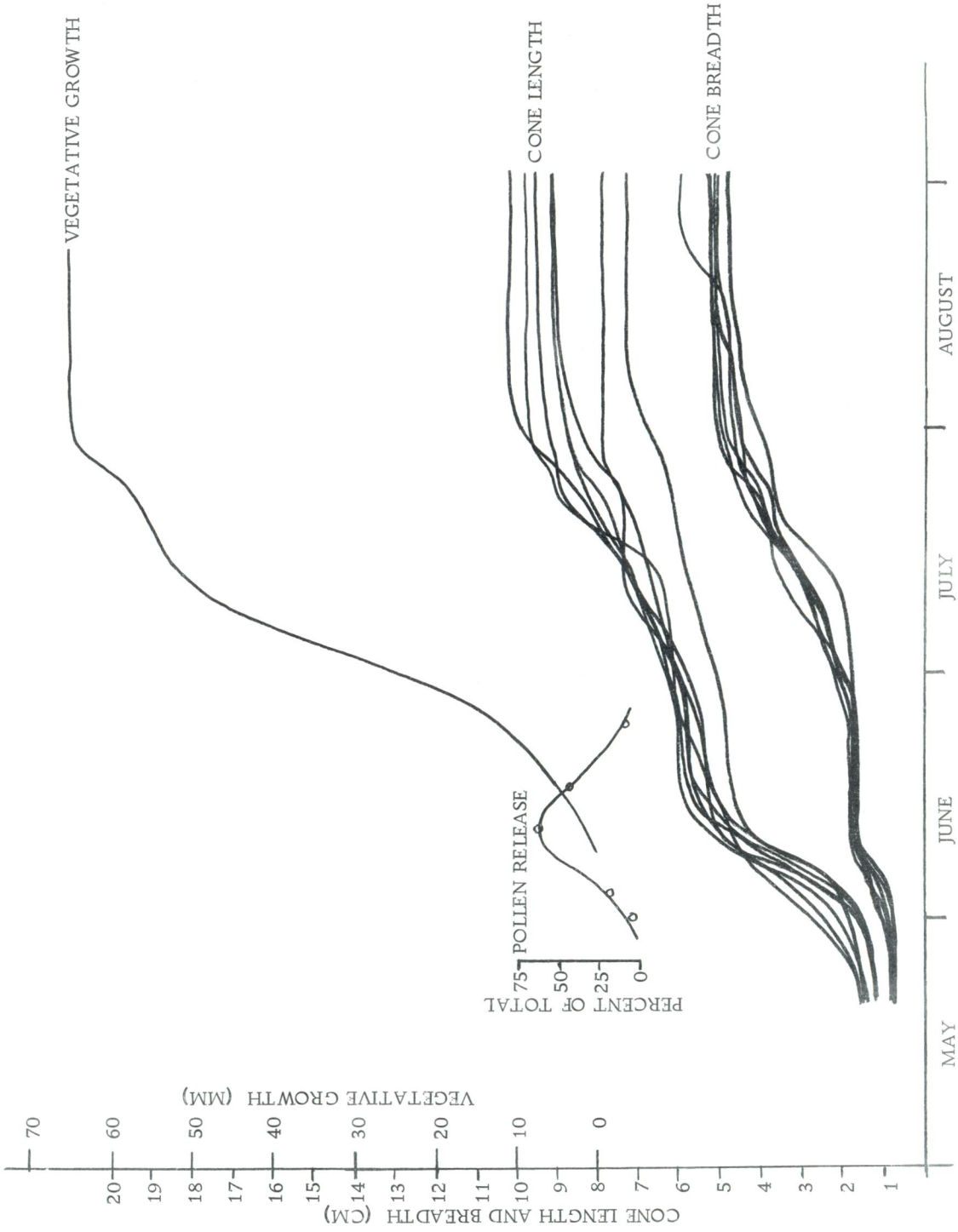


Figure 22: Seasonal growth of female cones and vegetative shoots and pollen release of silver fir No. 2.

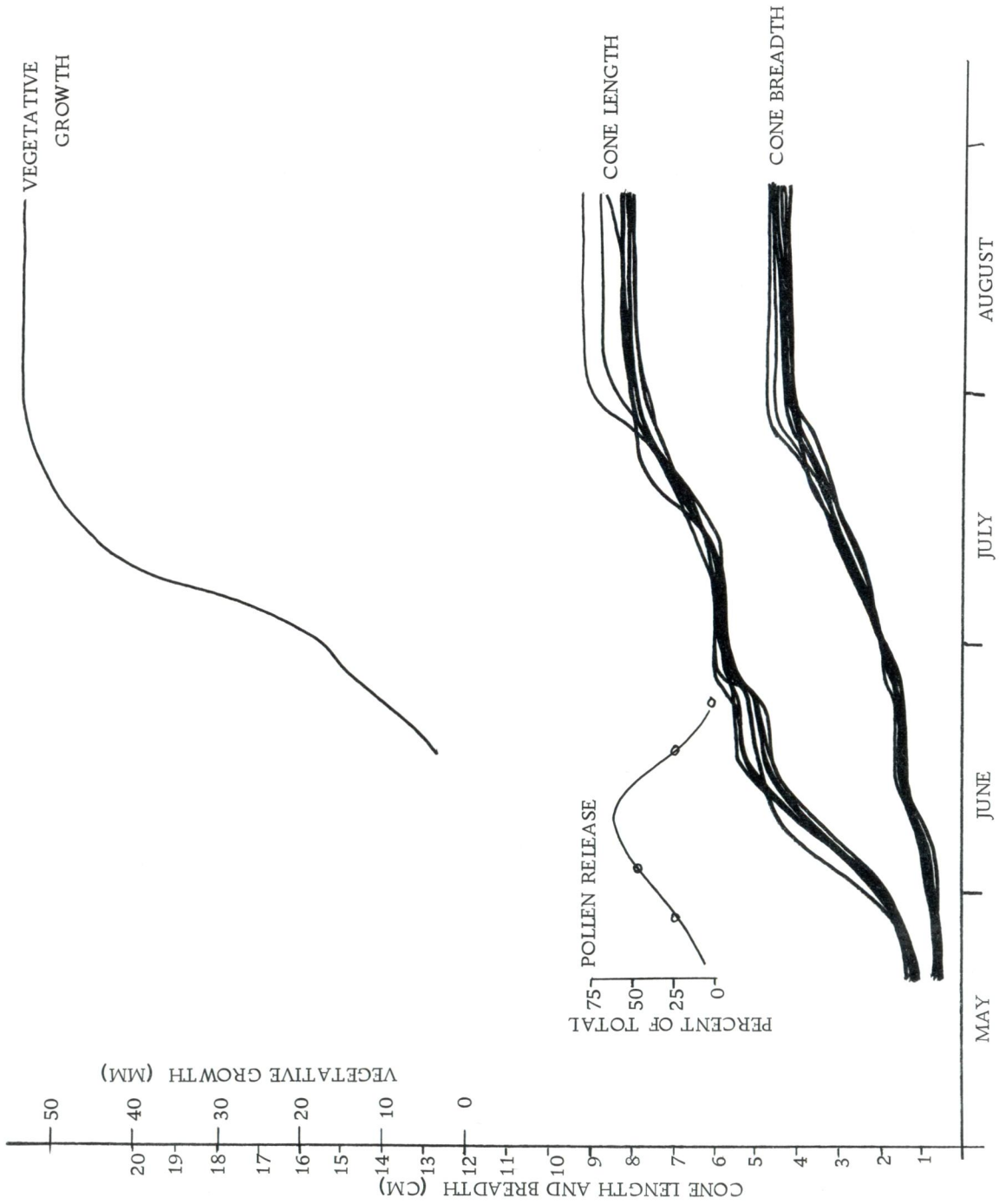


Figure 23: Seasonal growth of female cones and vegetative shoots and pollen release of silver fir No. 3.

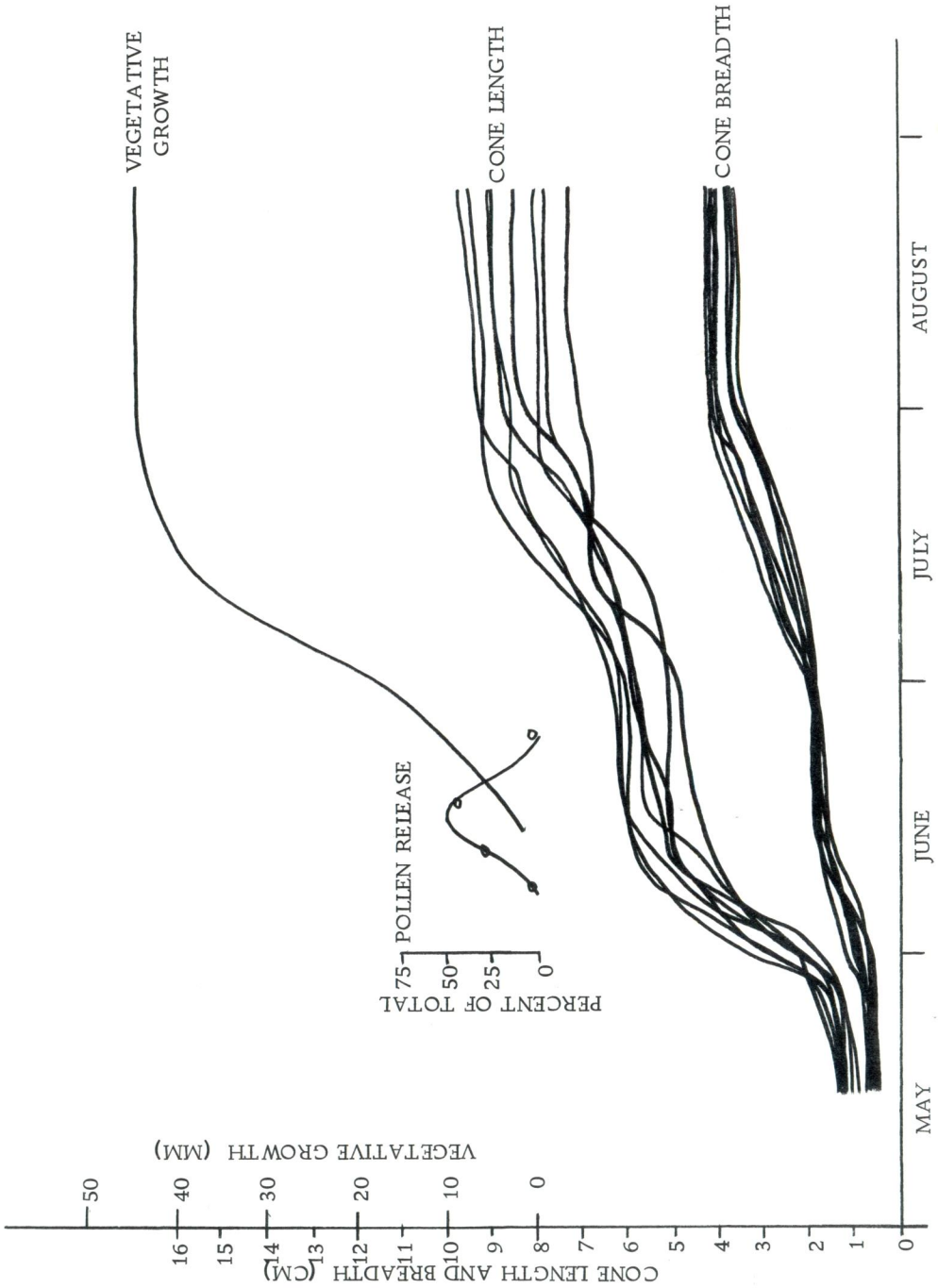
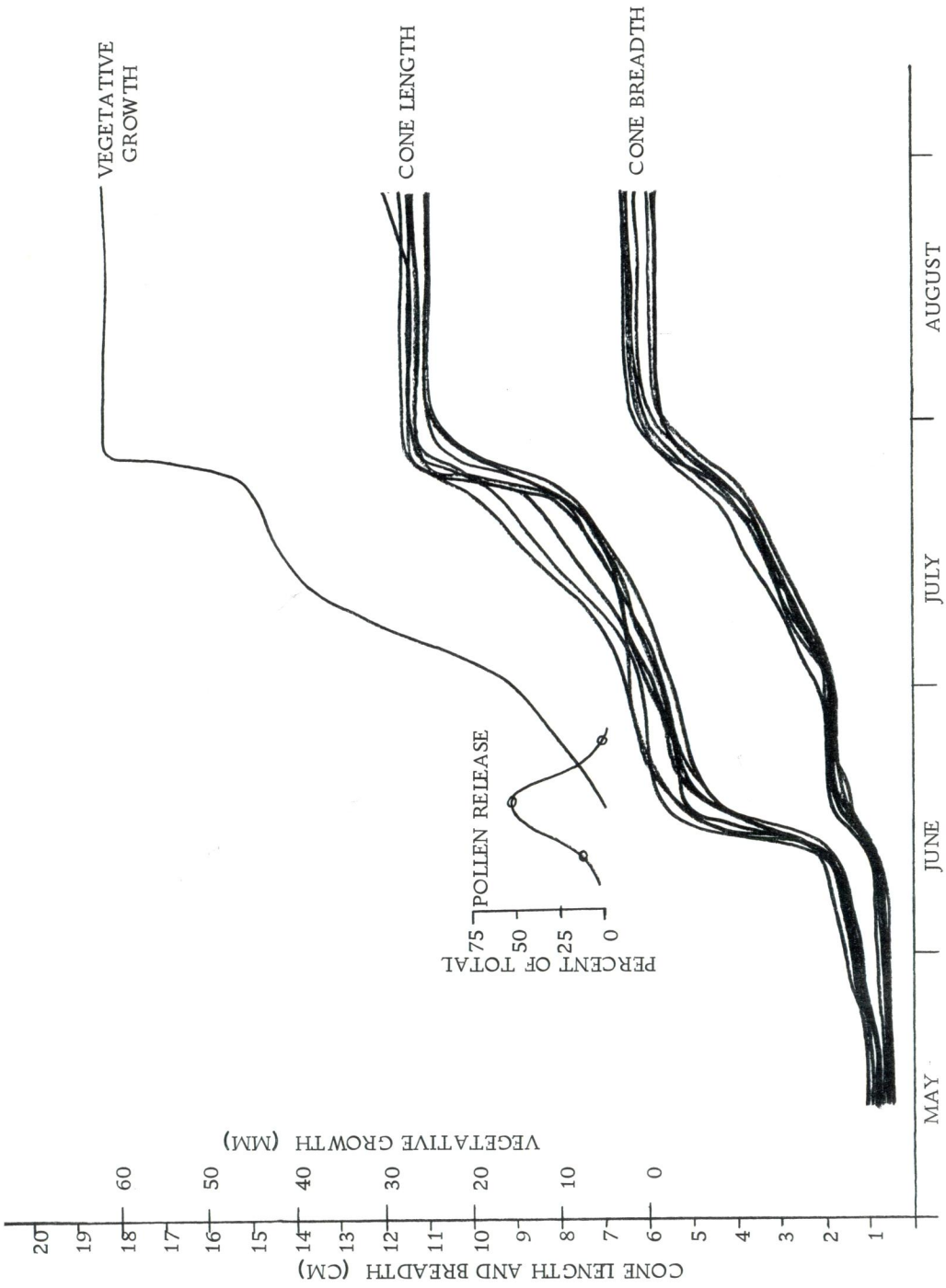


Figure 24: Seasonal growth of female cones and vegetative shoots and pollen release of silver fir No. 4.



on each tree were selected for measurement, however, during the season some of the selected buds failed to develop. In those cases measurements were not continued for these individuals and therefore every graph does not represent eight cones. Each graph, then, shows a growth curve for each successful cone on one tree. Breadth as well as width data were plotted. Also the period of pollen release of each tree was indicated; finally the vegetative growth curves for each terminal leader and a mean of 10 lateral shoots were included.

The curves indicate that each cone underwent a very definite growth sequence. This sequence has been logically subdivided into seven developmental stages by Franklin (1966) and these stages were described in the literature review. The growth curves obtained in the present study coincide with those of Franklin and his nomenclature will be adhered to in the following description.

Stage I is bud swelling (see Figure 25) during this stage the dormant buds (Figure 26A) resume internal development and a noticeable increase in their size can be measured. Bud burst, Stage II, begins when the first cone bracts can be seen projecting out from beneath their protective cover of bud scales (Figures 26B and 26C) and took place from about May 25 to June 5. The cones then enter a stage of growth called the First Elongation Stage (Stage III). During elongation the rate of growth exceeds that of any other time (Figures 25, 26D and 27A). At this period the noble fir cone is a light green with

Figure 25: Seasonal growth stages and rates for all female noble and silver fir cones.

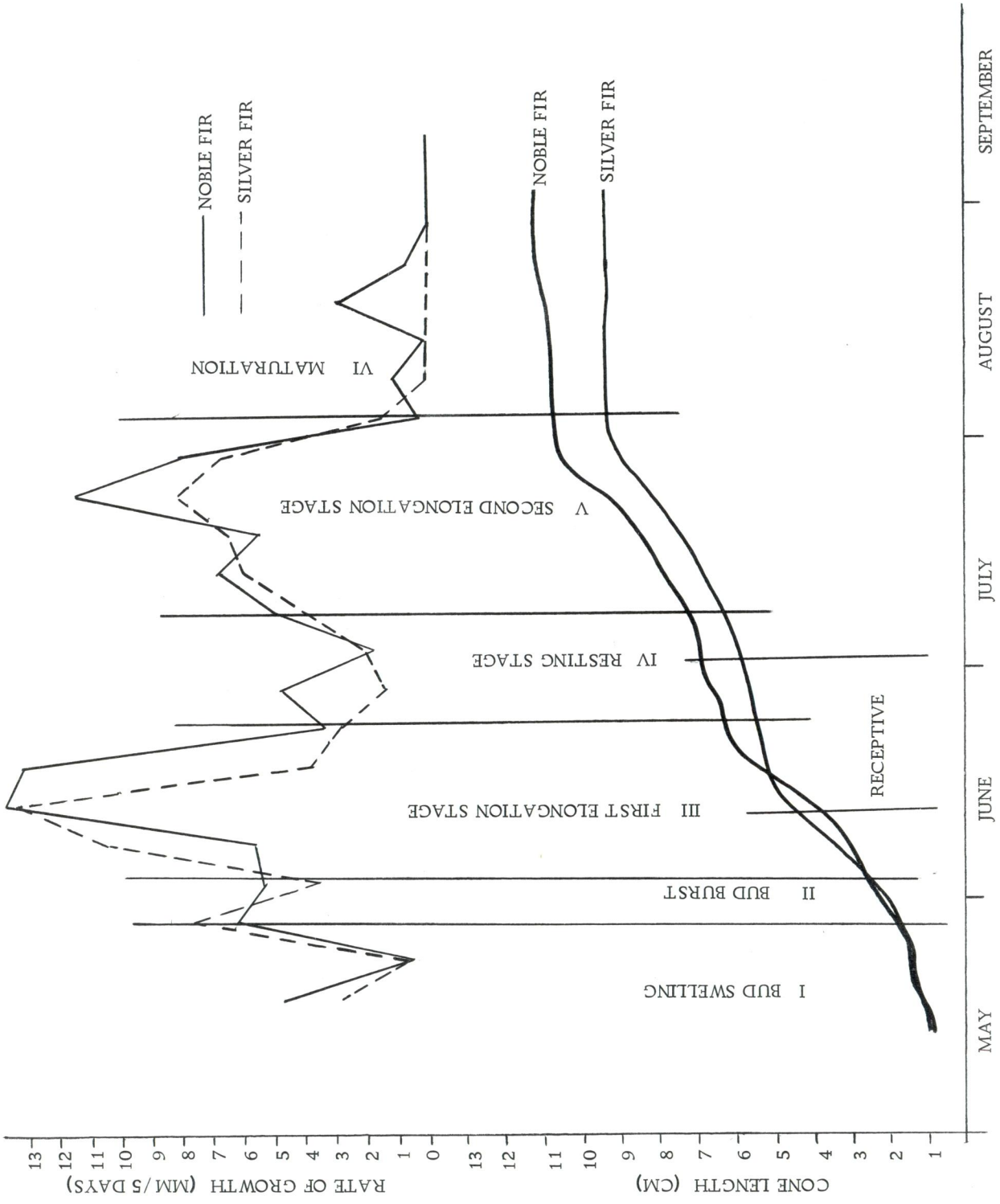


Figure 26: A, dormant winter female buds of silver (above) and noble fir collected on April 14, 1965; B, female buds of silver and noble fir just prior to period of bud burst, collected on May 20; C, female buds of silver and noble fir during period of bud burst, collected May 30, 1965; D, female buds of silver and noble fir just entering the first elongation stage, collected June 5, 1965.

reddish-brown bract tips, while the silver fir cones are a brilliant purple. This period of extreme rapid growth lasts for 10 to 15 days and culminates in what appears to be a period of receptivity to pollen (Stage IIIa). The limits of this period are determined by the opening and closing of the cone scales, presumably controlling pollen entrance (see Figure 28). During the latter part of the First Elongation Stage, the bracts become partially opened and pollination may also be possible at this time. Following the receptive stage, which lasts for about 15 days, a period of reduced rate of external growth occurs. This has been called Stage IV or the resting stage. When pollination has been completed the bracts on the silver fir cones bend upward and become tightly appressed to the sides of the cone. On the noble fir cones, the bracts bend downward and likewise become tightly appressed. This occurs during the first week of the resting stage while the cone scales are progressively enlarging and overgrowing the bracts (Figures 27B and 28). By about July 17 the scales have overgrown the silver fir bracts but have not yet completely covered the bracts on the noble fir cones (Figure 27C). From this time on the cones retain the same appearance and only size changes are undergone. Thus begins Stage V, the Second Elongation Stage, which last for another 15 days until the beginning of August (Figure 27D). During the second elongation stage the cones undergo another period of rapid elongation but not as rapid as the first. This growth phase comes to a distinct halt at the first of

Figure 27: A, silver (above) and noble fir female cones following the first elongation stage and during the receptive stage, collected on June 14, 1965; B, silver and noble fir female cones during the resting stage collected on June 23, 1965; C, female silver (left) and noble fir cones during the second elongation stage, collected on July 12, 1965; D, silver and noble fir female cones at the end of the second elongation stage, collected July 27, 1965.

Figure 28: Series depicting receptivity of female cone of Pacific silver fir; A, bracts beginning to open at beginning of first elongation stage; B, maximum receptivity, bracts extended outward approximately 90^{o}; C, receptive period nearing completion, bracts bending upward; D, receptive period over, bracts tightly appressed upward and resinous; E, F, scales growing upward between bracts marking the end of the resting stage; G, bracts almost totally concealed by scales beginning the second elongation stage; H, bracts are now totally hidden and cone has assumed mature appearance. Note: this sequence in noble fir is similar except at C bracts begin to turn downward and remain extended throughout the life of the cone.

August simultaneously with the halting of vegetative elongation. The cones have attained their final dimensions of about 95 cm in length for silver fir and 110 cm for noble fir (see Figure 29).

Figure 29: Female silver (left) and noble fir cones during the maturation stage, collected on September 1, 1965.

The remainder of the growing season is devoted to development of seeds within the cone and is referred to as Stage VI or maturation. Stage VII, cone shattering and seed dispersal mark the end of the life of the female cone. Here the two species, after having displayed remarkable synchrony throughout the early developmental stages, show a decided difference in phenology. Seed dispersal on the silver fir trees began on September 15 and continued until October 7, a full month earlier than noble fir which did not begin to break up until October 7

and were still in various stages of advanced disintegration on October 24 when the last observations were made.

The mode of disintegration also differed. Silver fir cone scales upon drying become greatly distorted and literally tear themselves off of the axis. On the other hand, noble fir cones merely dry, the scales dehising from the axis, but remaining undisturbed and in place until wind action or branch movement dislodges them and disperses the seed.

Pollen release by the male flowers was also plotted on these individual tree graphs and it can be seen that pollen release occurred consistently during the termination of the first elongation stage or the beginning of the receptive stage. Pollen release required periods of from 15 to 25 days depending on the individual tree, with the average period around 20 days.

Another consistency in these curves is the time of vegetative bud burst, and shoot growth termination. On all the study trees, vegetative bud burst occurred at the termination of the First Elongation Stage of the female cones, continuing through the Receptive, Resting and Second Elongation Stage and terminated at the first of August, coincident with the termination of cone elongation. This relationship was found to be consistent on all of the study trees.

The Phenological Calendar

In Figure 30 is depicted the sequence of growth of vegetative and repoductive structures of mature trees of both species and vegetative structures of 24 immature trees of both species. Because the vegetative buds undergo complete development in one growing season, one column on the chart is devoted to each of them. But the reproductive structures require two seasons for development and thus are assigned two columns on the chart. Keep in mind that this study was completed during just one growing season, thus the reproductive structures presented in their first year of development would have completed growth in the summer of 1966. The structures presented in their second year of growth underwent their first year in the summer of 1964, so they are from the reproductive cycles of two different growing seasons. This also means that at any one season, two different crops of reproductive organs are undergoing development, one in its first year, the other in its second. Another point to remember is that the dates of phenological events represent averages for each of the four trees of each species. Where a span of time was required for the completion of an event by a particular organ, this is indicated by the following symbol:

bud burst

Where the first of a series of similar events occurred it is represented as follows:

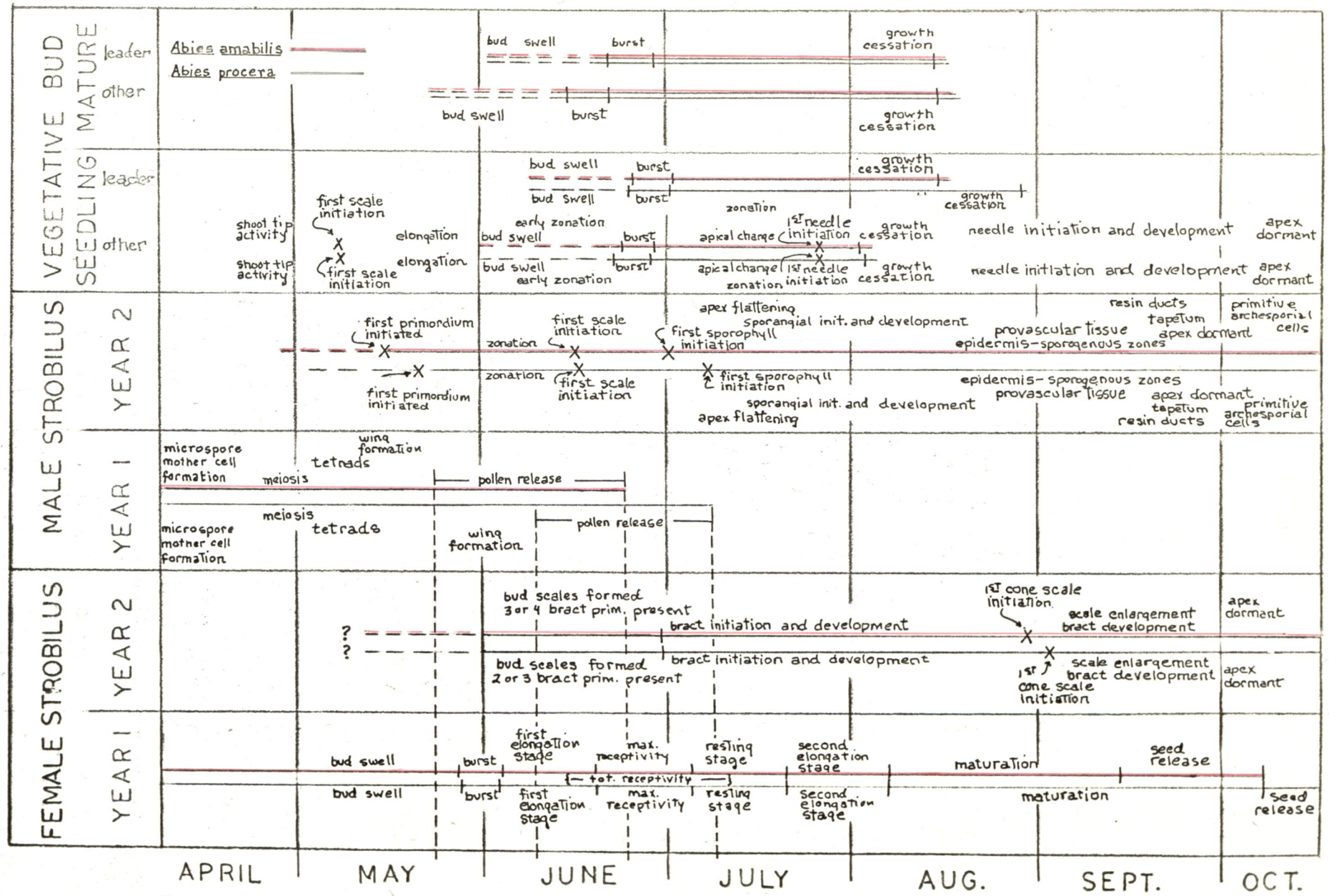


Figure 30: Phenological calendar for Abies amabilis and Abies procera --- 1965.

Perhaps the most striking trend is the synchrony between both species in a vast majority of events. Notable exceptions are the periods of pollen release which occurred approximately two weeks earlier in silver fir, and the time difference in seed dispersal noted earlier. Although the information on this calendar is self-explanatory and any comparison can quickly be made, a few interesting trends will be pointed out.

a. Bursting of vegetative buds occurred slightly earlier in seedlings than in mature trees.

b. In the seedlings, lateral buds burst about one week earlier than terminal leader buds, but elongation ceased simultaneously in both, the leaders growing for about 45 days and the laterals for 53 days.

c. Lateral vegetative buds on the mature trees grew for only 38 days, their growth ceasing one to two weeks earlier than the terminal leaders and the vegetative shoots of the seedlings.

d. The terminal leaders of noble fir continued to elongate for a period of about two weeks longer than the silver fir leaders.

e. On about May 1 internal activity begins in both the vegetative organs and the first year reproductive organs, but

sex cell activity in the second year male flowers has begun before mid-April.

f. The female cone buds burst on about June 1, followed directly by bursting of the second year male buds. Vegetative bud burst followed two weeks later during the period of pollen shedding.

g. The peak of pollen shedding occurred consistently at the end of the first elongation stage of the females and had a duration of 14 to 28 days on the individual trees.

h. All visible reproductive and vegetative growth ceased during the first days of August. At this time distinctive internal changes were also taking place in the vegetative and female buds. Apical shape changed and bud scale initiation ceased in the vegetative buds and the maturation stage began in the second year female cones. This date seemed to have no significance in the development of first year reproductive structures.

i. A close synchronization seemed to occur between the first year male and female structures, e.g., the dates of initiation were probably close, but scale initiation ceased the last week of June in both structures and bract and sporophyll initiation commenced together, cone scale initiation and sporogenous initial formation occurred

simultaneously; finally the apices became dormant around early October.

j. During the second year of growth of the female and male strobili this pattern persisted with meiosis perhaps occurring at the same time and pollen release occurring during the period of receptivity.

In general, the significant points seem to be the synchrony between growth of first and second year sexual parts, the synchrony between second year sexual parts and vegetative parts, and the common cessation of visible development at the first of August.

DISCUSSION OF RESULTS

Meristems and Zonation

Parke (1959) described four distinguishable zones within the apices of shoots of Abies concolor; a zone of apical initials, a central mother cell zone, a peripheral zone, and a zone of central tissue or rib meristem. These zones conformed to the so-called Ginkgoid pattern described by Foster (1938). Furthermore, his observations have demonstrated that this zonation pattern undergoes a series of changes during the growing season. He has recognized three distinct growth phases throughout the year and reports that changes in the zonation patterns in the shoot apices are related to these growth phases.

It is not surprising to find that this mode of apical growth is repeated in both Abies amabilis and A. procera. The deviations from Parke's findings recognized in the present study are deviations only in timing of these growth phases, as the phases themselves and the patterns of zonation resemble closely the findings made on A. concolor. At 4,000 feet elevation in the Sierras of California, Parke found that the Rest Phase occurred from September to early April, Growth Phase I began in early April and continued until mid-June. Growth Phase II lasted from mid-June to September. The Rest Phase for the noble and silver firs studied in the Western Washington Cascades occurred from

early October to early May. Expansion of the rib meristem signalling the beginning of Growth Phase I began around May 1. This was followed by initiation of scale primordia and further shoot elongation and a decided steepening of the apex. Growth Phase II did not begin until mid-July, a full month later than Parke's trees. But the changes in the apex, the cessation of elongation and cataphyll initiation, and the beginning of needle initiation occurred simultaneously as Parke reported. The Rest Phase also began a full month later. Latitudinal differences between the two study areas are probably sufficient explanation for the month lag in events. In all other respects, this study has borne out Parke's findings, re-emphasizing the importance of seasonal awareness with meristem investigations.

This study also supports the findings of several previous workers who stress the similarity between vegetative and reproductive apices during the early stages of growth (Clowes, 1961; Gifford and Wetmore, 1957). Owens and Smith (1964) noticed that in Douglas-fir, megasporangiate and vegetative lateral bud apices were distinguishable in the early stages only by their relative location and in other respects were very similar, and that the time and mode of initiation of these and the microsporangiate apex were also parallel. The microsporangiate apex was distinguishable from an early stage due to its smaller size. The observations made on A. amabilis and A. procera unfortunately did not illucidate the time nor mode of initiation of the megasporangiate

bud, however, in later developmental stages all three types of apices were compared, and certainly analogies can be drawn. Zonation patterns are apparent and dynamic in all three structures; initiation of cataphyll, needle, bract, and sporophyll primordia occurred in similar fashion and these primordia at the time of initiation are nearly identical.

Thus we are reminded of the concept of totipotency or the ability of young cells in a meristem to react to various biochemical stimuli to differentiate into or produce any one of several hundred types of specialized cells. And the question again arises; what are these biochemical stimuli, how do they act, and will it some day be possible to produce a given type of structure from an apical meristem by making the appropriate hormonal applications?

A phenomenon brought out by this study is the homology between the vegetative and reproductive organs in both structure and genesis. Apices of each structure initiate cataphylls in the same manner, at the same time, and undergo similar changes in their own shape and zonation during the preceeding initiation. In the overwintering state and with only casual examination, one might easily mistake the female for the vegetative bud when examined under low magnification (Figures 8A and 15D). Needle and bract primordia are very similar in size and shape and have undergone similar degrees of vascular formation and differentiation. Apices are practically identical in shape and zonation. A well developed crown is present beneath both structures and bud

scales originate in like fashion.

Their similarity is most striking at initiation and from that point progressively decreases with age; but while these structures are within the first year of their growth, they remain essentially very much alike.

During the second year of development, however, profound differences begin to appear. Where in needle primordia, functional vascular and mesophyllar cells begin to differentiate, haploid, sporogenous tissues emerge within the bract and sporangial primordia. While the vegetative apex again becomes active atop the elongating shoot, a dead apex is raised above the expanding cone axis. As the new vegetative shoot begins producing new bud scales and needles in preparation for the next season, the sexual organs are concerned only with the production of sex cells and reproductive tissue for the present season and make no provision whatsoever for next year, except the seeds themselves. Thus three organs which begin life virtually identical and whose overwintering forms retain much of this identity, become very different in structure and function by the end of the second season.

The Crown

We have found that that mysterious structure, the crown, not only subtends the vegetative buds as has been reported in the literature but is also associated with the reproductive buds. In both structures

it delimits the juvenile from mature tissue in the sense that no secondary wall thickenings become visible above the crown until the second year of development. At the same time, secondary vascularization has proceeded right up to the base of the crown in the two year old shoot. Although no secondary wall development in the vascular traces occurs above the crown, the traces themselves are certainly evident throughout the bud. They proceed from the lower shoot upward and around the crown and then slant back toward the center of the axis (Figure 10B).

There has been no previous literature dealing with the development of the crown. However, in this study its development in vegetative and reporductive buds can be observed. The first noticeable evidence of the genesis of this zone is seen in Figure 8C. Just below the base of the first scale primordium across the pith meristem perpendicular to the axis is a poorly defined yet distinguishable zone where cell divisions appear to be more frequent than in surrounding tissue. Subsequently (in Figures 8D and 9A) this region, although not more prominent, is still visible. As scale initiation begins to taper off and the apex assumes a more pointed shape preceeding needle initiation, this subapical region becomes increasingly distinct (Figure 9B). Below the crown, divisions have apparently ceased to occur, while in and above it mitosis proceeds. During the early stages of needle formation the crown becomes readily distinguishable in this zone (Figures 9C and 10A). Throughout the remainder of the growing season, the crown

is at an advanced state of development; beneath it is the shoot formed by the old terminal bud. In summary, the old crown persists beneath the completely formed terminal bud during the Rest Phase. During Growth Phase I, the period of cataphyll initiation and elongation of the telescoped shoot, the crown zone is beginning to form but is no more than a zone of cells which appear slightly different than the surrounding cells. At the end of Growth Phase I, however, the crown suddenly matures and has reached an advanced state of development by the beginning of Growth Phase II. At this point, elongation of the telescoped shoot stops, cataphyll initiation stops and needle initiation begins. During the remainder of the growing season, the crown remains fully matured.

Several investigators have inquired into the function of this tissue but experimental evidence has yet to substantiate any theories. Lewis and Dowding (1924) domonstrated that the crown is impermeable to upward movement of certain water soluable dyes. Romburger (1963) states that it is not without reason to expect a relationship between the crown and the control of domancy.

The evidence presented in this study certainly indicates that a chronological relationship exists between formation of the crown and cessation of meristematic activity in the old shoot. A re-examination of the photomicrographs presented by Parke (1959) reveals the same developmental sequence of the crown in A. concolor, but surprisingly

Parke has made no critical observation of the structure despite its close coincidence with his growth phases. Whether the formation of the crown is a cause or an effect of the tapering off of shoot elongation cannot be proved. However, the author feels that it is not unreasonable to suppose that this structure may act as a physical barrier for auxins produced in the apex and moving downward inside the vascular cylinder, and that it may be partially respnsible for the termination of meristematic activity within the telescoping shoot. Thus its formation coincides with cessation of shoot elongation.

Pollen Release and Female Receptivity

Figure 30 presents a rather misleading picture of pollen release and receptivity characteristics of each species. However, it does point out that flowers of both sexes of noble fir tended to have better synchronization than those of silver fir. When this function is examined on an individual tree-by-tree basis, a better picture is obtained of this relationship than is shown in Figure 30 where the data have been averaged until they have lost their meaning. Pollen release for each noble fir tree lasted for approximately four weeks and occurred during the period of total receptivity of the females, with the peak of pollen release occurring coincidently with the stage of maximum receptivity. See Figures 17 to 20. With silver fir (Figures 21 to 24) this was not always the case. Trees 1 and 2 each shed pollen for a

period of more than four weeks, but the males of tree 2 were in poor synchrony with the females. Trees 3 and 4 only shed pollen for two weeks but were in perfect coincidence with maximum receptivity.

These trends may be viewed in two ways. The evidence suggests that noble fir tends to be more inbred than silver fir, but both species are certainly selfers as is reflected by their ability to survice in the rigorous, narrowly defined environment of the high altitude. However, the weather must be considered when dealing with pollen release as wet weather during pollen shed can limit both the amount of pollen shed and the period of shedding. Franklin (personal communication) observed this very phenomenon in an investigation of pollen release in noble fir in 1961 as compared to 1965. A cold wet summer in 1961 restricted pollen release to a few days, while in the warm dry 1965 summer it lasted for about four weeks. Generally we may conclude that these species tend to self but that weather plays a vital role in the success and timing of pollination.

Cone Crop Prediction

It would seem that information concerning the numbers of male and female buds present on the tree during the dormant season would have value as an indicator of the following year's cone abudnance. In fact, the sexual buds of noble and Pacific silver fir are visible from the ground by August preceeding the dormant season. Allen (1941)

believes that counts of these structures on Douglas-fir, hemlock, and grand fir can provide a method of forecasting seed crops. Similarly, Lester (1963) assumed that the number of flower primordia initiated on red pine directly reflects conelet production.

Despite these reports, observations made on noble and silver fir in this study indicate that there may be a confounding factor involved which could reduce the value of this method. This factor is the degree of sexual bud abortion. It is quite possible to observe cone production history for several years in the past on the branches of the true firs, as the female and male flowers leave distinct scars on the shoots which bore them. By observing these scars it soon becomes apparent that many potentially good cone crops failed to develop; the primordia were there but aborted. Thus it seems that the number of primordia initiated per year remained reasonably constant and that the determinator of cone crop success was abortion rate and not initiation rate. Further investigation into this phenomenon could prove fruitful in the understanding of cone crop periodicity in the true firs.

Elevation

The study area was located at an elevation of 4, 800 feet. Because the environmental factors which determine the pattern of phenological events vary with elevation, we must expect an elevational gradient in the timing of the events which we have observed. In order

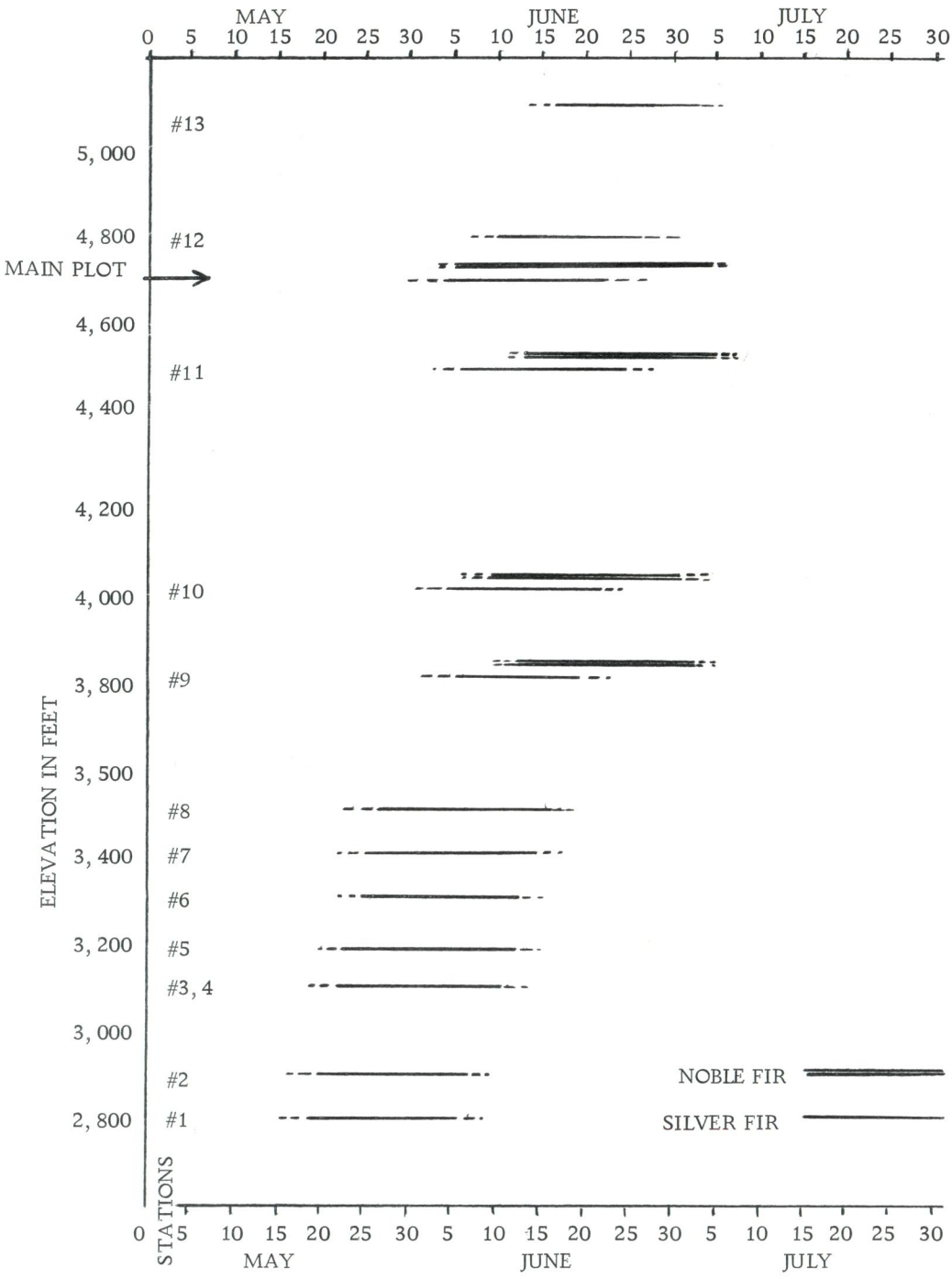
to observe and quantify this gradient, 13 plots were chosen at progressively higher elevations along the Paradise Road and weekly visits were made to these plots. Ground observations with binoculars were made of pollen and seed release. Figure 31 illustrates the delay in time of pollen release of both species with increase in elevation. A delay of about 25 days is evident from 2, 800 to 5, 000 feet in elevation. Noble fir released pollen about one week later at all elevations and the period of pollen shed tended to lengthen by about one week from lowest to highest elevation. There was no elevational gradient in times of seedfall.

Ecological Implications

The importance of a species' phenological patterns to its ecological success or failure can be profound. When the species in question is existing in an environment which imposes narrow limits in terms of seasonal changes and climatic factors, phenology determines which competing individual or species will open its buds late enough to escape late killing frosts or drop seed at the proper time to increase its chances of germination and survival.

Interestingly enough, noble and Pacific silver fir growing on the study area showed little variation in phenological characteristics. Bursting of the sexual and vegetative buds occurred simultaneously, pollen shedding and female cone development followed nearly identical

Figure 31: The elevational delay of pollen release in mature noble and silver fir from 2, 200' to 5, 000' elevation.



patterns and most internal phenomena showed remarkable synchrony. However, the aspect of phenology under investigation that varied to any significant degree was a very vital aspect indeed, that of seed release.

In the Canadian Zone forests inhabited by these two species snow comes early, often as early as late September. In a report by Stein (1951) it was demonstrated that the seeds of true firs, when deposited in the snow-pack, may germinate in the snow the next spring and die. The seeds of their associates, however, do not germinate until they are placed on the forest floor by the melting snow. It may therefore be an ecological advantage for a true fir to shed its seeds as soon as possible, especially in light of their very infrequent and undependable crop years.

During this growing season, silver fir began shedding its seeds a full month earlier than noble fir and shedding was nearing completion when noble fir cones began to disintegrate. Thus, had this been an early snow year the noble fir seed crop could have been partially destroyed. It is logical to assume that this trend may be evident every year due to the mode of disintegration of the female cones of each species as described in the RESULTS section. Silver fir cones undergo active disintegration, shedding their crop as soon as it matures. Noble fir, although the seed may be ripe, must have sufficient wind agitation to break the cones up. Should the first snow

storm preceed the first windstorm, the cones would remain unshattered and the seed crop would perish.

Practical Applications

Basic to the management of a tree species is a knowledge of its life cycle. An important part of the life cycle is the phenological pattern, and up until the present time no systematic study has been made of the phenology of these two species. Logging demands are increasing and as timberland becomes scarcer the high altitude forests are being extensively exploited. Successful regeneration will necessitate careful research. A good starting point for this research is a study of the phenology of the major species.

The data presented in this report will probably have most immediate value to the forest geneticist, who will be planning seed production areas, making artificial pollinations and flower induction trials. He will want to be able to predict good seed years and be able to assess the effects of environment on flower and cone production. Entomologists and pathologists must know the phenological patterns of host trees if they are to study life cycles of insect and disease pests. Forest managers must know phenology in order to plan their intermediate cuttings if they are aimed at stimulating seed production and harvest cuttings if they are to depend on natural regeneration. Silviculturalists require phenological information to perform fertilization experiments. In short,

prerequisite to the understanding of a species is a knowledge of its phenology.

CONCLUSIONS

Although this study was localized in time and place and interpretations must be made with this limitation in mind, certain trends and details of possible importance were observed. In conclusion, a list of these findings is presented:

1. Vegetative bud burst occurred at 4, 800 feet in the Western Washington Cascades during the week of June 15. Buds of mature and immature trees of each species burst within one week of each other, but grew for different lengths of time. In all cases, terminal leader buds were last to burst. Most shoot elongation had ceased by the end of the first week in August.
2. Internal meiotic activity began in the second-year reproductive buds during early April. In the first-year sexual organs and reproductive buds, internal development did not begin until May 1.
3. Female buds burst about June 1 and were followed immediately by bursting of the male buds. Pollen shedding and receptivity occurred about two weeks after sexual bud burst. Vegetative buds broke during the period of pollen release.
4. During the first week of August, second-year female cones stopped elongation and began the maturation stage. At the same time, vegetative shoot elongation ceased as cell division and elongation

in the rib meristem was terminated. From this date on all further development of sexual and vegetative buds was internal.

5. During their second year of development, the female cones pass through a sequence of seven growth stages. All female cones of each species followed this sequence very closely. The external phases are related to internal phenomena such as fertilization and seed maturation.

6. By mid-October, all further growth had stopped and the vegetative and reproductive buds became dormant.

7. Throughout the growing season, a very distinct syncronization existed between growing parts. This was apparent within organs on one tree, trees of a species and trees of both species. Very little variation in phenology was evident.

8. Zonation and periodicity of the apical meristems of both species were similar to that described by Korody (1937) and Parke (1959) for Abies concolor. There were distinct similarities between apical meristems of vegetative and sexual buds but they became less apparent as these structures matured.

9. Both species appear to be self-pollinating but climatic conditions influence the dates and duration of pollen release and receptivity.

10. High abortion rates were observed in both male and female buds and this may have an important bearing upon seed crop success and periodicity.

11. A delay in pollen release of 25 days was observed with an increase of 2,200 feet in elevation. Other phenological events probably display similar trends.

12. A different seed dispersal mechanism exists in each species. Noble fir cones undergo passive disintegration, depending on the wind to release the seed. Silver fir cones actively disintegrate and release their seed as soon as they have matured. This may have ecological significance in years of early snowfall, as deposition of seed on the surface of the snow can cause its premature germination and death.

13. Rapid development of the crown at the end of Growth Phase I suggests that it may be a mechanism related to the cessation of shoot elongation.

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APPENDICES

APPENDIX I

Descriptions of the Individual Study Trees

Species	No.	Age (years)	d. b. h. (inches)	Height (feet)	Remarks
A. procera	1	70	16.9	74.5	used for bud collection
	2	66	14.0	75.0	
	3	61	16.7	70.0	
	4	73	21.0	86.5	
A. amabilis	1	60	14.4	74.5	used for bud collection
	2	58	12.3	58.0	
	3	59	14.6	67.0	
	4	60	16.5	70.0	

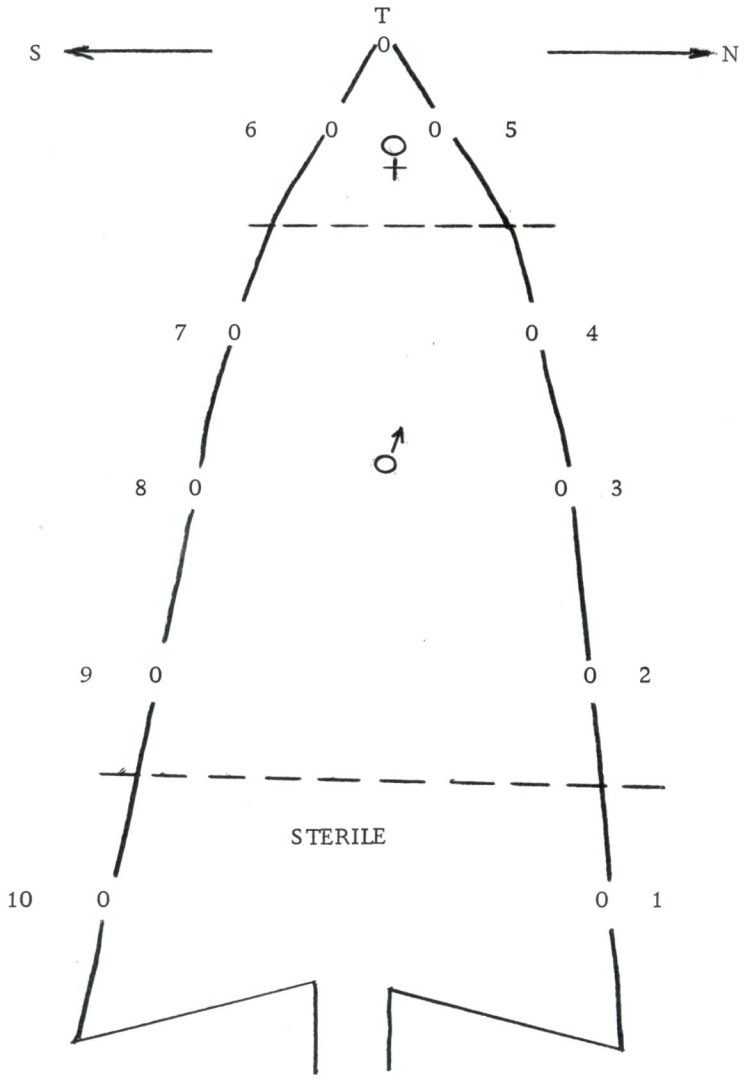
1. Ages were estimated by taking increment borings at breast height and arbitrarily adding 10 to each.

2. Diameters were measured with a diameter tape.

3. Heights were obtained with an abney level.

APPENDIX II

Crown subdivision, location of reproductive organs, marking and sampling procedures.



1. 0 = tagged vegetative buds (measured weekly)
2. ♀ = zone of female buds (collected weekly)
3. ♂ = zone of male buds (collected weekly)
4. eight female buds tagged and measured weekly
5. bud collections made on one mature tree of each species
6. measurements made on four mature trees of each species and 24 immature trees

APPENDIX III

Staining Schedule and Preparation of Stains

Safranin and Fast Green Counterstaining Schedule

1. Xylene (1) 1/2 hour
2. Xylene (2) 15 minutes
3. Xylene/Abs. Alc. 15 minutes
4. Abs. Alc. (1) 15 minutes
5. Abs. Alc. (2) 15 minutes
6. 95% Ethyl Alc. 15 minutes
7. 70% Ethyl Alc. 15 minutes
8. 50% Ethyl Alc. 15 minutes
9. Safranin 1% 2 hours (angiosperm material will require up to 48 hours)
10. Water Rinses until last rinse is clear
11. 2% Aqueous Tannic Acid 2 minutes (optional)
12. 30% Ethyl Alc. rapid change
13. 50% Ethyl Alc. rapid change
14. 70% Ethyl Alc. rapid change
15. 95% Ethyl Alc. + 1 drop HCl until safranin stained tissues are stained at desired intensity
16. 95% Ethyl Alc. dip
17. Fast Green dip
18. Clove Oil/Xylene/Abs. Alc. (1) 15 minutes
19. Clove Oil/Xylene/Abs. Alc. (2) 15 minutes
20. Xylene/Abs. Alc. dip
21. Xylene (1) 15 minutes
22. Xylene (2) 15 minutes
23. Permount

Preparation of Safranin O

1. Dissolve:
   4 gm. saffranin dye in
   200 cc. methyl cellosolve (2-methoxyethanol)
2. When solution is complete add:
   100 cc. 95% ethyl alcohol
   100 cc. distilled water

4 gm. sodium acetate
8 cc. formalin

3. Filter

Preparation of Fast Green

Mix:

1/2 gm. fast green dye
25 cc. absolute alcohol
25 cc. xylene
50 cc. clove oil