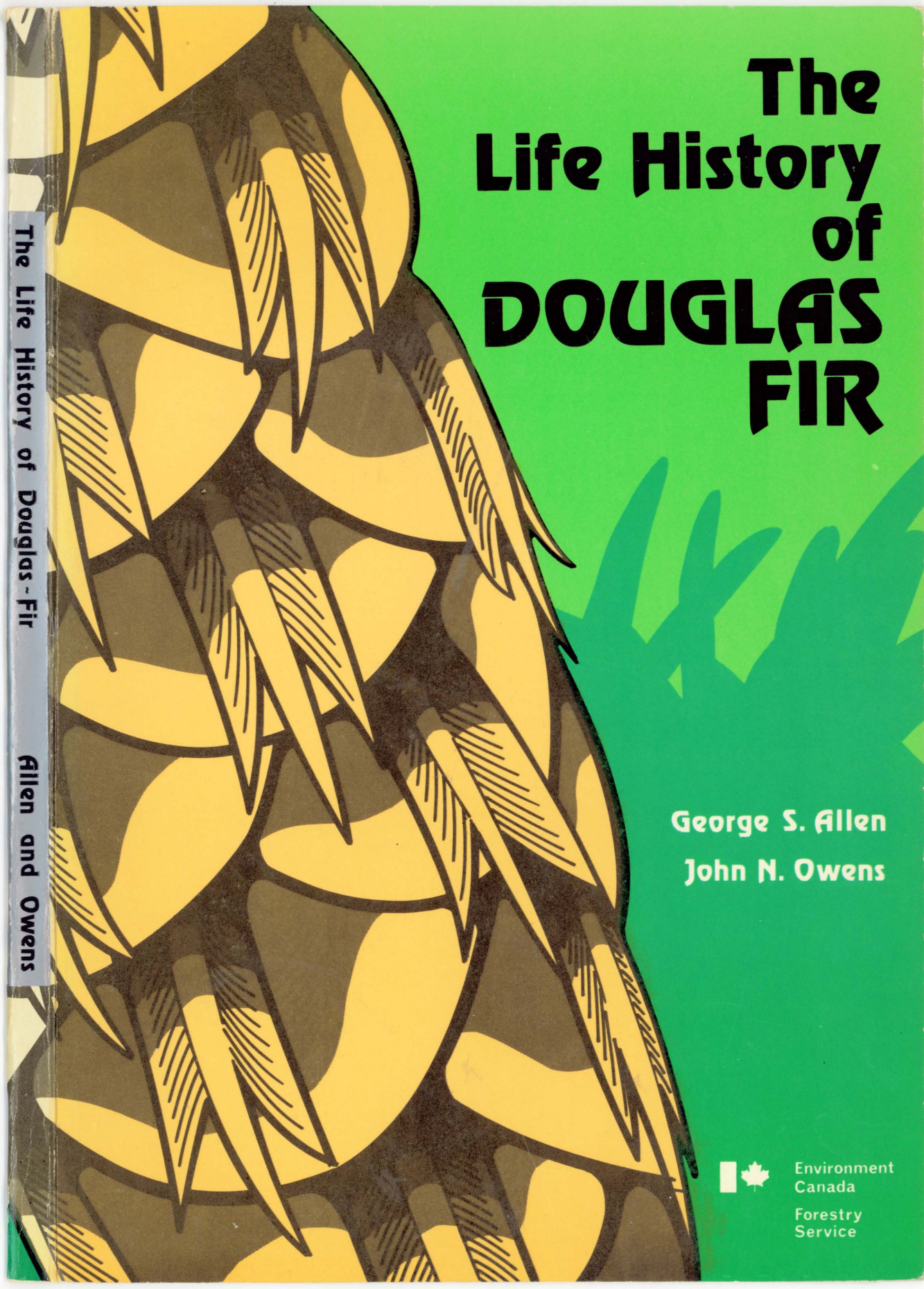

The Life History of DOUGLAS FIR

George S. Allen
John N. Owens

Environment Canada
Forestry Service

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Preface

This publication is intended as a tribute to the late George S. Allen who, from the earliest days of his professional career, demonstrated an active interest in portraying the life history of Douglas-fir. Dr. Allen was insistent all through his career that the information resulting from his work on this topic be of the highest order, so that it would consistently serve the best interests of scientists, teachers and students alike, in the years to come. The intensity of purpose that was exemplified in his work is hopefully recorded for all time in the present publication.

In studying the fruits of his work, the reader will be reminded of Dr. Allen's characteristic approach, both scientific and practical, to resolving biological questions. It would be well also for the reader to recall the major elements of Dr. Allen's career, as these events helped shape the pattern and purpose of his work. For example, his scientific abilities came to the fore in the course of doctoral studies at the University of California at Berkeley, and in the Ph.D. thesis he presented to that university in 1945. Dr. Allen had earlier made a lasting good impact upon forestry as a silviculturist while with the British Columbia Forest Service, in the period 1938-1945. He also left the mark of excellence upon the field of forestry education, having served first as forestry instructor with the now Faculty of Forestry of the University of British Columbia, from 1933-1937, later as Associate Professor and Professor of Silviculture at the same institution, during 1945-1953, and finally as Dean of the Faculty for the period 1953-1961. Dr. Allen re-entered forestry research on a full-time basis in 1961, on assuming the position of Director of Forestry Research at the Weyerhaeuser Company Research Centre at Centralia, Washington. In 1966, he returned to British Columbia and joined the present Pacific Forest Research Centre of the Canadian Forestry Service to pursue his longstanding interest in the life history of Douglas-fir. He was active in this work up to the time of his death in 1968, at age 56.

Among the records left by Dr. Allen was a set of photographic slides which he had entitled "the life history of Douglas-fir", many of which dated back to his graduate study days. While some of the slides had been used to illustrate his various publications, and others had been given to various educational institutions, the majority have until now been relatively

inaccessible. Through this publication, therefore, we desire to make Dr. Allen's slide material available to everyone, and in the process to incorporate with it references to other information on the life history of Douglas-fir that appears in the literature generally. The publication, then, is a much needed summary of the entire life cycle, from initiation of the seed and pollen cones to shedding of the mature seed.

It was with some humility, but with confidence as well, that Dr. John N. Owens, Associate Professor, Biology Department, University of Victoria, Victoria, British Columbia, agreed to undertake the task of selecting illustrative material from Dr. Allen's unpublished work and from the literature, and of writing captions and a descriptive text. Dr. Owens had already published extensively, and had maintained an active research program in a similar field to that of Dr. Allen. He was therefore in a position to contribute some of his own material and concepts to the publication. But because of the late Dr. G. S. Allen's considerable contribution to this publication, by providing its foundation, we felt that he should be the senior author; Dr. Owens was in full agreement. From this introduction, the reader will realize that Dr. Allen did not have the opportunity to add his seal of approval, but we trust that the publication will be of lasting credit to his memory.

G. P. THOMAS, *DIRECTOR*
Pacific Forest Research Centre,
Canadian Forestry Service,
Victoria, B. C.

November, 1971

Acknowledgments

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Sources of the illustrative materials used are acknowledged in the detailed listing of photographic credits. Many of the illustrations of Dr. Allen's work are reproduced, with the permission of the journals concerned, from his published work. A few were produced while Dr. Allen was a member of the Canadian Forestry Service staff, and others were selected from Dr. Allen's own photographic or original microscopic slides which were loaned by his widow, Mrs. Dorothy Allen.

Gratitude is extended to Mr. John Wiens who prepared the illustrations, and Mr. E. Chatelle and Mr. Alec Craigmyle for their help in preparing the photographic plates. I am also grateful to Miss Marje Molder, of the Biology Department, University of Victoria, for her technical assistance throughout the project, especially in preparing the photographic plates, and to Dr. Richard H. Falk, of the University of California, Davis, for his help in obtaining the scanning electronmicrographs.

Appreciation is extended to the following individuals for their review of the first draft of the manuscript and many helpful suggestions: Dr. Frank H. Smith, of the Botany and Plant Pathology Department, Oregon State University; Dr. Alan Orr-Ewing, of the British Columbia Forest Service; Dr. Roy R. Silen, of the U.S. Department of Agriculture, Forest Service; Dr. Ernest M. Gifford, of the Botany Department, University of California, Davis; Dr. Richard P. Pharis, of the Biology Department, University of Calgary; and Mr. F. B. Armitage, Dr. Holger Brix and Dr. George Puritch of the Canadian Forestry Service.

John N. OWENS

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Chapter 1

Introduction

The conifers, a group of plants with a well-documented evolutionary history, originated over 300 million years ago, before the flowering plants. Today, they are of tremendous economic importance for lumber and wood pulp and are represented by such familiar trees as pine, fir, spruce, cedar, redwood and yew. Conifers are members of the gymnosperms — meaning naked seeds, which denotes that the seeds are usually borne on the surface of scales rather than enclosed in fruits as in the flowering plants. Modern conifers are represented by 53 genera and about 500 species (Bierhorst, 1971). They have worldwide distribution and in temperate regions are the dominant trees, forming vast forests in Western North America and Asia. A few are strictly tropical in distribution.

Several attempts have been made to organize the conifers into a classification acceptable to all. Many, however, are still poorly understood and any classification system must be regarded as tentative. The conifers are comprised of six (Engler, 1926), seven (Pilger, 1926) or ten (Buchholz, 1933) families according to the classification system selected. Not all conifers bear cones and, as a result, they can be separated on this basis into two broad categories: Phanerostrobilares, which refers to all conifers with conspicuous seed cones and includes most of the familiar North American species; and Aphanostrobilares, represented by those having inconspicuous seed cones which are usually fleshy or berry-like. For a more complete discussion of conifer classification and morphology, C.J. Chamberlain's (1957) excellent book on gymnosperms is recommended.

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, is a member of the Pinaceae, the largest family of conifers. The number of species of *Pseudotsuga* is currently thought to be six (Orr-Ewing, 1966; Thomas and Ching, 1968), but some authors believe it exceeds ten (Martinez, 1963). Species are native to China, Formosa, Japan and Western North America. *P. menziesii* is the most familiar and important species, having an enormous north-south range of over 3,000 miles, and extending from the Pacific coast to the eastern slope of the Rocky Mountains (Fowells, 1965). It is distributed throughout most of the Pacific Northwest and reportedly extends southward along the Rocky Mountains well into Mexico at higher elevations. It has also been established in many other regions of the world. *P. macrocarpa* Mayr., Bigcone Douglas-fir, is found in southern California and northern Baja, California (Sudworth, 1908). At least four additional species have been recognized in Mexico, *P. flahaulti, P. guinieri,* Flous., *P. macrolepis* Flous., and *P. rehderi* Flous. (Martinez, 1963); however, their status as species is questionable and they may only represent extremes in ecotypic variation

of *P. menziesii*. Four species are currently recognized from China, Formosa and Japan, *P. forrestii* Craib., *P. japonica* Beissner, *P. sinensis* Dode and *P. wilsoniana* Hayata (Henry and Flood, 1920).

The wide distribution of Douglas-fir in the Pacific Northwest and its desirability as a lumber species make it the most important economic species of this region. This creates a continuous demand for seed used in reforestation. The need for more controlled cone and seed production is beset with problems, not the least of which is the unpredictable, slow, cyclic nature of cone crops. Cyclic cone production occurs in all conifers, as does fruit production in many of our common fruit trees. A good or heavy cone crop in Douglas-fir generally occurs about every 5 years but varies from 2 to 7 years, which is unsatisfactory if seed orchards are to be successful.

Control of cone production is also important to the forest geneticist in order for him to hybridize and select individual trees over several generations. Another complicating factor in such programmes is the long period of juvenile growth before reproduction is possible within most conifers. This need not always be the case for the conifers, however, because in some members of the Cupressaceae the juvenile stage has been reduced to a few months by hormone treatment, and artificial stimulation of cone formation is relatively simple. This, unfortunately, does not apply to economically more important conifers.

Several approaches can be taken to help unravel the perplexing problems of reproduction in conifers. Physiological studies of nutritional and hormonal requirements for reproduction are necessary, as well as morphological studies of the origin and development of reproductive structures, pollination, fertilization and seed development.

This morphological approach may be referred to as life history, life cycle or reproductive cycle. These terms are used in various ways but generally refer to that portion of the reproductive process from pollination through seed formation. Elementary texts have established this precedent over many years and quite justifiably, since their purpose is to describe the reproductive cycle in relation to that found in other plants.

In this book, however, the purpose is to present the phenology and details of the life history of Douglas-fir in a broader sense, including the initiation and early development of cones, as well as the more familiar later stages of cone and seed development. A brief over-view of the entire cycle is appropriate as an introduction for the reader unfamiliar with either the cycle in Douglas-fir or the terminology commonly used. The phenology will vary as a result of normal variation between individual trees (Griffith, 1968) and geographical distribution of the species. For instance, bud burst in the spring may occur several weeks later in plants from higher elevations than in plants from lower elevations (Silen, 1963). The dates given in Figure 1.1 are for plants growing at lower elevations of coastal British Columbia and the Pacific Northwest and represent the earliest times when changes occur within the trees rather than average times. As the season progresses, developmental stages become telescoped and changes occur more rapidly at higher than

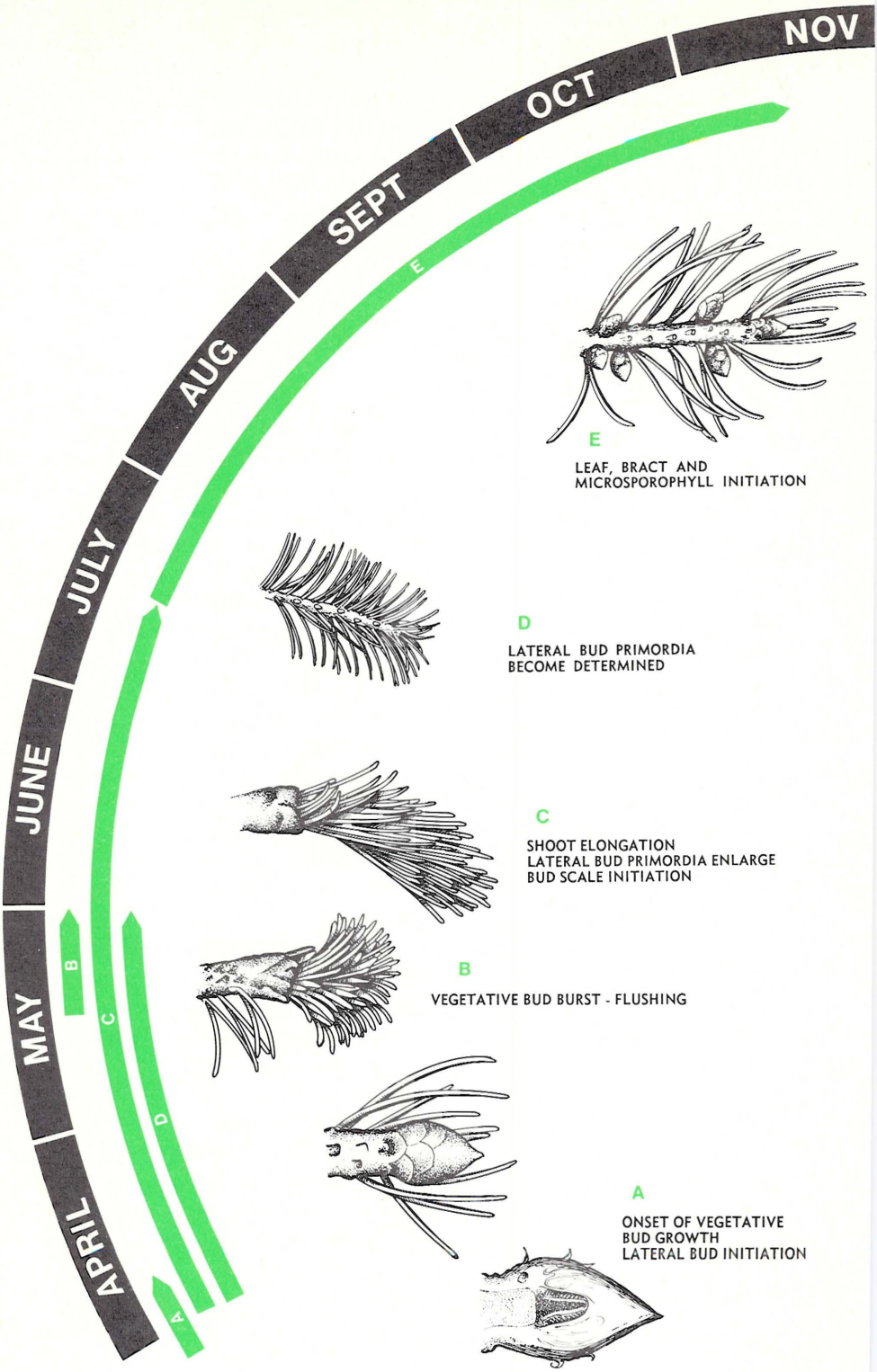
at lower elevations. Developmental stages within the buds and cones will, wherever possible, be correlated with conspicuous external changes familiar to the reader. Most of the externally visible developmental stages chosen as guidelines represent critical steps in reproduction, e.g. pollination, while others have been selected simply because they are easily recognized, e.g. vegetative bud burst. Details of stages will be considered in subsequent chapters. Here, only an over-view is intended. Throughout the discussion reference should be made to Figure 1.1.

The Life History of Douglas-Fir

Seasonal growth is commonly thought to begin when the vegetative buds burst (flush) in the spring, about mid-May at lower elevations in much of the Pacific Northwest and coastal British Columbia. However, considerable growth occurs within the bud scales prior to flushing and, in fact, it is this growth that is responsible for flushing. The bud scales are passive in this process, simply being pushed apart by the expanding shoot within. Histochemical tests made on the vegetative buds throughout the winter and early spring indicate that there is a marked increase in enzymatic activity during the last week of March (Owens, 1968). This enzymatic activity precedes and indicates the beginning of rapid cell division, which begins about the first of April, so there is considerable growth and development with the vegetative bud for a month or more before flushing. Lateral or axillary bud initiation is one of the most important changes to occur during this time (Fig. 1.1A). Several bud primordia arise, each from a few dividing cells, about the first of April. The primordia are initiated in the axils of leaf primordia, the axil being the juncture of the leaf primordium and the shoot axis. Considerable lateral bud enlargement and bud-scale development occurs before the vegetative buds, in which they are formed, burst (Fig. 1.1B). These newly formed primordia are the lateral buds along the shoot which will enlarge and burst the following spring to produce branches or cones (Owens, 1969).

The types of buds into which the primordia will develop cannot be distinguished during the first ten weeks following their initiation. Potentially, they can develop into either vegetative buds, seed-or pollen-cone buds. At the time of vegetative bud burst in mid-May (Fig. 1.1B), each primordium consists of only a tiny mound of rapidly dividing (meristematic) cells forming an apex. This apex is enclosed in several bud scales; the outer ones soon begin to turn brown and form the characteristic leathery bud scales so familiar in mature buds. Subsequent enlargement and development of lateral buds occurs while the shoot on which they are borne elongates. This elongation is usually complete by late June (Fig. 1.1C) (Owens, 1969).

The type of bud that develops from each primordium can be predicted to some extent, based on the position of the primordium along the shoot. The proximal primordia, those closest to the base of the shoot, tend to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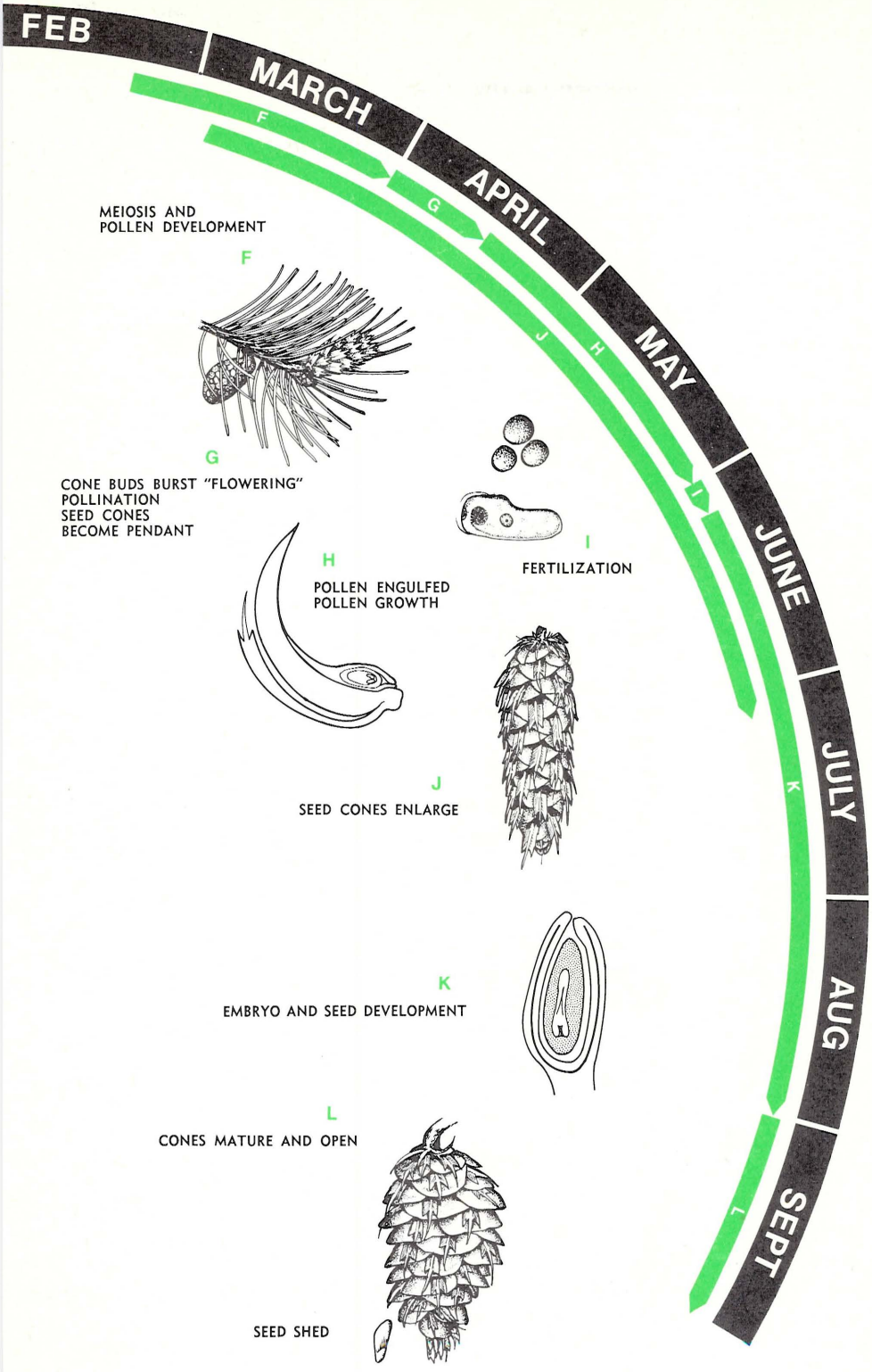
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Figure 1.1 Reproductive Cycle of Douglas-fir.

The entire reproductive cycle of Douglas-fir extends over 17 months. Axillary buds first become visible in April and differentiate into vegetative, pollen or seed-cone buds during the ensuing ten weeks. Pollination of the seed cones occurs the following April and the mature seeds are shed in September of the second year. Letters A-L represent various stages and a brief description of these stages. The approximate time each stage occupies in the reproductive cycle is represented by the colored arrows.

become pollen cones while more distal primordia become either seed cones or vegetative buds. At the end of the first ten weeks of development (Fig. 1.1D), histochemical differences can be detected between apices of different bud types. By early July, 15 weeks after initiation, lateral bud primordia begin to show morphological differences: all bud scales have been formed and the three bud types begin to form their characteristic foliar (leaf-like) organs (Fig. 1.1 E). Vegetative buds initiate a spiral series of leaf primordia, while pollen-cone buds initiate a spiral series of microsporophylls or leaf-like structures that produce the pollen sacs and pollen. Seed-cone buds initiate a spiral series of bract primordia which develop into the trident-like bracts characteristic of Douglas-fir seed cones. In addition, a series of ovuliferous scales are initiated, one in the axil of each bract. The ovuliferous scales bear seeds on their upper surfaces the following season (Owens and Smith, 1964, 1965; Owens, 1969).

Buds are completely formed by October or November and become dormant early in December. Dormancy is usually considered to be a period of reduced physiological activity, but is frequently used with reference to any bud not undergoing visible external changes. As a result, Douglas-fir buds are frequently referred to as "dormant" during late summer and early fall, because they appear completely formed externally. Actually, cell division and growth continue within the buds until late fall, long after they first appear to be dormant. Physiological activity continues within the buds throughout the winter but is considerably reduced. Differences exist between bud types in that reproductive buds (seed and pollen cones) are more active during winter months than are vegetative buds (Owens, 1968).

All three bud types are initiated and develop during the spring, summer and fall of the year prior to the spring when they burst and become visible as either vegetative shoots or cones. The beginning of growth, resulting in bud burst, varies in vegetative and reproductive buds. Both seed and pollen cones begin growth and expand within the scales throughout March (Fig. 1.1F). Bud burst occurs about the first of April and coincides with the onset of growth within the vegetative buds on the same tree (Fig. 1.1G). Seed-and pollen-cone buds usually burst simultaneously and on a given tree this may extend over a period of two weeks (Owens and Smith, 1965).

Pollination usually occurs in early April and involves the shedding of pollen from the pollen cone and the transfer of pollen by wind to the receptive seed cones. The pollen cones are pendant during pollination, dry out, and most fall from the tree with the first strong wind. Seed cones are upright

and receptive during the period of pollination.

Ovuliferous scales and ovules are small and not apparent at this time unless the cone is dissected. The female gametophyte, which contains at least one egg within each ovule, has just begun to develop at the time of pollination (Fig. 1.1 G) (Allen, 1943, 1963).

Pollen grains, upon entering the micropyle or pore leading into the ovule, elongate, then form a tube-like structure that penetrates the nucellus, or megasporangium, and releases its two gametes into the fully developed female gametophyte. These processes take about nine weeks, from early April until early June (Fig. 1.1H). No external changes mark the time of fertilization but seed cones by this time have almost completed their enlargement (Fig. 1.1I). Following pollination, ovuliferous scales and ovules enlarge many times while bracts enlarge only slightly. The cone takes on the appearance of a mature seed cone but is still green in color. Cone enlargement is complete by early July (Fig. 1.1J), and is followed by a period of maturation during which few external changes occur (Fig. 1.1K), but considerable tissue differentiation takes place within the cone (Allen, 1943, 1946b; Owens and Smith, 1965).

Embryo development takes place within the female gametophyte tissue of the ovule and begins immediately after fertilization (Fig. 1.1K). Embryonic stages are similar to those found in most other conifers. Embryos are well developed by late July but continue to enlarge slightly and mature about the end of August. The ovule develops into the seed. Seed-coat and seed-wing development occurs throughout late spring and summer, and seeds are generally mature by early September (Allen, 1946b, 1947a; Owens and Smith, 1965).

Cones begin to dry and turn brown by late August. Continued drying causes the cones to open and the seed is released in September (Fig. 1.1L). The opening of the cone is a result of the drying of specialized mechanical tissue at the base of each ovuliferous scale (Owens and Smith, 1965). Thus, considerable variation exists in the time of cone opening. In general, a very dry summer results in early cone opening. Seed cones are retained on the tree, often for several years, following seed dispersal.

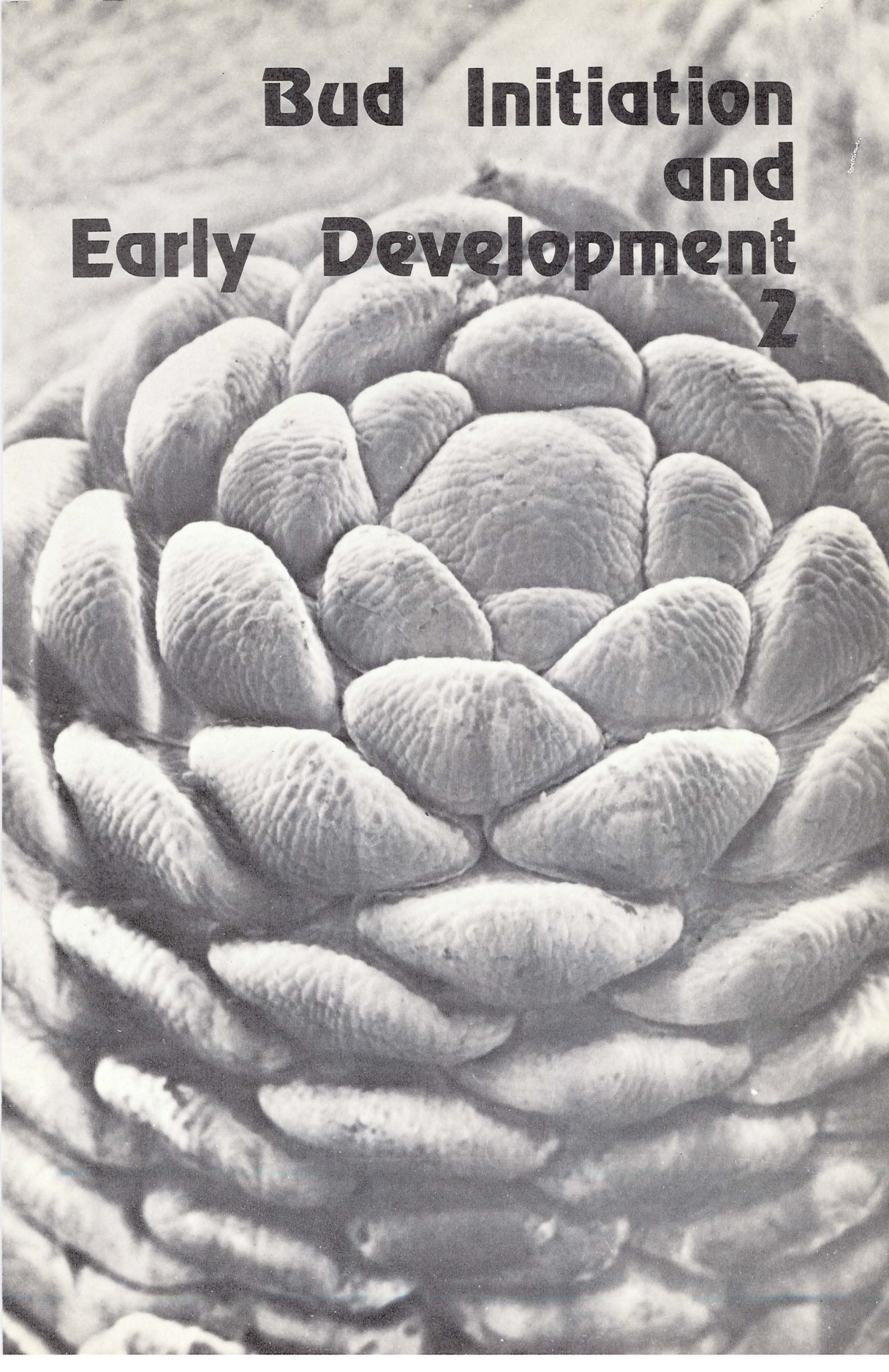
The entire reproductive cycle takes 17 months from the time of cone initiation in April to the release of mature seed in September of the following year (Allen, 1943; Owens, 1969). The period most frequently discussed in literature is that from pollination in April of the second year to seed release in September, or a period of only six months. However, the entire cycle must be considered, since much can happen during the first 11 months of cone development and dormancy that can drastically affect the final number of cones produced. This topic is discussed in Chapter 2. Other separate factors, covered in Chapters 3 to 7, affect seed cone and ultimately seed production during the final six months of the cycle. In such a long reproductive cycle, it is impossible to designate one stage as being the most critical.

The life history of Douglas-fir represents only one example found in con-

ifers and it should be viewed with reference to other conifers. This is not as complicated as it may first appear, for most conifers studied thus far have a life history similar to Douglas-fir, with cones being initiated in the spring or summer of the year before pollination and seed release. Cone development prior to pollination has been described in very few species, making comparisons difficult. In contrast, ovule and seed development have been described in many conifers so that comparisons can be made. Species of *Pinus* have been thoroughly described in this respect and have been used as examples in elementary texts to describe the typical conifer life history. This practice will no doubt continue, but it should be kept in mind that the phenology in pines is atypical in that fertilization occurs the year after pollination. Consequently, the life history extends over three years rather than two, as in Douglas-fir and most other conifers.

Scanning electron micrograph of the dormant stem tip of Douglas-fir showing the apex and leaf primordia. x180.

Bud Initiation and Early Development 2



Chapter 2

Bud Initiation and Early Development

Variation in cone crop from year to year is apparent even to the casual observer. In some years nearly all conifers in a locality have a heavy cone crop while in other years there are very few cones. When all species in a mixed stand produce cones abundantly, we become intrigued as to what environmental factor or factors trigger cone formation. Many factors are no doubt involved but since the time of cone initiation and phenology of cone development are not the same for all species in a mixed stand, it is difficult to imagine a single factor triggering all species.

Attempts have been made to correlate environmental factors with cone production (Lowry, 1966; Vredenburch and Bastide, 1969). Temperatures, rainfall and sunshine with all their complex interrelationships are the common parameters used. Few conclusive results have been obtained by these studies but some qualified generalizations can be made. Weather conditions appear to be very important for a good cone crop. For example, good cone crops in Douglas-fir are positively correlated with precipitation preceding and at the time of cone initiation; a warm, sunny summer during early cone bud development, and low temperature and sunshine in the spring and summer preceding cone initiation or 27-30 months before seed release (Lowry, 1966). This information is very difficult to interpret because of all the interactions between temperature, sunshine and precipitation.

Cone formation can be artificially stimulated but many of the methods are not practical. Even though cones are produced, the treatment may be fatal to the trees. Stress crops in conifers are well documented and may result from numerous causes, all of which drastically affect physiological processes within the plant. Precisely what processes are affected, however, is not yet clear. Damage by girdling of stems from various causes; diseases affecting roots or root pruning affecting water availability, and extreme drought are some of the more familiar causes of stress crops (Ebell, 1967). Many of these can be simulated under laboratory conditions and yield good results, but are not practical commercially because of the often fatal consequences to the trees. However, application of specific fertilizers (Stoate, Mahood and Crossin, 1961; Ebell, 1962; Griffith, 1968) and certain hormones (Pharis, Ruddat, Phillips and Heftman, 1965) have stimulated cone formation in some conifers without lasting, harmful effects to the tree. Although some of these techniques show great promise in certain species, no one technique has yet appeared that can successfully and safely be applied to all conifers.

Correlations with environmental conditions, knowledge of the physiology of the trees and methods of artificial stimulation of cone formation are all

indispensable to our ultimate understanding and control of cone production. However, the phenology of vegetative growth and cone formation in different species is also important so that all these factors can be related in time and space. The cyclic nature of cone production within each species is not yet understood so the cyclic pattern in a mixed stand is impossible to grasp. Perhaps the years of abundant cones in a mixed stand result from the cycles of various species coming into phase. If differences between species are ignored, one might assume that there is a single critical stage or developmental factor for all species. Likewise, low cone production in a mixed stand may not result from the absence of a decisive stimulus but from the fact that the cycles of the species are out of phase. Even if we consider a forest of only one species where all the individuals have a particular cycle of abundant cones every 2, 4, or 7 years, these also will eventually get into phase and there will be years when nearly all will produce abundant cones. Conifer reproductive cycles involve many months, and the absence of cones at the end of this long period may result from no cones being initiated or from failure of those initiated to develop to maturity.

The Distinction Between Cone Initiation and Development

Cone initiation refers to the earliest stages of cone primordium formation, which may occur in different ways in different species. Each species, however, is not distinct — certain patterns exist. Cones are normally formed by the conversion of a vegetative shoot into a reproductive shoot in cedar, hemlock, larch and spruce. The vegetative shoot may have undergone a few days to several years of normal vegetative growth, forming leaves and perhaps lateral branches as well (Owens and Pharis, 1971). Others such as pines (Doak, 1935; Mergen and Koerting, 1957; Owston, 1969), *Abies,* (Eis, 1970), *Pseudotsuga* (Owens, 1969) and spruce, initiate new axillary shoots that may develop into cone buds rather than first functioning as vegetative shoots. Other less obvious differences in methods of cone initiation further separate the conifers into perhaps five patterns of cone initiation.

Time of cone initiation varies in different species and even within a species in different environments. Cone initiation normally occurs in the season preceding pollination. The exact time, however, varies with the species from early spring (Douglas-fir) or early summer (Western red cedar) to fall in certain pines. Unfortunately, only a few species have been studied in this regard. Following initiation, cone development covers many months in all species, and up to 27 months in pines. The developmental stage most susceptible to abortion or damage is uncertain for most species, and factors favorable for cone initiation may not be favorable for cone development.

A clear distinction must be made between the processes of cone initiation and cone development, and an evaluation made of their relative importance in cone production in each species. Considerable work has been done on Douglas-fir and it is one of the few conifers, representing only one of the several reproductive patterns, where the phenology of the entire reproductive

cycle is known. To understand the cycle, we must first look at the normal growth periodicity of the vegetative shoot, then at the axillary bud initiation and subsequent cone development. Throughout this discussion, caution must be taken when generalizing from the Douglas-fir pattern to that of other conifers.

The Annual Growth Cycle of the Vegetative Shoot in Douglas-Fir

The annual growth cycle of vegetative shoots of Douglas-fir has been described for different localities (Allen, 1943; Sterling, 1946; Owens, 1968, 1969). The sequence of morphological changes does not vary with environment but the time at which developmental events occur (the phenology) varies greatly. As a result, the approximate dates assigned, as in Chapter 1, represent those for the Victoria, British Columbia area, but they may also be applied with some adjustment to the rest of coastal British Columbia and much of the Pacific Northwest. The phenology is summarized in Fig. 2.1.

The growth periodicity of the shoot passes through the same cycle each year. The dormant vegetative winter bud is quite conspicuous, with large bud scales which are easily removed. The entire following year's shoot, lacking axillary bud primordia, is enclosed within the bud scales (Fig. 2.2 A). At the tip of the shoot is the apex, an embryonic dome of cells characterized by precise cellular arrangement. Allen (1947b) and Sterling (1946) have described the organization of the vegetative apex, based on cell size, cell appearance, and frequency and plane of cell division. Since the dormant apex does not undergo cell division, any description must be based on patterns present during periods of active growth at other times of the year. It is characterized by a small group of apical initial cells at the summit which divide periclinally (parallel to the surface), giving rise to a central mother cell zone below and anticlinally (perpendicular to the surface), giving rise to the protoderm (Fig. 2.3I). Cell divisions are infrequent in the interior of this zone but peripheral divisions produce a mantle-like peripheral zone of small, densely staining cells. This zone gives rise to cortex, procambium and foliar organs. Divisions at the base of the central mother cell zone form a central rib meristem. The rib meristem and the uppermost cells of the peripheral zone are sometimes grouped into one zone called the eumeristem (Sterling, 1946; Esau, 1953).

When growth resumes in late March, cell divisions become frequent and zonation gradually becomes more apparent. The shoot axis and leaf primordia elongate, causing the bud to enlarge (Fig. 2.3A). Buds slowly enlarge for about seven weeks, at which time the elongating shoot pushes through the bud scales causing buds to burst (Fig. 2.3B). Throughout this time, the apex initiates a spiral series of new bud scales which will eventually enclose the bud of the following year. Bud-scale initiation is complete by mid-July but bud-scale enlargement and development continue until late in the summer. The apex enlarges gradually during bud-scale initiation and zonation

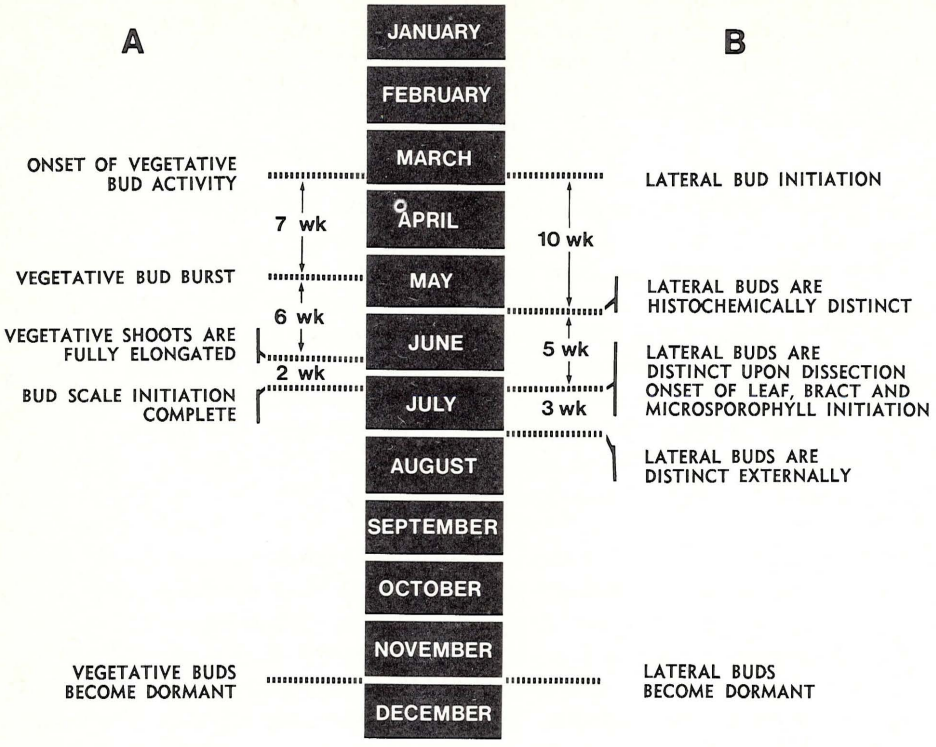


Figure 2.1. Growth periodicity in Douglas-fir.

A diagrammatic representation of the growth periodicity in Douglas-fir near Victoria, B.C. Sequence A represents vegetative shoot activity of the current season, while sequence B represents initiation and early development of axillary buds for the following season. Redrawn from Owens (1969).

becomes more evident. Toward the end and after the period of bud-scale initiation, the apex enlarges more rapidly. Because the rate of increase in height of the apex exceeds the rate of bud-scale initiation, the apex takes the form of an elongated cone above the bud-scale primordia (Fig. 2.3 F,I).

Leaf initiation begins after all bud scales are initiated. As a result, bud scales are all at the base of the bud and grow upward, over-arching and enclosing the upper portion of the bud (Fig 2.2A). Leaves are initiated spirally upward from the base of the enlarged conical apex. A series of many, almost equal-sized primordia rapidly appear along the flanks of the apex. The basal leaf primordia are slightly larger at any given time since they are initiated first. The rate of leaf initiation thus surpasses the rate of apical elongation and the conical apex is rapidly "used up" as leaf primordia are initiated nearer the summit (Owens, 1968). Apices from large terminal or lateral branches are very broadly conical and, after two-thirds of the

leaf primordia are initiated, assume a form characteristic of many other gymnosperms. The apical initial and central mother cell zones become pronounced as the peripheral region below widens and a characteristic "mammary apex" appears (Sterling, 1946). This does not always appear, especially in apices of less vigorous branches that undergo less growth in width.

The rate of leaf initiation slows in August but still slightly exceeds the rate of apical elongation. Leaf primordia are gradually initiated up to just below the summit of the apex and nearly all of the peripheral portion of the apex gives rise to leaves. Only the apical initial-central mother cell zone and a small portion of the peripheral zone remain above the last-formed leaf primordia. Leaf primordia elongate, frequency of cell divisions slows, apical zonation diminishes in October and November, and the dormant bud for the following year is again present by mid-December (Fig. 2.2 A, D). No branch primordia are formed in the axils of leaves before or during dormancy (Owens, 1968, 1969).

A crown region begins to form in August after most leaf primordia have been initiated. It consists of thick-walled cells and extends across the pith between the procambial strands and into the cortex at the base of the bud (Fig. 2.2 D). Cell walls of the crown region are impregnated with unusually high quantities of pectic substances. The crown region thus forms an anatomical and perhaps a physiological separation between the growth of one year and the bud that will develop the following year. Although the crown region appears in most conifers that possess distinct buds, a complete survey of all conifers has never been made and no known function has been demonstrated for this structure.

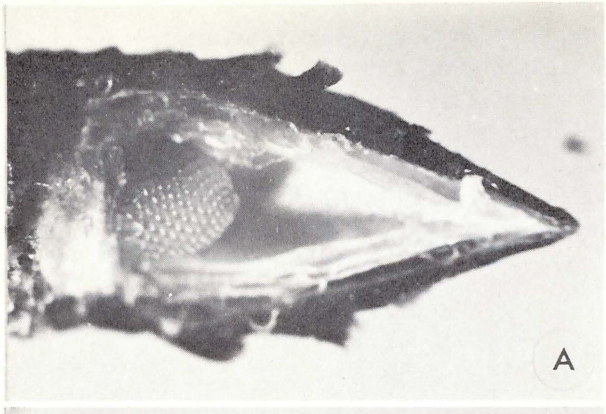
Axillary Bud Initiation

Axillary bud initiation in Douglas-fir coincides with the onset of vegetative bud growth in the spring (Fig. 2.1). The earliest stages can be detected only by microscopic observation of transverse sections of vegetative buds (Fig. 2.2). Sections of fresh or frozen buds can be stained to show the presence of an enzyme, succinic dehydrogenase (Jensen, 1962). An increase in succinic dehydrogenase precedes and indicates sites of frequent cell division (Owens, 1969). The precise site and time of axillary bud initiation can thus be determined. This is one of the first activities to occur in the vegetative bud after dormancy. Cell divisions appear at these sites within one week so that, anatomically, axillary bud initiation in Douglas-fir occurs by the first of April. These primordia develop and become visible axillary buds during spring and summer.

Axillary bud primordia are initiated in the axils of only some of the young leaves along the length of the shoot axis. The arrangement is somewhat predictable. Primordia are initiated primarily laterally and most abundantly in the axils of the basal and distal leaves. All primordia are initiated at the same time. That is, basal primordia are not initiated first and distal

Figure 2.2 Initiation of axillary bud primordia.

Dissected and sectioned vegetative buds of Douglas-fir collected early in April showing the initiation of axillary bud primordia. A. Vegetative bud with half the bud scales removed to reveal the telescoped vegetative shoot within. All leaf primordia that will develop during the current spring and summer were formed the previous summer. X7. B. Vegetative shoot tip with all bud scales removed. The terminal portion, containing the apical meristem (apex), has been sliced off to reveal the appearance of the shoot tip as seen in transverse section. X 10. C. Microscopic view of a transverse section of the shoot tip showing the spirally arranged leaf primordia and the initiation of an axillary bud primordium, as is shown in the square just above the center of the section. X 12. D. Longitudinal section of a vegetative shoot tip (as shown in A above) with all bud scales removed. All leaf primordia (lp) are present and the shoot apex (a) is reduced to only a small dome of cells. A distinct crown region (cr) has differentiated at the base of the shoot tip. X 50. E. An enlarged transverse section of the shoot tip (as in B and C above) showing the earliest stage of axillary bud initiation. Enlarged, lightly staining cells in the axil of the leaf primordium at the surface of the shoot axis are beginning to divide rapidly to form an axillary bud primordium (lb). X 250. F. A slightly later stage of axillary bud initiation (second week of April). Rapid cell division has produced an axillary bud primordium seen here as a slight swelling in the axil of the leaf primordium. X250. G. Axillary bud primordium at mid-April. The primordium now consists of an apex (a) flanked on two sides by small swellings that develop into the first two prophylls or bud scales (bs). X 250.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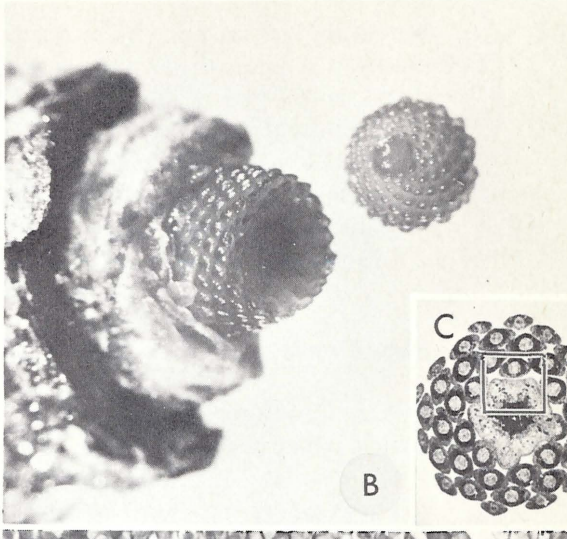
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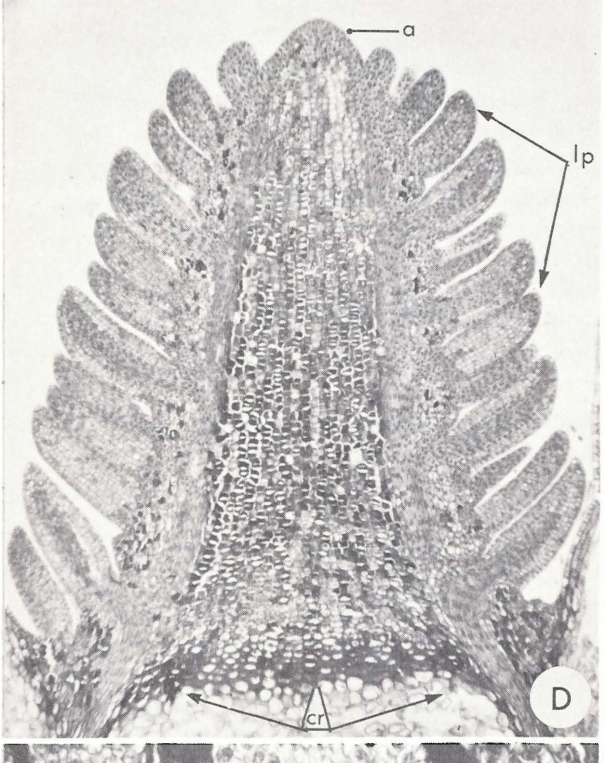
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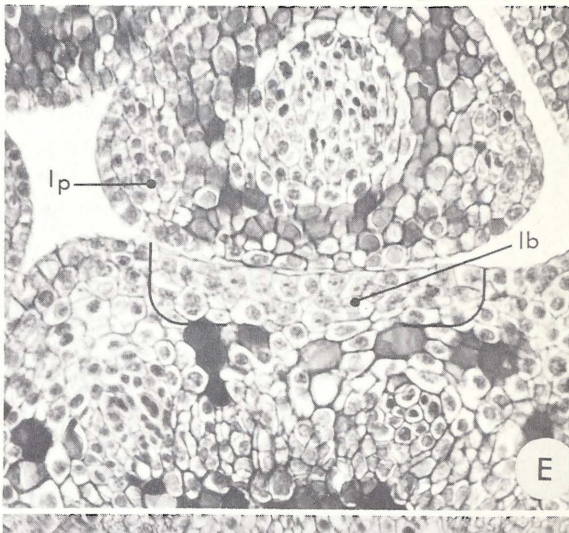
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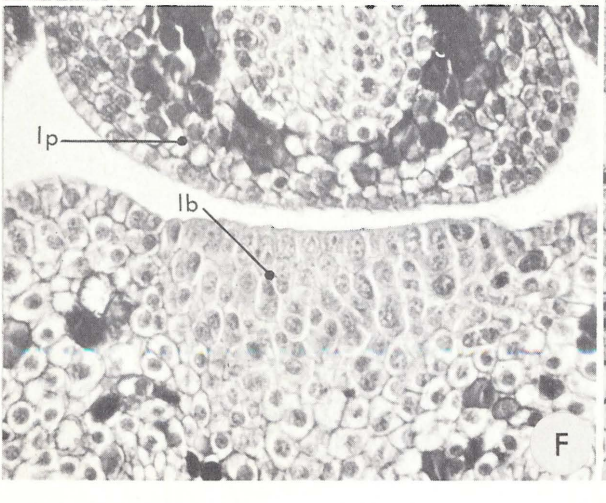
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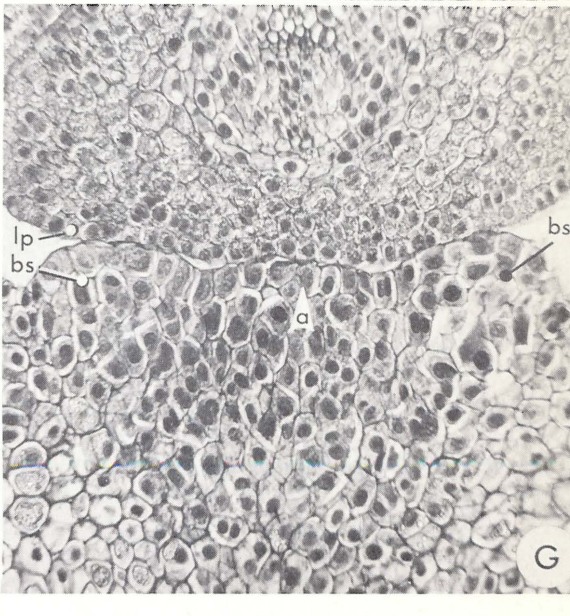
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primordia last, as appears to occur in pines (Owston, 1969). Thus, differences in day-length do not occur between time of initiation of basal and distal lateral bud primordia along the shoot. The type of bud into which a primordium can develop may be related to its position along the shoot. Vegetative and seed-cone buds normally develop from distal primordia, while pollen cones normally develop from more basal primordia. Primordia in intermediate positions may develop into any of these three types (Fig. 2.5 D-J).

Early Development of Axillary Buds

Early development occurs during a ten-week period following initiation during which time primordia cannot be distinguished as vegetative, seed cone or pollen-cone buds (Fig. 2.1). Primordia are visible with a dissecting microscope by mid-April as very tiny, green apices with two prominent prophylls that develop into the outermost bud scales (Fig. 2.2G; 2.5A). A distinction can usually be made ten weeks after initiation on the basis of histochemical staining, which shows apical zonation patterns not visible when using standard anatomical staining techniques (Owens, 1969). An earlier distinction could possibly be made with more complete or precise histochemical studies. The first ten weeks is the period during which axillary primordia are most plastic with regard to their future development and when environmental or internal changes could determine the pathway along which a primordium will develop.

The changes that make a primordium recognizable as a distinctly vegetative, seed-cone or pollen-cone bud are gradual, and anatomical differences are not apparent until early July, or about 15 weeks after initiation. Vegetative lateral apices at that time are about 270 μ high and 370 μ wide (Fig. 2.3 F,I). They show a distinct zonation with conspicuously enlarged, vacuolate central mother cells, a narrow peripheral zone of actively dividing cells, and a rib meristem with the future pith region below. The larger seed-cone apices are about 300 μ high and 530 μ wide (Fig. 2.3 E, H) and have a less conspicuous zonation. Central mother cells are smaller and divide more frequently than in vegetative apices. More frequent divisions are also evident in the rib meristem and future pith which stains more lightly than in vegetative apices. The peripheral zone is twice as wide as that in vegetative apices. Pollen-cone apices are smaller than seed-cone apices — 170 μ high and 270 μ wide — but otherwise appear similar in shape and zonation (Fig. 2.3 D, G). Shortly thereafter, differences in the three types of axillary bud primordia become visible by removal of the bud scales. Vegetative lateral apices are sharply conical and light green due to the presence of abundant chlorophyll in the future pith region (Fig. 2.3F). Both seed-and pollen-cone apices have a more rounded, conoid shape and are light yellow because of less chlorophyll in the future pith region (Fig. 2.3 D, E). No chlorophyll is evident in the peripheral or central mother cell zones. Differences in chlorophyll content can be readily compared in frozen sections with a

fluorescence microscope (Owens, 1969).

Early anatomical differences, as well as preliminary tests of various enzymes present in vegetative and reproductive apices, suggest a very early divergence in metabolism of the different bud types (Owens, unpublished). These differences are no doubt related to morphogenetic changes within the apices, but much more work is necessary to determine which are causal and which are manifestations of morphogenesis.

Pathways of Bud Development

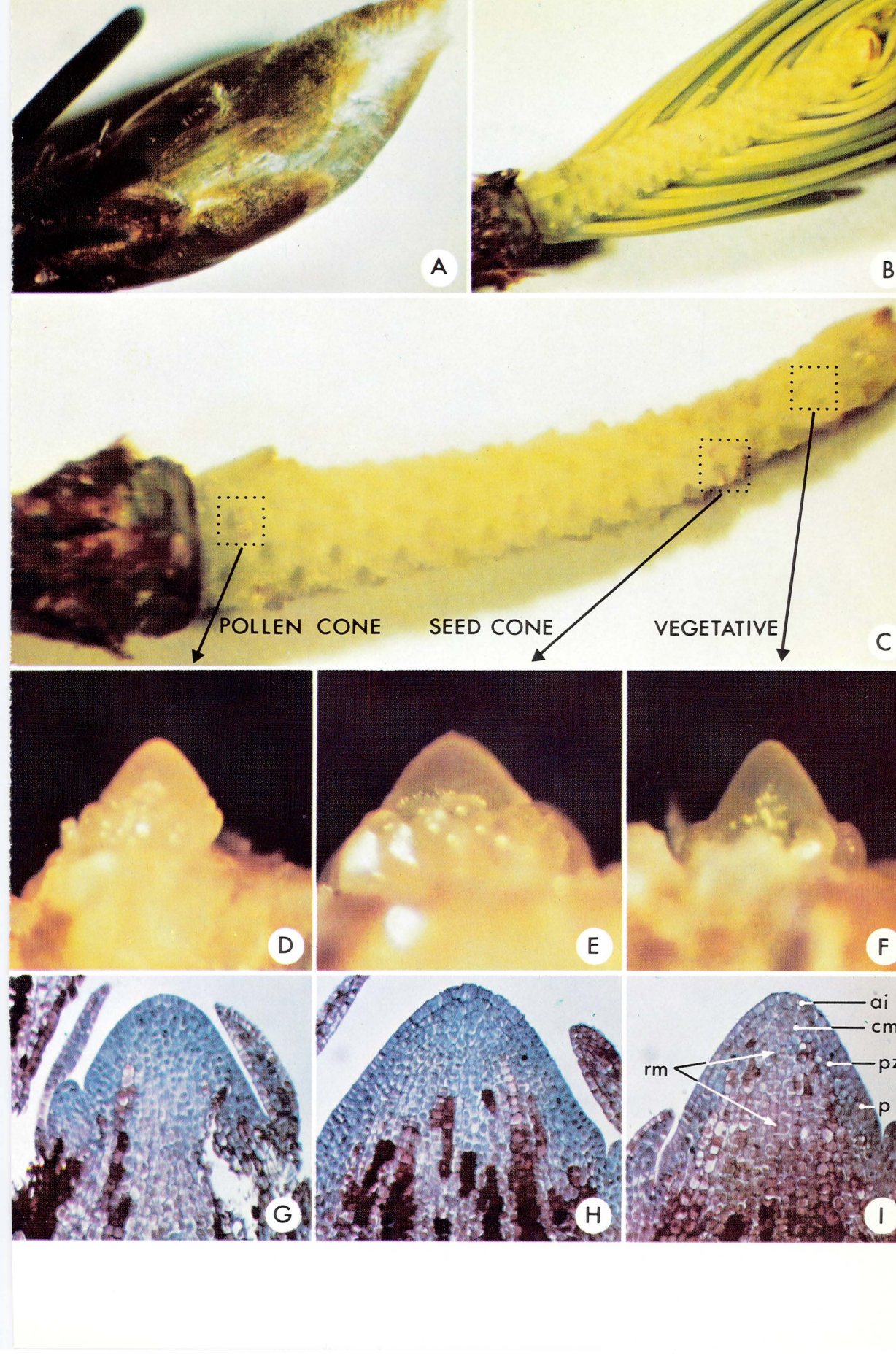
Primordia, at the time of initiation, are not predestined to form a particular bud type. Rather, they are undetermined but, because of their position on the shoot, may have a tendency to develop along a particular pathway.

Studies of early development of axillary primordia into the distinct vegetative, seed-or pollen-cone buds showed that not all bud primordia develop to the same extent or at the same rate (Fig. 2.4) (Silen, 1967; Owens, 1969). Some begin to lag within a few weeks after initiation and cease development. These soon degenerate and, in the mature shoots, leave no evidence of their previous existence. They are herein referred to as aborted buds. Other primordia form most of their bud scales, then cease to develop and become quiescent or latent buds. These buds have not become determined as vegetative or reproductive at the time of growth cessation, and consist of a small apex surrounded by numerous bud scales but with no additional foliar primordia (Fig. 2.5 A-C). Latent buds can be stimulated to develop by removal of the terminal vegetative bud on the twig. If this is done, they usually develop into vegetative buds. Bud primordia, which abort or become latent, are commonly the most basal primordia while those more distal are more likely to develop fully. Thus the development of axillary primordia may follow any one of five pathways: (l) abort early and disappear completely within a few weeks of initiation; (2) partially develop and then become undetermined latent buds; (3) develop into vegetative buds; (4) develop into seed-cone buds, or (5) develop into pollen-cone buds (Fig. 2.4).

The process of a primordium becoming aborted or latent is a gradual rather than an abrupt change in development. Primordia cease development and become latent when the apex is still quite small and before a very distinct zonation pattern is established (Fig. 2.5C). The size of latent apices varies considerably because some develop for a longer time than others. The central mother cell zone is not distinct; the peripheral zone shows few cell divisions and takes on the staining characteristics of the less-meristematic pith region. This unusual staining appears first in the basal peripheral cells and progresses acropetally until the central mother cells eventually stain in a similar manner. The staining appears first in the nucleus, then throughout the cytoplasm. The nature of the material that accumulates and stains in this manner in all the cells of the latent apex is as yet unknown. By late June, a uniform staining pattern is present throughout the latent apex and no cell divisions are evident (Owens and Smith, 1964).

Figure 2.3 Early Stages of Axillary Bud Development.

A. A large vegetative bud collected in mid-May, just before vegetative bud burst. The elongating shoot within has pushed the bud scales apart. X 6. B. Vegetative bud as shown in A but with bud scales and half of the leaves removed. X 6. C. Enlarged vegetative shoot axis as shown in A and B with all leaves removed to show the number and general location of axillary bud primordia as outlined by the squares (see text for details concerning the location of various bud types on the shoot). X12. D-F. Apices of pollen-cone, seed-cone and vegetative buds, respectively, with all bud scales removed. Collected in mid-July when buds can first be distinguished by carefully removing the bud scales. Pollen-(D) and seed-cone (E) apices are pale yellow and dome-shaped at this stage, while vegetative apices (F) are green and conical. All bud scales have formed by mid-July and leaves, bracts and microsporophylls have begun to be initiated. X60. G-I. Median longitudinal sections through the apices of pollen-cone, seed-cone and vegetative buds shown in D-F directly above. X100. Pollen-cone (G) and seed-cone (H) apices at this stage have similar but less-distinct apical zonation than vegetative apices (I). The apex consists of the following zones: apical initial cells (ai) that give rise to the central mother cell zone below and laterally to the protoderm; protoderm (p) which forms the surface layer of the apex; central mother cell zone (cmc) which consists of a group of relatively inactive cells that divide infrequently, giving rise below to a rib meristem and laterally to a peripheral zone; rib meristem (rm) consists of a column of cells that divide frequently, forming the future pith cells below; the peripheral zone (pz) consists of a cylinder of rapidly dividing cells which encloses the rib meristem and developing pith.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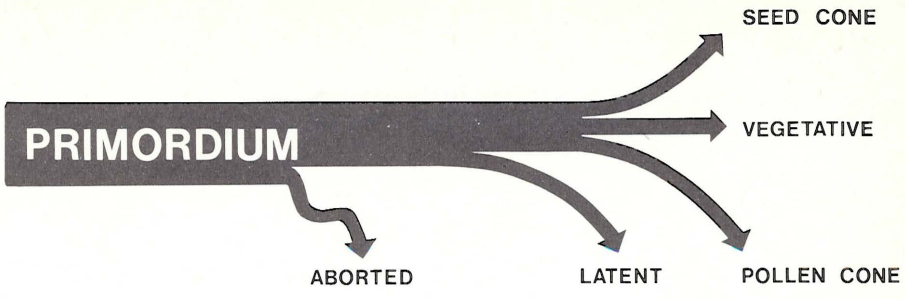


Fig. 2.4. Alternative Pathways of Axillary Bud Primordial Development.

Redrawn from Owens (1969).

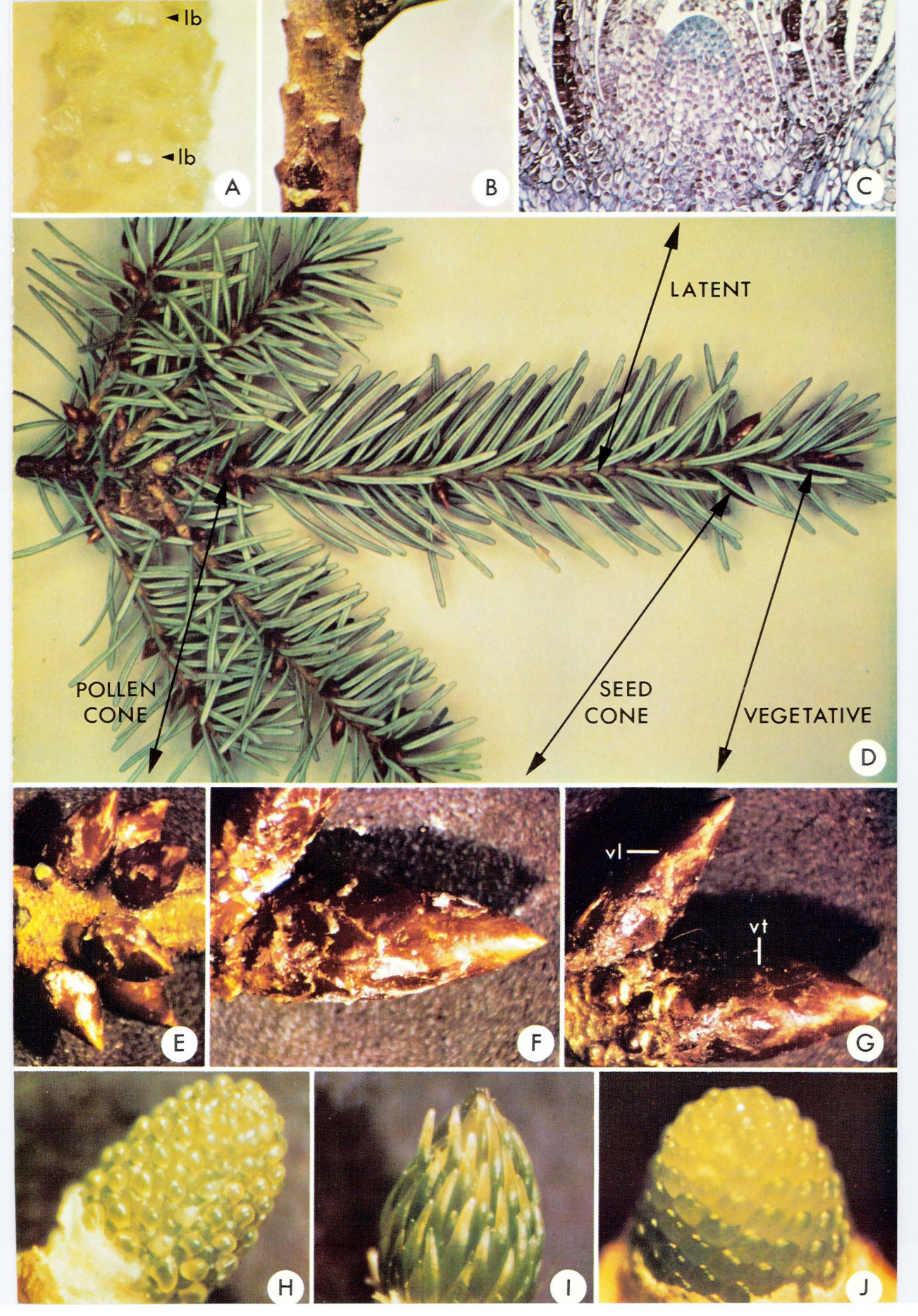
Initiation Versus Development

It was believed for many years that the number of cones produced in Douglas-fir and other conifers was determined by the number of cones initiated and that if one wished to increase cone production one would have to increase cone initiation. This has been shown to be incorrect, at least for Douglas-fir, by comparing the number of bud primordia initiated and the number of mature buds formed in successive years (Silen, 1967; Owens, 1969). In one study (Owens, 1969), collections were made from three sites, three trees being chosen on each site: one tree was about 15 years old and could be expected to bear no cones; a second was about 30 years old, and a third was about 50 years old. Trees in the last two categories had produced cones in previous years. A summary of the data from this study is shown in Fig. 2.6. Three comparable branches were collected in spring and fall from each of the trees, in both 1967 and 1968.

The number of axillary primordia initiated in corresponding trees did not vary significantly between 1967 and 1968, but the number of reproductive buds produced did vary significantly. Abundant cones developed in 1967 while none developed on the trees studied in 1968. The variation in cone formation can be explained by the high proportion of buds that ceased development and became latent, 59% in a year when no cones developed (1968) compared to 28% in a year of abundant cone development (1967). The difference between the proportion of buds developing in a good year (1967) as opposed to a poor year (1968) is 31%, equal to the proportion of pollen-cone buds that develop in a good year. This is supported by observations that show that in a poor year the primordia most likely to become latent are found in the proximal region of the shoot, and are those that would otherwise develop into pollen-cone buds. More distal, potentially seed-cone primordia are also initiated, but these usually become vegetative rather than latent, or abort. Observations show that although both aborted and latent primordia do occur along the entire length of the shoot,

Figure 2.5. Late Stages of Axillary Bud Development.

A. Enlarged view of developing axillary bud primordia (lb) as they appear in mid-May just before vegetative bud burst. Two developing bud scales overarch and obscure each apex. The larger, upper primordium is developing normally. The smaller, lower primordium shows early signs of retarded development and will either completely degenerate and become an aborted bud or will develop partially and form a latent bud. X15. B. Latent buds as they appear on the shoot in the fall. X8. C. Longitudinal section through a latent bud. Development of the apex stopped before the cells became organized into the recognizable zonation pattern shown in Fig. 2.3I. X300. D. A branch collected in the fall showing vegetative, seed-cone, pollen-cone and latent buds in their typical arrangement along the shoot. X3/4. E. A cluster of basal mature pollen-cone buds as they appear in the fall. Each bud occurs in the axil of a separate leaf so they are actually arranged in a very tight spiral. X3. F. A mature seed-cone bud collected in the fall. Seed-cone buds are the largest axillary buds present and, like the pollen-cone buds, are generally lighter brown than the vegetative buds. X6. G. A mature vegetative lateral bud (vl) commonly found just below the vegetative terminal bud (vt) of the branch. X6. H. A dormant pollen cone with bud scales removed to show microsporophylls. Dormant pollen cones are commonly more yellow than dormant vegetative buds or seed cones. X12. I. A dormant seed cone with bud scales removed to show very prominent pointed bracts. X10. J. A dormant vegetative shoot with bud scales removed showing small, rounded, spirally arranged leaf primordia and the shoot apex. X20.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they occur infrequently in distal regions. Also, fewer vegetative buds develop in years when abundant cones are formed. Thus it appears that distal undetermined primordia develop as seed cones only under favorable conditions. With unfavorable conditions, a few abort or become latent but most develop into vegetative buds.

A similar conclusion prevails if only young, essentially vegetative trees are considered. In 1967, seven percent of the primordia initiated on young trees developed into reproductive buds (2% seed cone, 5% pollen cone) and 31% became vegetative. All other primordia aborted or became latent. In 1968, no reproductive buds developed on young trees and 36%, or all of the buds that developed, became vegetative. Young trees thus showed about the same proportion of buds developing in both years and those that became reproductive represented primordia that otherwise would probably have become vegetative. Counts of primordia initiated on 3-year-old seedlings show that they also initiate numerous proximal primordia but these usually abort, with a few developing further only to become latent. The proportion of buds developing in older trees is much more variable from year to year (30-60%) than in juvenile trees. This variation is a result of the development of reproductive buds from primordia that otherwise would probably become latent or abort. Therefore, variation in cone production in Douglas-fir is possibly a result of the proportion of primordia that develop and the pathway along which they develop rather than variation in the number of primordia initiated (Silen, 1967; Owens, 1969).

This conclusion not only helps account for the tremendous variations in cone production that can occur on a given tree from year to year, but it also provides a means of early forecasting of the cone crop. Early forecasting of cone crops depends upon a reliable means of sampling relative numbers of seed-cone buds. Early sampling, the season before pollination, can accurately forecast a crop failure though not necessarily a successful crop. Insects, frosts, poor pollination or cone abortion can seriously reduce a potential cone crop between August, when seed cones are easily recognized, and a year later, when the seeds are shed (Allen, 1941). Silen (1967) developed a means of very early forecasting based on relative numbers of seed-cone and pollen-cone buds. He found that the number of developing bud primordia in the potentially pollen-cone position along the shoot in May (Fig. 2.3C) is positively correlated with the relative number of seed-cone buds present that fall (Fig. 2.5D). This finding is extremely important in planning seed procurement since it allows an additional summer, the year before seed release, to prepare for cone collection and seed extraction if there is potentially a good cone crop for the following year; or, more important, to realize 16 months ahead of seed release that there will be no cone crop.

Factors Influencing Bud Development

Few conclusive statements can be made regarding how many environmental, physiological and morphological factors influence bud de-

velopment in Douglas-fir. The reason is that little is known of the physiological condition of the tree during cone development, so we cannot understand all of the interactions that might occur. However, it is worthwhile at this point to present some of the possibilities that may exist.

The pathway along which a primordium will develop is closely related to its position along the shoot. Primordia found proximally on the shoot will nearly always become pollen-cone buds if they develop fully, while more-distal primordia more often become seed-cone or vegetative buds (Fig. 2.5 D-J). Similar observations have been made in other conifers, including *Pinus sylvestris* (Wareing, 1958), *Chamaecyparis* (Courtot and Baillaud, 1955), *Thuja plicata* (Owens and Pharis, 1971) and in certain angiosperms (Nitsch, Kurtz, Liverman and Went, 1952).

Hormones

Generalizations have been made in the angiosperms with regard to position of various bud types along the shoot and the auxin and gibberellin concentrations as they relate to "sex determination" (Heslop-Harrison, 1957; Galun, 1959; Hillman, 1962). High auxin levels favor pistillate and reduce staminate expression in angiosperms, while gibberellin causes a trend toward staminate and delayed pistillate expression. As in many other conifers studied, Douglas-fir shows a pattern consistent with results found in the angiosperms. Seed-cone buds develop in distal regions of the shoot where higher auxin concentrations would be expected, and pollen-cone buds usually develop in more proximal regions that might have an auxin-gibberellin balance more favorable for pollen-cone development. At the present time, this remains speculative since no studies have been made of Douglas-fir or any other conifer that shows that the auxin-gibberellin balance is different in distal and proximal positions of the shoot. However, since primordia in Douglas-fir are undetermined at the time of initiation, these factors may affect differentiation of primordia during the early developmental period.

Although several studies have been made on the effects of plant hormones on cone initiation and development in conifers (Hashizume, 1959, 1960, 1961; Saito, 1957; Pharis *et al.*, 1965; Pharis and Morf, 1968; Owens and Pharis, 1967, 1971), relatively little is known about the endogenous hormones present in the various tissues of conifers. Native gibberellins in the Cupressaceae (Kato, Purves and Phinney, 1962; Ruddat, Pharis, Aoki and Crozier, 1968; Hashizume, 1969) and several species of *Pinus* (Krugman, 1967; Michalski, 1967; Michniewicz, 1967) as well as Douglas-fir may eventually allow us to relate this growth hormone to reproduction. An early study by Dinus (1963) on auxin levels in Douglas-fir included only relatively juvenile non-reproductive trees. Young trees showed increased auxin and decreased β -inhibitor levels as they matured, but the absence of mature trees in the study makes it impossible to generalize regarding cone development. Other endogenous plant growth regulators have not yet been studied in conifers.

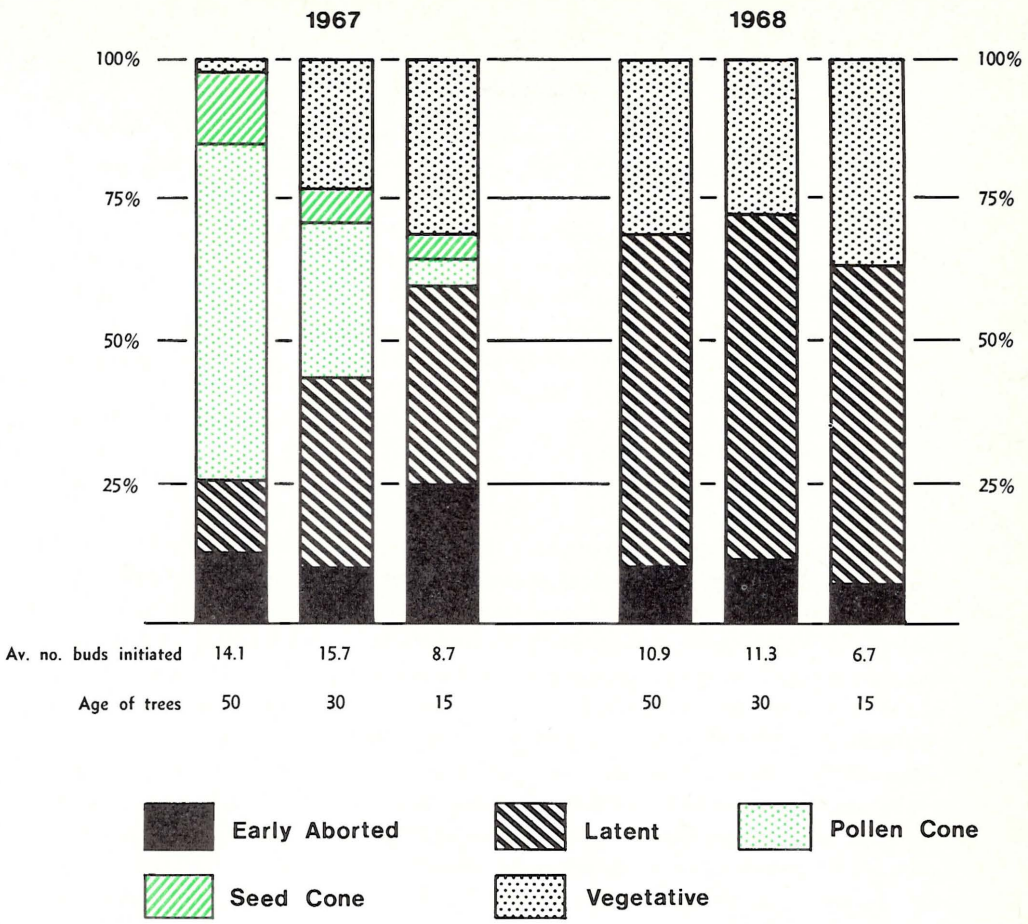


Figure 2.6 Percentage of Axillary Bud Primordia that develops into various bud types.

Summary of the three ages of trees on three sites showing the average number of primordia initiated per branch and the percentage of the primordia that developed into the various bud types in 1967 and 1968. Redrawn from Owens (1969).

Juvenility

The problem of juvenility and reproduction in conifers is no doubt tied closely to types and amounts of growth hormones. Most conifers, before producing cones, pass through a juvenile period of several years. Gibberellin treatment of seedlings in certain species of the Cupressaceae and Taxodiaceae has overcome this problem and has stimulated cone formation

in some seedlings within three months of seed germination (Pharis *et al.*, 1965). The bypassing of the juvenile period in these species, however, is temporary, existing only as long as gibberellin treatment continues. The potential of these results in the area of forest-tree breeding is considerable but, unfortunately, similar results have not been obtained in the more economically important species.

Photoperiod

In some conifers where there may be a time lag of several weeks between initiation of pollen- and seed-cone primordia along the shoot, the suggestion has been that this could provide enough of a day-length difference to explain sex determination on a photoperiodic basis (Giertych, 1967). This is not the case in nature in Douglas-fir because the exact time of initiation, as determined by histochemical tests, does not vary from the proximal to distal end of the shoot axis.

Japanese workers first demonstrated a relationship between day-length and type of cone produced in *Cryptomeria japonica* (Hashizume, 1961). Cones were artificially induced by treating the trees with the plant hormone gibberellin A_3. They found that plants treated under long days produced about an equal proportion of pollen and seed cones, while plants treated under short days produced a higher proportion of seed cones. Similar results have since been demonstrated in several members of the Cupressaceae (Pharis, Morf and Owens, 1969). The generalization has been advanced that, at least in some conifers, long days promote pollen-cone development, while short days promote seed-cone development.

More recent work in *Thuja plicata* under natural conditions shows that in this species both pollen and seed cones are initiated under long days but pollen cones are initiated and begin development under increasing day-length, while seed cones are initiated and develop under decreasing day-length (Owens and Pharis, 1971). Conclusive evidence that day-length causes differences in cone development has not been shown, except in the case where initiation of cones in *T. plicata* is accomplished with gibberellin application (Pharis and Morf, 1968). More precise determination of time of initiation and sequence of development in many species will help determine whether day-length appears to have an effect on the type of cone formed.

Nutrition

A factor that has not received adequate attention is the nutritional condition of the expanding shoot on which the developing primordia are found. Mineral content (Rehfuess, 1970) and food reserves (Allen, 1942d) within expanding shoots may vary greatly from year to year and this may affect growth and development. Cone maturation has been shown to reduce cambial growth

substantially in *Picea abies* (Matthews, 1963; Eklund, 1954), *Abies grandis*, *Pinus monticola* and Douglas-fir (Eis, Garman and Ebell, 1965) and leaf production in *Abies balsamea* (Morris, 1951). The diversion of carbohydrates from vegetative growth to cone and seed development in Douglas-fir was reported by Ching and Ching (1962) and Ching and Fang (1963). Starch grains appeared in cones in early April and filled all the parenchyma cells by June 1. Ovuliferous scales served as temporary storage sites for food needed for seed growth.

Girdling experiments have been performed on double-stemmed, older Douglas-fir trees where one stem served as a control (Ebell, 1971). Best results were obtained when girdling was done in the spring at the time of axillary bud development. The number of cones produced the following spring on the girdled stems was 7.4 times that of control stems and correlated with a greater proportion of buds that developed, i.e., fewer latent buds. Girdling increased starch levels during the critical period of early development, indicating a relationship between high starch content of twigs and the proportion of primordia developing into cones on the twigs. The presence of many developing seed cones on the previous year's growth subtending the expanding shoot has an important effect on the vigor of the expanding shoot in Douglas-fir and, therefore, on the development of primordia on the expanding shoot. Shoots subtended by developing cones generally have reduced shoot and leaf elongation (Owens, 1969), as well as reduced carbohydrate concentration and a lower percentage of primordia that develops fully (Ebell, 1971).

Nitrogen, like carbohydrate, was shown long ago to be diverted to growing reproductive tissue in fruit trees (Murneek, 1925). It has recently been found in Douglas-fir that nitrate nitrogen applied during early bud development increased cone production two to seven times, whereas ammonium nitrogen produced no response. Both forms of nitrogen gave similar shoot growth and increases in total nitrogen within twigs. Nitrate treatment, however, increased total free amino acids and, more important, the proportions of several amino acids. Large accumulations of basic amino acids, notably arginine and guanidino substances, resulted from nitrate treatment and were quantitatively associated with increased cone development (Ebell and McMullan, 1970).

Analysis of nitrogen, phosphorus and carbohydrate contents of expanding and 1-year-old shoots in Douglas-fir seedlings showed that year-old shoots supplied substantial amounts of these materials to new growth in the spring and abundant reserves were necessary for vigorous new shoot growth (Krueger, 1967). Chlorosis, suggesting a nitrogen deficiency, is present in elongating shoots subtended by expanding seed cones for a much longer time and is much more extreme than in shoots not subtended by expanding seed cones (Fig. 7.1 J). The more rapid period of growth of subtending cones, which would serve as a sink for nutrients, minerals and hormones, occurs from early March to July and more than covers the critical period of development of lateral buds on the elongating shoot (Owens and Smith, 1965;

Ching and Ching, 1962). Removal of all subtending developing seed-cone buds from a branch results in the development on that branch of reproductive buds and a decrease in the number of latent buds. This could explain, in part, why, in Douglas-fir, abundant cone crops do not occur in successive years (Isaac, 1943; Lowry, 1966; van Vredenburch and Bastide, 1969).

Why Is There a Cycle?

Explaining why abundant cone crops do not occur every second year is speculative, but it is apparent from Isaac's (1943) and Lowry's (1966) records for the years 1909-1956 that the time between abundant or medium cone crops in a region is from 2 to 7 years and is commonly about five years. These data give only the abundance of mature seed cones produced and do not distinguish between those that failed to develop during the critical early weeks of development and those that were lost as a result of numerous possible causes during the subsequent 14 months. An explanation of why the expected 2-year cycle would actually seldom occur and a longer cycle result must take other environmental factors and at least the entire 17-month period of development into consideration. Good cone crops have even been correlated with a cool cloudy summer 24-26 months previous (Lowry, 1966; van Vredenburch and Bastide, 1969). Another study (Silen and Copes, 1972) shows an effect 30 months before seed release. How these environmental influences affect the tree physiology as it relates to bud development has not been explained. More studies must be made of physiological requirements for cone development that can be related to additional ecological and morphological information.

Although it is not possible to relate all of the information pertaining to cone development in order to give a complete explanation of the cyclic nature of cone production in Douglas-fir, certain factors are related in an intelligible manner. Reproductive buds appear to have higher nutrient requirements than do vegetative buds. Vegetative buds in turn have higher requirements than partially developed latent buds or primordia that abort. Both carbohydrate, especially reserve starch, and certain amino acids appear to be required for cone-bud development. An elongating shoot on which primordia develop obtains most of its nutrients from the subjacent 1-year-old shoot. When the subjacent 1-year-old shoot bears expanding seed cones, the elongating shoot is usually chlorotic, not vigorous, and lateral bud development is poor. Few or no reproductive buds develop and the vegetative buds that do develop are neither large nor vigorous. Expansion of the vegetative buds the following spring usually results in shoots that are not vigorous. This does not provide optimal conditions for bud development during the year of the good cone crop, nor in the following year. The second spring after a good cone crop would likely be the first time when conditions within the tree would again be suitable for cone-bud development. This assumes that environmental factors are favorable. As a result, it would normally be at least 3 years between heavy cone crops in Douglas-fir. This, un-

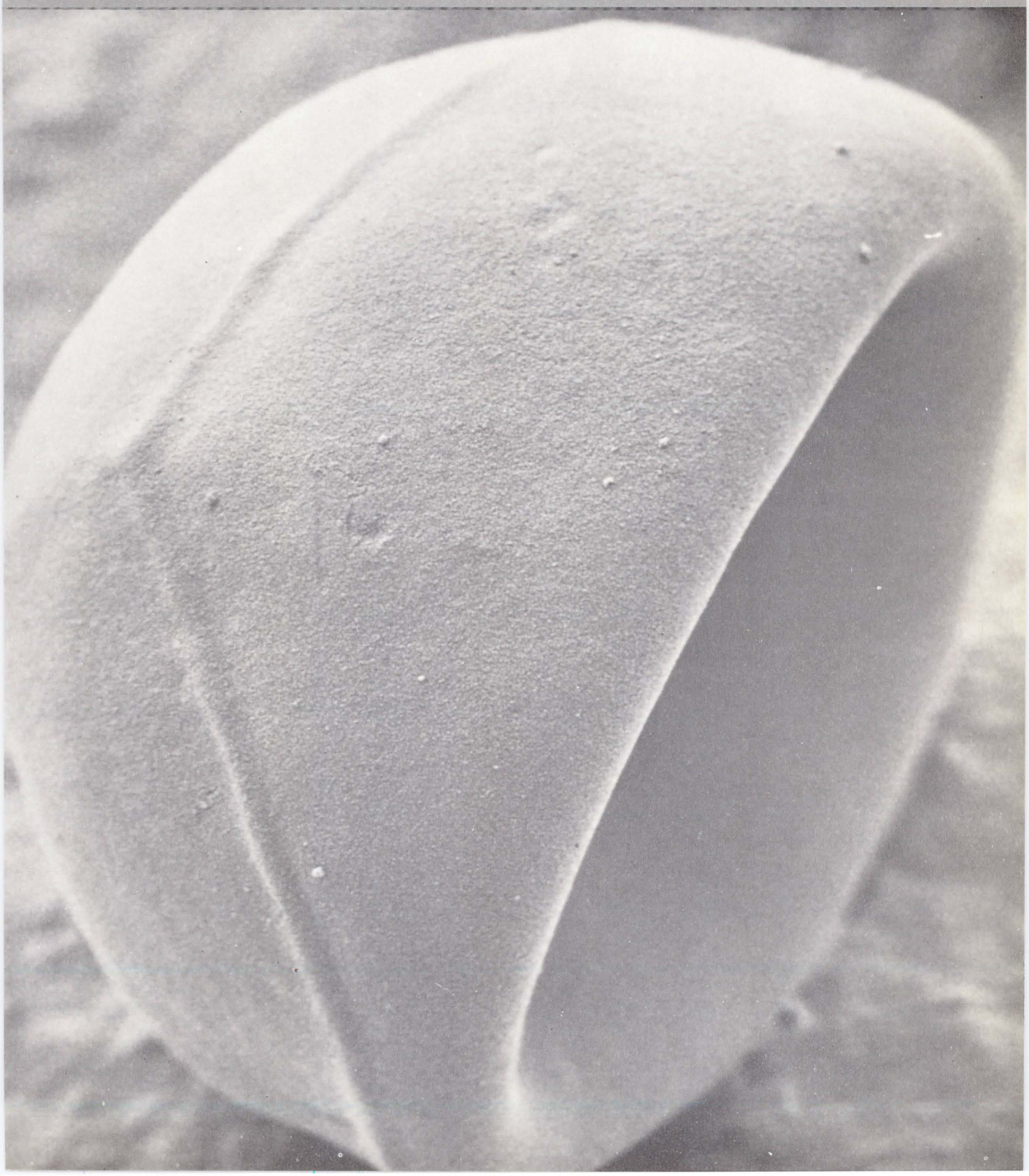
fortunately, does not take into consideration all of the factors affecting the last 14 months of cone development, but simply the early development of cone buds. However, a cycle approaching 3 years should be possible under carefully managed seed orchard conditions where fertilizer application, pollination, frost and insect damage can be partially controlled.

How hormones affect cone development in Douglas-fir is not known. Hormones may prove to vary considerably with environment, age of tree, position of shoot on the tree and position of primordia on the shoot. The influence of hormones would probably be affected by nutrient levels within the tree. When more information becomes available on nutritional and hormonal requirements, it may be possible to relate all of these factors in a meaningful way and arrive at a more complete understanding of cone development in Douglas-fir.

An understanding of the cause of a cyclic pattern of cone production in Douglas-fir may help explain similar cycles in other conifers if the phenology of the reproductive cycle is known in the other conifers. Conifers, such as *Abies,* have a similar pattern of axillary bud initiation and cone development. Therefore, the cyclic pattern is similar to Douglas-fir and possibly the problems and solutions also. Conifers that initiate cones by the transition of a previously vegetative shoot such as the Cupressaceae, *Tsuga* and *Picea,* also have a cyclic cone production. The problems here may be more complex because of possible differences in day-length during the transition of vegetative apices to either seed or pollen cones. Pines are likely to be less cyclic since three years are involved in cone development rather than two, and growth of cones extends over a longer period of time than in other conifers. Position of cones along the stem, pollen cones at the base and seed cones more distal, is similar to Douglas-fir but, in pine, these primordia are initiated at different times. This again may relate to day-length and its possible relationship with hormones and type of cone produced. It is evident at this point in our understanding of conifer reproduction that generalizations should not be made from one species to another unless the pattern and phenology of cone initiation and development are similar. The distinction should also be made between problems involving initiation and cone development.

Scanning electron micrograph of Douglas-fir pollen. x2250.

The Pollen Cone
3



Chapter 3

The Pollen Cone

Pollen cones, or microsporangiate strobili, are less familiar than seed cones because of their small size and short life. In most species they are enclosed within bud scales for most of the year, become visible at the time pollen is released, and remain on the tree for only a few weeks or a few months thereafter.

All conifers bear pollen cones in the form of simple strobili. The pollen cone, a simple strobilus, consists of a single axis bearing a series of usually spirally arranged pollen-forming appendages, the microsporophylls. No structures form in the axils of the microsporophylls so it is not a compound structure (Doak, 1935; Allen, 1946a; Owens and Smith, 1964). The pollen cone has been referred to as microsporangiate strobilus, staminate strobilus, male strobilus, male flower or male cone. Microsporangiate strobilus is morphologically the most accurate but seldom-used term, and male flower has for years been in common usage. The use of male and female when referring to sporophytic (spore-producing) structures such as cones is incorrect. It is only the gametophytic (gamete-producing) structures, pollen grains and female gametophytes, that can correctly have a particular sex attributed to them. They are the only structures that ultimately produce male and female gametes. The misuse of male and female in this manner, however, seems too well-established to be easily overcome.

Pollen cones vary in shape from globose in many Cupressaceae to the more familiar cylindrical shape in most other conifers. Their appearance is largely determined by the nature of the microsporophylls. Some appear very leaf-like, as in certain species of *Araucaria* and *Picea,* while in most other conifers, including Douglas-fir, they are very reduced, blunt, sac-like structures. In Douglas-fir, the leafy blade of the microsporophyll is much reduced and only the tip is apparent beyond the swollen microsporangia (Fig. 3.1 C, K). The microsporangia are commonly borne on the abaxial (lower) surface of the sporophyll. The dominant number of microsporangia is two, being consistent throughout the Pinaceae, but many other coniferous species have more (Chamberlain, 1957; Foster and Gifford, 1959).

The distribution of pollen cones on a tree varies with the species. They are normally restricted to, or more abundant on, the lower branches, although in some species they also form on upper branches. On a particular branch, pollen cones are generally more proximally located than seed cones. Pines commonly bear pollen cones in a cluster (actually a tight spiral) at the base of a branch. Douglas-fir frequently bears a basal spiral of pollen cones (Fig. 2.5, D, E); but, more commonly, they are evenly distributed

in axils of leaves along the proximal portion of the branch. Still other conifers, e.g. hemlock and members of the Cupressaceae, bear pollen cones, usually singly, at the tips of proximal branches. Many anomalies have been recorded, including bisporangiate strobili and proliferated cones, in a wide variety of conifers, but these appear infrequently.

Phenology of the Pollen Cone in Douglas-Fir

Unlike seed cones, the life cycle of the pollen cone is only slightly more than one year in duration. During this time they become conspicuous for a few weeks during pollination in the spring. Pollen-cone buds are initiated by the first of April as undetermined axillary bud primordia — the same as vegetative and seed-cone buds. Generally, by early June, pollen-cone apices can be distinguished from other apices only by histochemical means, but by early July, a distinction can be made by carefully removing the bud scales and observing the apex (Fig. 2.3 D-F). At that time microsporophylls begin to be initiated and the apex continues to enlarge (Fig. 3.1 G-I). Microsporangia develop on the microsporophylls throughout the summer and all microsporophylls and microsporangia are formed by early fall (Fig. 2.5H). Pollen-cone buds can usually be distinguished externally from seed-cone buds by the end of July. Buds appear dormant by early December. Although the earliest stages of meiosis begin early in the fall, mature pollen does not form until spring. Cones enlarge during March and burst through the bud scales about the first of April (Fig. 3.1 A-C). Pollination may continue on a tree for about 2 weeks. The pollen cones become completely dry and usually fall from the tree within a few weeks. This cycle is similar to that in most other conifers in that pollen cones are initiated approximately 1 year before pollination. The precise time of pollen-cone initiation and pollination, however, may vary considerably among species.

Pollen-Cone Development

Early development of the pollen-cone bud involves frequent cell divisions in all planes, which causes a small, dome-like apex to become visible in the leaf axil (Fig. 2.2 E, F). Bud scales begin to be initiated within a few days after lateral bud initiation, when the bud primordia are only a few cells in height (Fig. 2.2 G). The apex enlarges more slowly than potential vegetative and seed-cone apices. As a result, the zonation pattern described for pollen-cone apices (Chapter 2) does not become apparent as early as in the other types of apices. The apex gradually assumes a more conical appearance, while bud scales continue to be initiated along the flanks of the apex, elongate, overarch and enclose the apex (Fig. 3.1 D-F). Toward the end of the period of bud-scale initiation, about mid-July, the pollen-cone apex shows a zonation pattern similar to but less distinct than that described for vegetative apices (Fig. 2.3 G, I). Bud scales enclosing the pollen cone

are fewer in number than in vegetative buds. Whether this has any influence over pollen-cone development or is simply another manifestation of development has not been determined.

The base of the developing bud, where it attaches to the branch from which it originated, broadens during apical enlargement. This results from both the broadening of the pith and the formation of a meristematic region, a receptacular meristem, in the cortex of the developing bud. Similar growth occurs in all lateral buds, but to a lesser extent in pollen-cone buds. As a result, pollen-cone buds attach less firmly to the branch. It is this region of the cone axis that breaks so readily after pollination and this partially explains why Douglas-fir pollen cones seldom remain long on the tree following pollination.

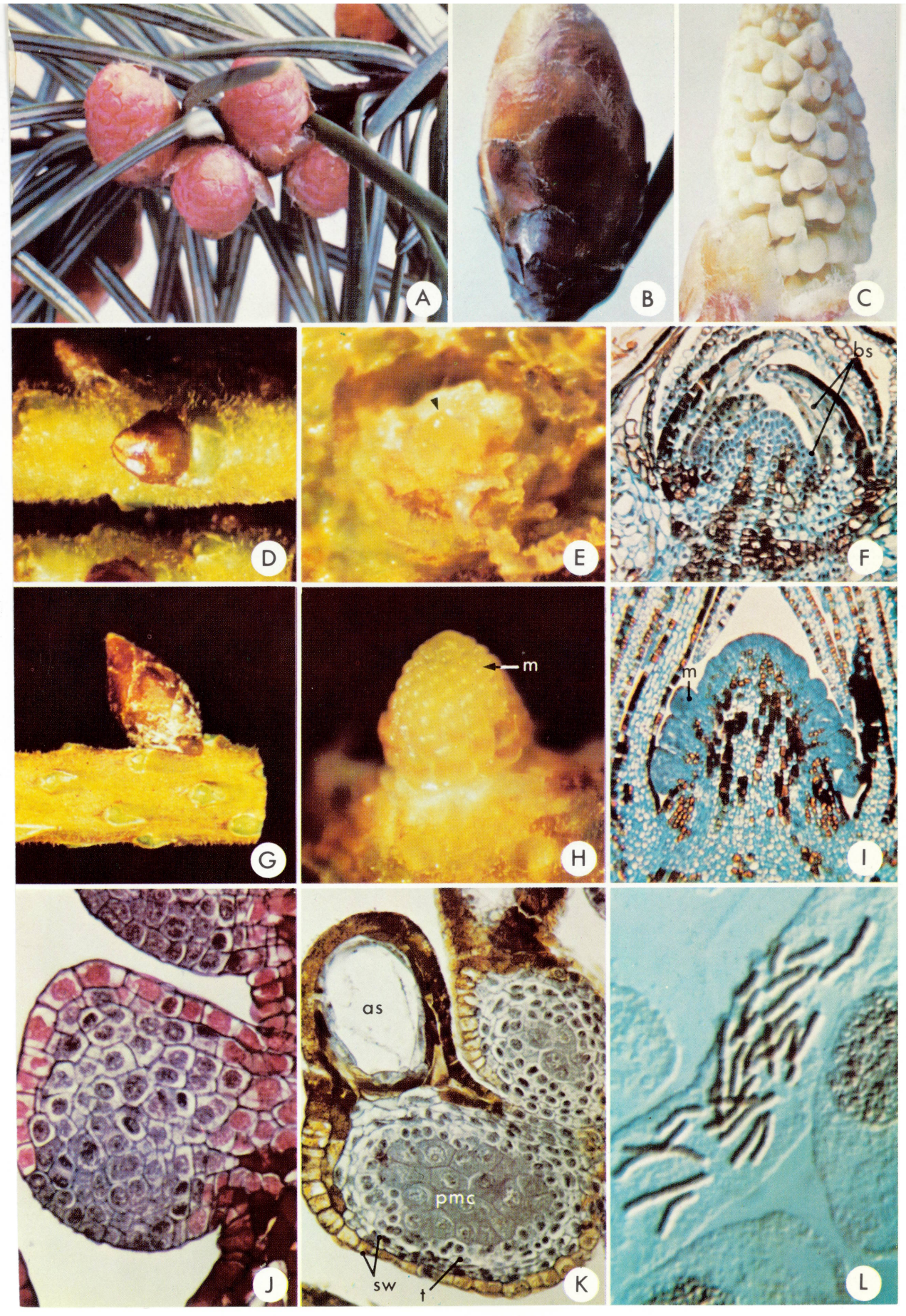
Microsporophyll initiation begins after all bud scales have been initiated, about mid-July, and is complete by early fall. The pollen-cone apex at the onset of microsporophyll initiation is slightly smaller, about 200 μ high and 200 μ wide, than seed-cone or vegetative apices at the same stage of development (Fig. 2.3 G-I). The entire bud enlarges during microsporophyll initiation but because microsporophylls are initiated in rapid succession up the flanks of the apex, the apical dome is continually "used-up" and gradually diminishes in size (Fig. 3.1 H, I). When microsporophyll initiation is complete, the apex is reduced to a flattened dome — 60 μ high and 200 μ wide.

The method of initiation and early stages of development are very similar in microsporophylls and leaves (Allen, 1946a; Owens and Smith, 1964). Microsporophylls are first evident as a group of surface cells that elongate radially, divide periclinally and produce a symmetrical, hemispheric protuberance — the primordium. Continued divisions become organized in a predictable manner and, as in leaves and bracts, an apical form of growth occurs. Microsporophylls, however, do not elongate nor grow upward as much as leaves or bracts but form blunt foliar appendages. This is a result of equal growth on both surfaces and especially abaxial cells dividing in all planes, resulting in increased volume rather than causing elongation. The microsporophyll thus remains short, more massive, and stands more perpendicular to the cone axis than bract or leaf primordia (Fig. 3.1 I-K). Due to continued rapid growth, the abaxial side swells and gives rise to two microsporangia, one on either side of the midrib, completely joined along their inner surfaces (Fig. 3.1 C). The midrib extends beyond the adjacent microsporangial regions and forms a pointed, upturned tip that contains a large air space (Fig 3.1 K). The mature microsporophyll then consists of a reduced foliar structure with a single vascular bundle anatomically similar to that in leaves and bracts. On the abaxial surface are two large, medianly fused microsporangia that give the structure a blunt, pouched appearance (Fig. 3.1 C).

Microsporangial initiation occurs when the newly formed microsporophyll is only about 75 μ long. Microsporangia originate by the division of several superficial (protodermal) cells on the abaxial surface of the microsporophyll

Figure 3.1 Pollen-cone Development.

Pollen cones as they appear shortly before pollination (early April). Pollen cones have just burst through the bud scales but pollen has not been shed. X4. B. Close-up of a pollen cone just before bud burst. X6. C. Close-up of a pollen cone after bud burst to show pointed microsporophylls each bearing two sac-like microsporangia. Elongation of the cone axis has caused the separation of microsporophylls. X6. D-F. Pollen-cone buds collected at the end of June when nearly all bud scales have been formed but before any microsporophylls are initiated. D. Whole pollen-cone bud with bud scales intact. X12. E. Apex (arrow) of pollen-cone bud shown in D with all bud scales removed to show appearance of the small conical apex. X36. F. Longitudinal section of a pollen-cone apex as shown in E. Bud-scale (bs) initiation is apparent along the flanks of the apex. X100. G-I. Pollen-cone buds collected early in August, after about two-thirds of the microsporophylls (m) have been initiated. G. Whole pollen-cone bud. X7. H. Pollen-cone bud with all bud scales removed to show characteristic color, yellow, of pollen cones at this stage. X45. I. Longitudinal section of a pollen-cone apex as in H to show the structure of the dome-shaped apex and the numerous blunt microsporophylls as they appear just before the initiation of microsporangia. X65. J. Longitudinal section through a microsporophyll to show the origin of the microsporangium from superficial cells on the abaxial surface X400. K. Longitudinal section through a microsporophyll collected in October showing the microsporangium and large sporogenous cells (pollen mother cells, pmc), tapetal layer (t), sporangial wall (sw) and large air space (as) at the tip of the microsporophyll. X 180 L. The 26 (diploid chromosome number) of Douglas-fir prepared from the vegetative shoot apex by the squash technique; stained with aceto-orcein and photographed with an interference constrast microscope. X 1400.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(Allen, 1946a). A considerable part of the growth in volume of the microsporangia results from divisions of surface cells producing abundant cells to the inside. Eventually these inner cells form the sporogenous tissue and the inner layer of the sporangial wall, while the surface cells from which they were derived form the epidermis of the microsporangia (Fig. 3.1 J, K). The mature sporangial wall, including the epidermis, is three-cells thick. The outer layer is not considered to be a true epidermis as it is in most other conifers, e.g. Cupressaceae, where sporangial initiation occurs from cells embedded deep within the microsporophyll and the epidermis is continuous over the microsporangia (Owens and Pharis, 1967). Sporogenous tissue completely fills each microsporangium before dormancy in the fall. Although other tissues of the pollen cone are inactive during November through mid-February, as indicated by certain enzyme levels, the sporogenous cells remain active throughout the winter but do not divide (Owens and Molder, 1971b). A single layer of cells, the tapetum, separates the sporogenous cells from the microsporangial wall. The tapetum is most visible in fall and winter as one or two layers of large cells with granular nuclei and dense cytoplasm. After meiosis is complete in February, the sporangium enlarges, causing the tapetal cells to show signs of separation. As pollen grains mature in March, tapetal cells become widely separated, the cytoplasm begins to degenerate, but the nuclei remain intact for a short time. The nutritive tapetal cells degenerate by the end of March when pollen grains are mature. Tapetal degeneration in Douglas-fir coincides with maturation, cell-wall formation and starch accumulation within pollen grains (Owens and Molder, 1971a).

The dormant pollen cone can be distinguished without dissection from vegetative lateral and seed-cone buds. They are equal in size or somewhat smaller, more globose, lighter brown, have fewer bud scales and less white resin on the surface of bud scales than vegetative lateral buds. They are similar in shape and coloration but smaller than seed-cone buds (Fig. 2.5 D-G).

Pollen Formation

The pollen grain when shed is the immature male gametophyte — the gamete-producing plant. In Douglas-fir, it usually consists of five cells, each of which is haploid, containing only one set of 13 chromosomes rather than two similar sets (26 or 13 pairs), as is found in the vegetative tissue of the tree (Fig. 3.1 L). The process by which the haploid pollen is produced is complex and involves many steps basically similar in all conifers. Two processes are actually involved: (1) Microsporogenesis, the formation of haploid microspores by meiosis; (2) the development of the mature multicellular pollen grain or male gametophyte from the one-celled microspore.

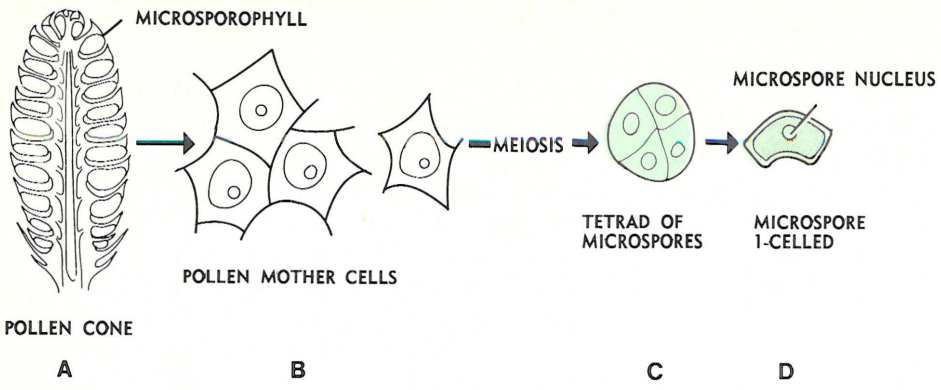


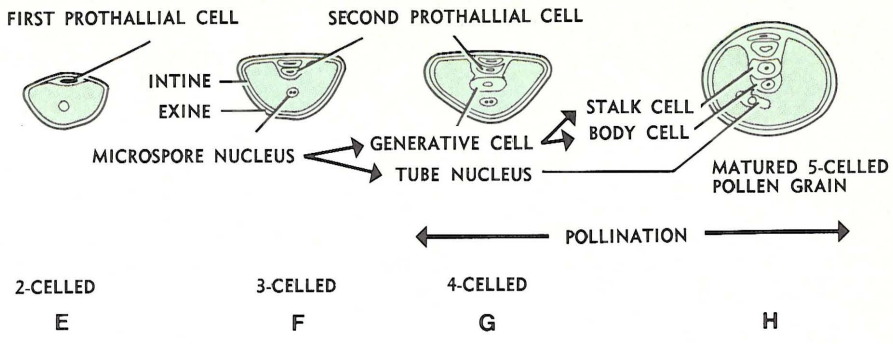
Figure 3.2 Meiosis and Pollen Development

A. The pollen cone is differentiated during summer and fall before pollination. Gametophyte (haploid) tissue is shown in color. B. Microsporophylls bear two microsporangia, each of which is filled with sporogenous tissue. Cells of the sporogenous tissue enlarge, forming the distinct pollen mother cells which begin meiosis in October. Meiosis becomes arrested at the diffuse diplotene stage during the winter and resumes in the latter part of February. C. Each pollen mother cell forms a tetrad of single-celled, haploid microspores. The microspore develops into a pollen grain during March. D. The one-celled stage. The microspore rounds out slightly, separates from the other members of the tetrad, the exine thickens and the cell accumulates starch. E. The 2-celled stage. The microspore divides, forming the first lens-shaped, prothallial cell around which forms the intine layer of the spore wall. F. The 3-celled stage. A second division produces a second, thicker lens-shaped, prothallial cell adjacent to the first. Intine forms within the exine and around both prothallial cells. G. The 4-celled stage. The third division produces a generative cell adjacent to the second prothallial cell and a large tube cell. H. The mature 5-celled pollen grain. The generative cell divides equally to produce a stalk cell adjacent to the second prothallial cell and a body cell. Both are enclosed within only a thin membrane except the base of the stalk cell where there forms a cup-shaped portion of the intine. Starch is abundant in the mature pollen grain. Pollen is usually shed at this 5-celled stage early in April. When the pollen germinates following pollination, the body cell divides to form the two male cells or gametes.

Microsporogenesis

Sporogenous cells within the microsporangia of a pollen cone divide by meiosis to produce microspores about mid-February at lower elevations. Stages of meiosis are reasonably synchronous in all microsporangia of a pollen cone but stages may vary considerably in different cones on a given tree and even more on different trees.

Zenke (1953) gave the first detailed description of meiosis in Douglas-fir. The course of meiosis was described and illustrated, the characteristic stages demonstrated, and the number and morphology of chromosomes during meiosis was given. Several partial accounts have been reported — most for the purpose of obtaining accurate chromosome counts (Sax and Sax,



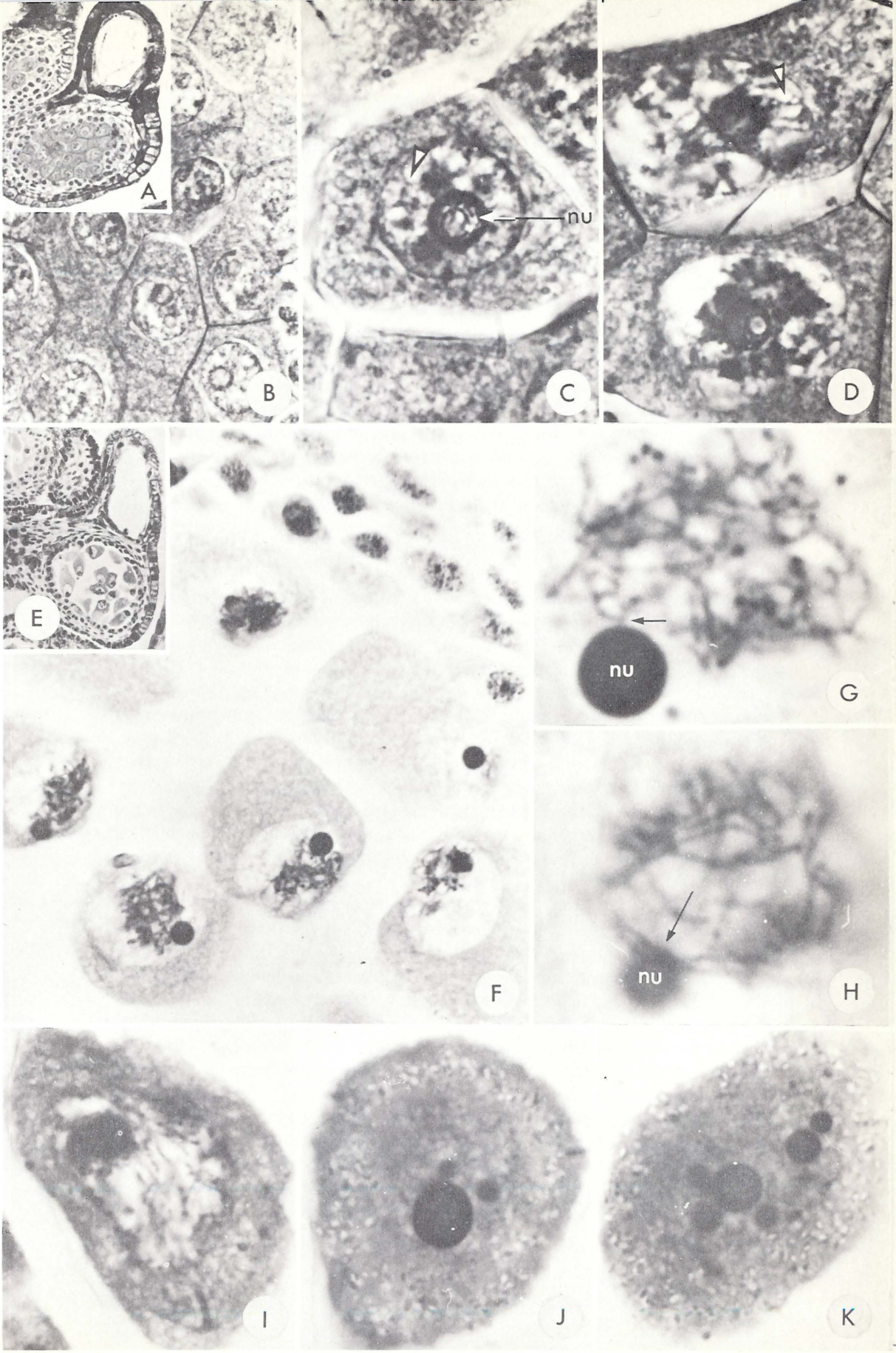
1933; Barner and Christiansen, 1962; Livingston, 1969). In all these reports, pollen cones were collected during late winter and studied in squashed preparations (Johansen, 1940).

In these preparations, it is often difficult to stain adequately and observe subtle nuclear changes during certain stages. Pollen cones collected from August to April, embedded in paraffin and sectioned have proven superior to squashed preparations for observing certain nuclear changes and have revealed that meiosis actually begins in the fall rather than in February, as previously thought (Fig. 3.3) (Owens and Molder, 1971b).

Early in the fall, pollen mother cells (pmc) appear as thin-walled, compact, polyhedral cells, about 40 μ in diameter. Cytoplasm is dark and granular. The nucleus is large (15 μ in diameter), distinct, and has a 2C amount of DNA. Characteristically, two very large, darkly stained nucleoli (1 μ in diameter) occur in each nucleus (Fig. 3.3 A, B). By early October, the chromosomes appear as very fine strands; there is evidence of at least some pairing and nuclei have a 4C amount of DNA, indicating that duplication has taken place. The chromosomes are arranged randomly throughout the nucleus and nucleoli remain large and distinct (Fig. 3.3 C). By mid-October, chromosomes become quite distinct and pairing is more evident (Fig. 3.3 D). Pachytene occurs by late October and chromosomes appear as thick, fuzzy strands that may be evenly distributed throughout the nucleus but are usually clumped in a tangled mass, filling only a portion of the nucleus near the large, distinct nucleolus (Fig. 3.3 E-H). Although this clumping appears much like synizesis (Swanson, 1957) which occurs in leptotene of many species, in Douglas-fir the chromosomes have already paired. A similar stage has been referred to as schizonema by Moens (1964) because homologues appear to separate along much of their length but centromeres remain paired. The next typical stage of meiosis (diplotene) is when chromosomes normally continue to shorten and homologous chromosomes repel, but in Douglas-fir a modified type or diffuse diplotene behavior is found. The chromosomes become quite diffuse and appear much as they did in

Figure 3.3 Early Prophase and Diffuse Diplotene Stages of Meiosis in Pollen Mother Cells

A. Longitudinal section through a microsporophyll collected early in October showing location of pollen mother cells. X90. B. Pollen mother cells in metabolic condition, before meiosis begins, as shown in A. X600. C. Early prophase stage of meiosis collected October 17 showing chromosomes as very fine strands after some pairing has occurred (arrow). The very large nucleolus (nu) is evident. X1150. D. Early prophase stage of meiosis, collected October 21, showing pairing (arrow) of relatively thick distinct chromosomes. X1150. E. Longitudinal section through a microsporophyll collected on October 28 showing the characteristic appearance of the pollen mother cells at pachytene. X100. F. Enlarged view of pollen mother cells shown in E at the pachytene stage of meiosis after chromosomes have duplicated and paired. Chromosomes and nucleoli commonly clump to one side of the nucleus at this stage. X600. G, H. High magnification of pollen mother cell nuclei at pachytene showing large nucleoli and the apparent nucleolar attachment to chromosomes (arrow). Alternating light and dark regions on the chromosomes are where the paired chromosomes coil about one another. X2400. I. Pollen mother cells collected in November showing the early diffuse diplotene stage when chromosomes increase in length and appear as very faint fuzzy lines filling the nucleus. X1250. J. Diffuse diplotene stage of meiosis of a pollen mother cell collected in February. The nucleus is indistinct and the chromosomes appear only as very faint lines. The small, clear dots outside the nucleus are starch grains which have accumulated during the winter "dormant" period. X1250. K. Diffuse diplotene stage of a pollen mother cell collected in February just before meiosis resumes. Starch is abundant in the cytoplasm and numerous nucleoli appear in the nucleus. This is the stage previous workers have mistaken for the leptotene stage. X1250.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early leptotene, i.e., very faint lines filling the nucleus (Fig. 3.3 I). Nucleoli also follow an unusual pattern and form many small nucleoli — 15 per nucleus is not uncommon. Nucleoli vary in size from 0.5 — 6.0 μ in diameter and are distributed throughout the nucleus (Fig. 3.3 J, K). Douglas-fir pollen mother cells remain in this diffuse diplotene stage from early in November through mid-February. During this time, pollen mother cells accumulate starch (Fig. 3.3 J, K). Other possible changes have not been studied. The typical diplotene phase follows during the latter half of February, which was previously thought to be the time when all stages of meiosis occurred (Owens and Molder, 1971b).

Similar diffuse diplotene stages have been found in *Larix* (Eriksson, 1968), in several angiosperms (Moens, 1964), in the oocytes of certain amphibians and fishes, and in the spermatocytes of certain insects (Swanson, 1957). In most cases, diffuse diplotene is correlated with a growth of cytoplasm. The phenomenon is more generally observed in eggs that go through a long period of development during which reserve food materials are stored in the form of yolk. A similar correlation exists in the sporogenous cells of Douglas-fir which remain in this prolonged diplotene stage from early in November to mid-February. During this time, there is no increase in cell size but there is an accumulation of starch (Owens and Molder, 1971a,b).

The later stages of meiosis are based primarily on the work of Zenke (1953). When meiosis resumes in February, the chromosomes move rapidly into the typical diplotene phase. The apparent attraction of homologous chromosomes gives way to repulsion, and they tend to separate. The longitudinal separation reveals that each bivalent consists of four chromatids. The separation is not complete, however, as the paired chromosomes remain held together at one or more points along their length. Each point of contact is a chiasmata that represents a point of exchange between chromatids and is the cytological expression of genetical crossing over. At this stage it is usually easy to determine which chromosomes serve as nucleolar organizers but, in Douglas-fir, the nucleoli and the nuclear membrane disappear at the end of diffuse diplotene, long before chromosomes shorten enough to be identified.

Diakinesis follows and is characterized by a more contracted state of the chromosomes and an even distribution of bivalents throughout the cell. Chromosomes continue to shorten, forming tight coils, loops or crosses, depending upon chromosome length and number of chiasmata present (Fig. 3.4 A). Thirteen bivalents become visible and show a characteristic shape for Douglas-fir which indicates that the position of the chiasmata is somehow controlled and extremely localized. As in other Pinaceae, very little terminalization, movement of chiasmata during prophase from an interstitial to a terminal position, occurs in Douglas-fir (Zenke, 1953). Infrequent crossing over, the small amount of terminalization, and stability of chiasmata in conifers have been suggested as reasons for the evolutionary stability of conifers and the rare appearance of chromosome aberrations and polyploids (Sax and Sax, 1933; Zenke, 1953).

The thirteen bivalents, now contracted to their shortest length, assemble across the center of the cell (Fig. 3.4 C). The bivalents separate and homologous chromosomes, each consisting of two chromatids, pass to opposite poles of the cell at anaphase I (Fig. 3.4 B). Each anaphase group consists of a haploid number of chromosomes, each consisting of two chromatids. The chiasmata apparently lose their retentive influence at the start of polar movement and free the separating chromosomes. The two chromatids constituting each chromosome then flare apart widely, being held together only at the centromeres to which the spindle fibers attach. Telophase I in plants commonly shows the reformation of nuclei at each pole and the formation of a new cell wall between the nuclei (Swanson, 1957). This usually occurs in Douglas-fir but occasionally the chromosomes rapidly orient at the poles and pass directly into metaphase of the second division. A cell plate partially forms between the two cells of the diad (Fig. 3.4 C). The chromosomes uncoil, become more thread-like, nuclear membranes form and several nucleoli usually reappear in each cell of the diad.

The chromosomes of the diad remain in this interphase condition for a brief period, then nucleoli and nuclear membranes again disappear and chromosomes shorten, but to a lesser extent than during the first division. The chromosomes reassemble across the center of each cell. Chromatids appear quite long and joined at the centromere with the ends widely separated. The second division results in separation of the two chromatids of each chromosome. Chromatids move to opposite poles, resulting in the production of four haploid nuclei from the original diploid cell (Fig. 3.4 D, E). Each chromatid might be quite different genetically from its condition at the initiation of the meiotic process, depending upon the number of times each chromatid was involved in crossing over, and, as a result, chiasmata formation. Nuclear membranes and nucleoli reform and chromosomes uncoil again, becoming thread-like. Thin cell walls appear between the cells, forming a tetrad of microspores enclosed within the thin wall of the original microspore mother cell (Fig. 3.4 E). Each microspore forms its own wall, and as microspores enlarge in March they burst through, freeing themselves from the original microspore mother cell wall.

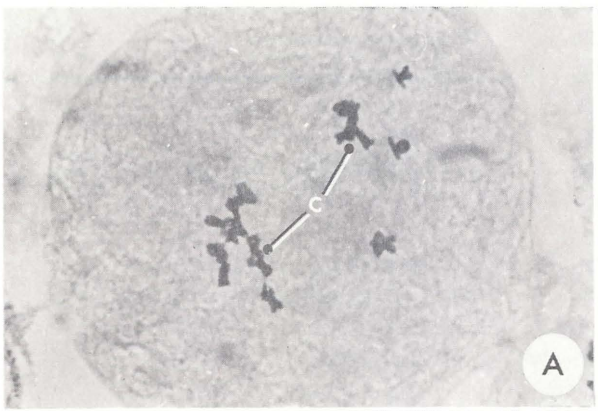
Irregularities in meiosis have been observed in Douglas-fir and other conifers (Zenke, 1953; Barner and Christiansen, 1962). Lagging chromosomes and chromosome bridges sometimes occur in Douglas-fir, which can result in aberrations in chromosome number and chromosome structure. However, the frequency of meiotic irregularities, a form of mutation, has not been estimated for Douglas-fir.

Development of the Pollen Grain

Development of the conifer pollen grain and subsequent development of the mature male gametophyte is described in elementary botany texts. It has been suggested that Douglas-fir pollen development differs in the sequence of cell divisions from that of *Pinus* (Christiansen, 1969), which

Figure 3.4 Late Stages of Meiosis in the Pollen Mother Cells.

A. During diakinesis, which follows the diplotene stage, the chromosomes (c) reach their most contracted state and the bivalents usually become evenly distributed throughout the cell. Thirteen bivalents are frequently visible and show a characteristic shape for Douglas-fir. X1000. B. Anaphase of the first division of meiosis showing homologous chromosomes passing to opposite poles of the cell. The chromatids separate and flare apart (arrow) since they are held together only at the centromere. X1000. C. Three stages in the first division of meiosis. Metaphase I (mp) shows the thirteen bivalents assembled across the center of the cell. Telophase I (tp) shows the chromosomes clumped at opposite poles of the cell following separation of the paired homologous chromosomes. Interphase (ip) actually represents the brief period between the first and second divisions when chromosomes uncoil and nuclei reform. No cell wall separating the two daughter cells is visible. X900. D. Anaphase of the second meiotic division where the paired chromatids of each chromosome separate and pass to opposite poles, resulting in four haploid daughter nuclei containing 13 chromosomes each. X1000. E. Cell walls form, separating the four haploid daughter cells which constitute the tetrad of microspores still enclosed within the wall of the pollen mother cell. Meiosis is normally complete by the end of February. X900.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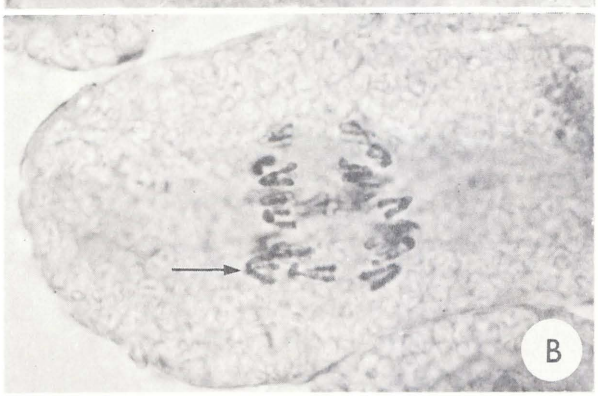
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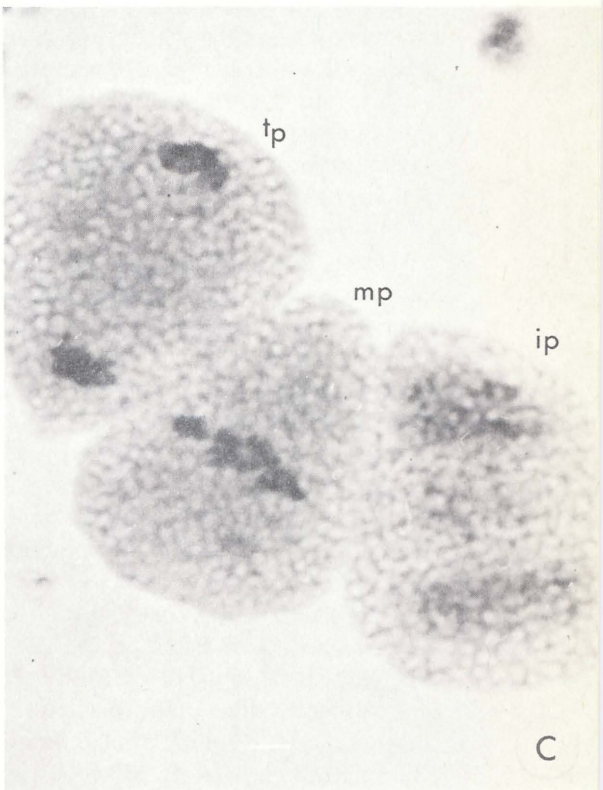
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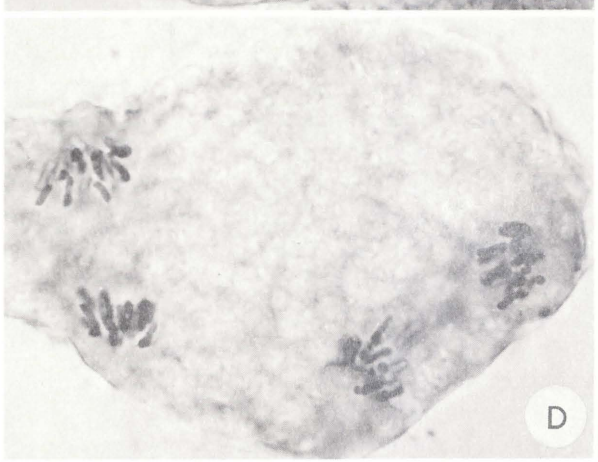
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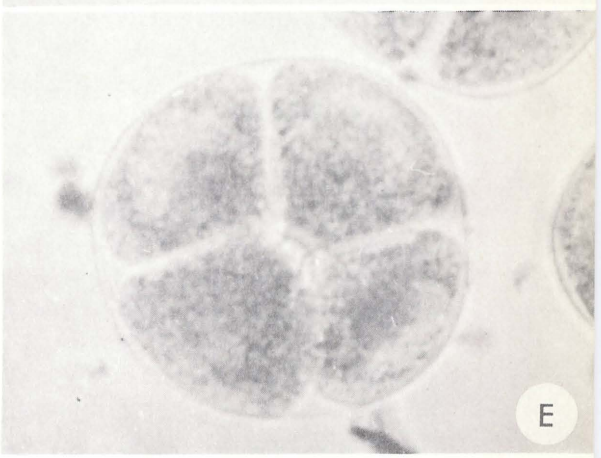
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is representative of most conifers (Chamberlain, 1957; Foster and Gifford, 1959; Bierhorst, 1971). More recent work, however, has shown that the early pollen development differs only slightly from that of *Pinus* (Owens and Molder, 1971a). The confusion concerning the sequence of division and formation of the mature pollen grain resulted from a lack of observation of actual cell divisions and from the use of only whole mounts of pollen or slides prepared by the squash technique. The early development of a thick cell wall on the pollen grain and the densely packed starch grains within, mask cytological detail and cell divisions. Also, the inadvertent squashing of pollen grains in the preparation of whole mounts produces many artifacts. Pollen cones sectioned at 10 μ during March when pollen develops, show very clearly all stages of development within the pollen grain (Fig. 3.2).

Meiosis is usually completed in Douglas-fir by the end of February and each microsporangium is filled with several hundred tetrads of haploid microspores borne in a watery fluid (Fig. 3.5 A, B). Each single-celled, haploid microspore develops into a pollen grain during March, within a few weeks following meiosis. Each microspore of the tetrad is angular and the four fit compactly together to form a sphere within the microspore mother cell wall. Each microspore contains a single, haploid nucleus and little starch. For the first three weeks following meiosis no cell divisions occur within the microspores. The cell wall of the microspore rapidly thickens equally on all surfaces. Microspores enlarge slightly but remain together within the microspore mother cell wall. Rapid accumulation of starch occurs until the cytoplasm of each microspore is densely packed with large starch grains. The outer wall or exine of the developing pollen grain thickens to about 2 μ . By the last week of March, microspores have enlarged sufficiently and rounded out enough to rupture the thin microspore mother cell wall, the fragments of which appear scattered throughout the microsporangium. Microspores, however, still retain an angular appearance (Fig. 3.5 C). The microspore nucleus then divides. One daughter nucleus remains in the center of the pollen grain and the other presses against the cell wall. A new cell wall forms, dividing the cytoplasm unequally, and the nucleus and a small amount of cytoplasm adjacent to the wall form the lens-shaped, prothallial cell (Figs. 3.5 D; 3.2 E). The wall that now forms inside the exine and around the first prothallial cell is the intine. It stains lighter than the exine and is separated from the exine by a distinct, thin, dark line. The microspore nucleus in the center of the developing pollen grain divides a second time, and a second prothallial cell is formed tightly against the first. The cytoplasm is again divided unequally but the second prothallial cell, though still lens-shaped, is thicker than the first (Figs. 3.5 E; 3.2 F). A wall, continuous with the intine, forms around the second prothallial cell. The intine at this three-celled stage becomes almost equal in thickness to the exine, with the wall around the second prothallial cell remaining slightly thinner. A third mitosis and unequal cell division result in the production of a generative cell adjacent to the second prothallial cell, and a larger tube cell, the nucleus of which remains in the center of the developing pollen grain and is termed

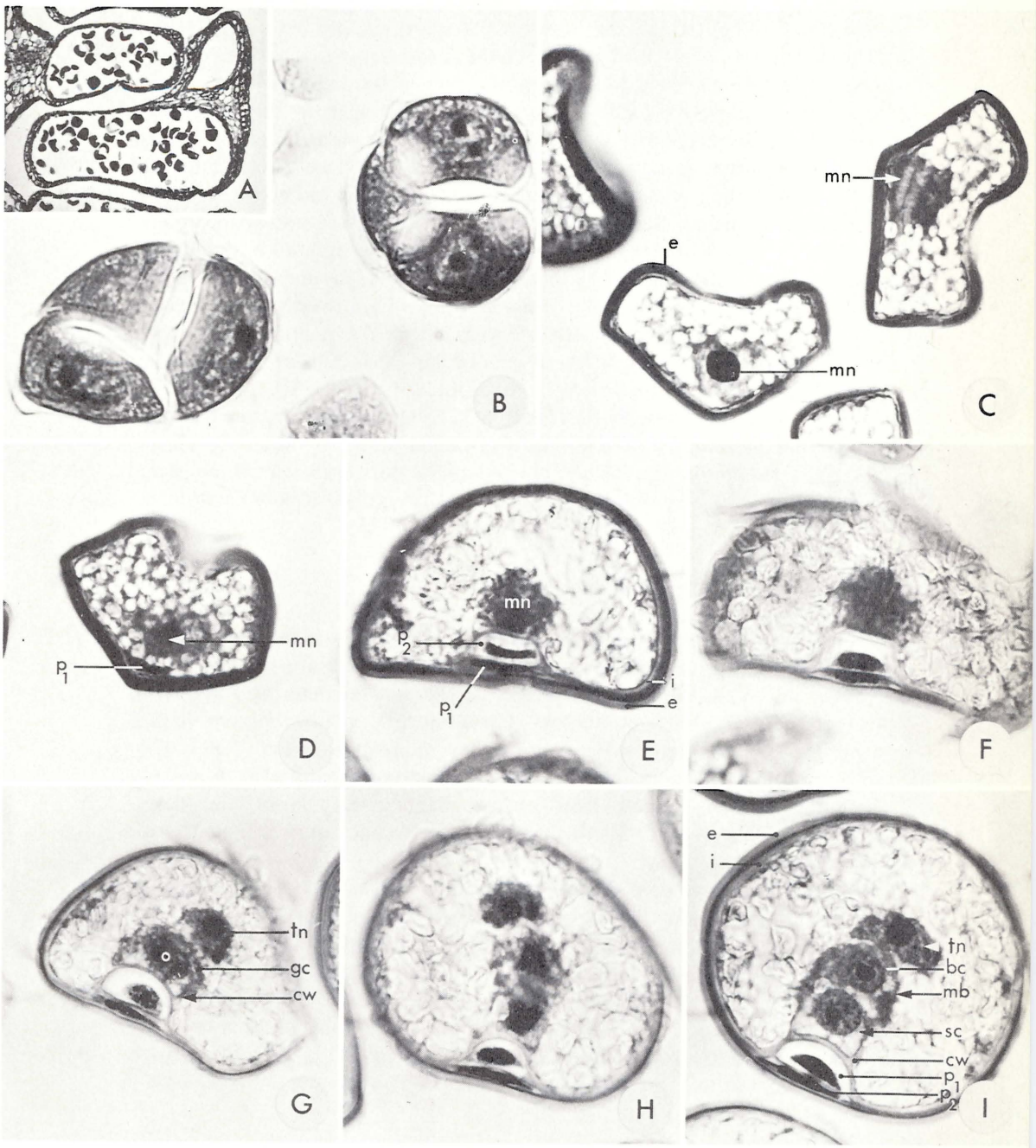
the tube nucleus. No special wall forms around the tube nucleus since the wall of the developing pollen grain, exine and intine, represents the wall of the tube cell (Figs. 3.5 G; 3.2 G). No thick cell wall forms between the generative and tube cells — only a thin membrane. The portion of the generative cell adjacent to the second prothallial cell does form a rudimentary cell wall indistinguishable from the intine. This wall extends slightly out around the generative cell and forms a shallow cup-shaped wall projecting from the wall of the second prothallial cell into the center of the developing pollen grain (Fig. 3.5 F-I). The thin membrane enclosing the generative cell is continuous with this cup-shaped cell wall. The generative cell then divides equally to produce a stalk cell adjacent to the second prothallial cell and a body cell adjacent to the tube nucleus (Figs. 3.5 H, I; 3.2 G, H). No thick cell wall forms around these cells but they remain enclosed within only a thin membrane. Whether this membrane consists only of the cell membrane or includes an extremely thin cell wall has not been determined.

The mature pollen grain of Douglas-fir at the time of pollination usually consists of five cells: two small lens-shaped prothallial cells; a stalk cell; a body cell, and a large tube cell. Mature pollen is 90-100 μ in diameter, approximately spheroid, and is usually indented on one side. Unlike some other conifers (Chamberlain, 1957), it lacks bladders (wings) and conspicuous pores or furrows (Barner and Christiansen, 1962). The thin microspore cell wall thickens during microspore enlargement and pollen grain development. The exine or outer wall layer is thick and its surface is very smooth except for a very faint triradiate ridge indicative of the mutual contact among members of the spore tetrad. The intine, or inner wall layer, is about equal in thickness (approximately 2 μ) to the exine (Figs. 3.5 I; 3.2 H).

It has been suggested that the generative cell does not occur in Douglas-fir (Christiansen, 1969a) and that a pore exists at the proximal pole of the pollen grain, adjacent to the prothallial cells (Barner and Christiansen, 1962). The pore was interpreted from whole mounts of mature pollen and was described as an aperture surrounded by a thickening of the intine. Upon squashing, two small discs, a flat one and a concave one, were observed by these authors near the proximal pole. These were interpreted as degenerated prothallial cells or membranes covering the pore. The pore was interpreted as the orifice of a membrane enclosing the body and stalk cells. It now appears that the two discs were prothallial cells extruded when pressure was applied and the wall separating the second prothallial cell and the stalk cell probably broke open and was interpreted as the pore. Moreover, the apparent pore, visible in whole mounts under the light microscope as a ring, is probably the cup-shaped, outer and partial wall of the stalk cell formed adjacent to the second prothallial cell. No pore is visible under the scanning electron microscope.

Figure 3.5 Pollen Development

A. Nearly mature microsporangium collected early in March showing many separate microspores and tetrads of microspores. X90. B-I. All are magnified 500 times to show relative changes in size and shape of the pollen grain during development. B. Tetrads of microspores as they appear early in March just after meiosis is complete. Each microspore contains a single haploid nucleus and little starch. C. Separate microspores after the pollen mother cell wall has ruptured. Microspores rapidly accumulate starch, shown here as small white discs, the exine (e) thickens and the microspore nucleus (mn) divides to form the first prothallial cell. D. Division of the cytoplasm is unequal in the formation of the first prothallial cell (p_1). The microspore nucleus remains in the center of the larger cell which divides a second time, forming the second prothallial cell. E. The second prothallial cell (p_2) is lens-shaped but thicker than the first. By this time, late in March, the pollen grain has enlarged, rounded out somewhat, and the intine (i) becomes almost equal in thickness (2μ) to the exine. Intine forms around both prothallial cells. F. A third mitosis occurs in the developing pollen grain. Abundant, large starch grains are quite visible at this stage. G. The four-celled stage of the pollen grain. The third unequal cell division has produced the generative cell (gc) adjacent to the second prothallial cell. No thick cell wall forms around the generative cell — only a thin membrane. The portion of the generative cell adjacent to the second prothallial cell forms a rudimentary cell wall (cw) that extends slightly out around the generative cell. A large tube nucleus (tn) is found in the center of the pollen grain. H. The generative cell divides equally to form the stalk cell and body cell. I. The mature five-celled pollen grain as seen at pollination consists of: two lens-shaped, prothallial cells enclosed by the intine; a stalk cell (sc) whose base is partially enclosed by the cup-shaped intine (cw); the body cell (bc) enclosed only by a thin membrane (mb); and the large tube cell containing the tube nucleus (tn). Extine, intine and starch grains are clearly evident.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Pollen of Other Conifers

Pollen grain development and structure are variable within the conifers (Chamberlain, 1957; Bierhorst, 1971). All conifers are wind pollinated and two-thirds of the genera, including *Pseudotsuga,* have pollen with no wings or bladders. When wings are present, there are usually two, as in *Pinus*, but pollen grains of several genera in the Podocarpaceae have two to six wings. The wall of the pollen grain has two distinct layers — the exine and intine. Usually the exine is thicker, but in Douglas-fir they are equal. Like *Pinus*, Douglas-fir is an example of the prevalent course of pollen grain development in conifers. Prothallial cells are a constant feature of the Pinaceae, while in all the Taxaceae, most of the Taxodiaceae and many of the Cupressaceae, they are lacking. Wherever there are no prothallial cells, pollen development occurs as in the angiosperms and is interpreted as being more advanced. In most of the Podocarpaceae and all of the Araucariaceae, many prothallial cells develop. This results from division of the two or three prothallial cells formed from the microspore nucleus. The male gametophyte of different genera can be found at various stages of development when the pollen is shed but, for a given species, this will vary little, if at all. Douglas-fir pollen may be shed at the four-celled stage but is more commonly shed at the five-celled stage. In some species of *Cupressus* and *Juniperus,* the uninucleate microspore is shed and subsequent development occurs within the seed cone before pollen-tube formation.

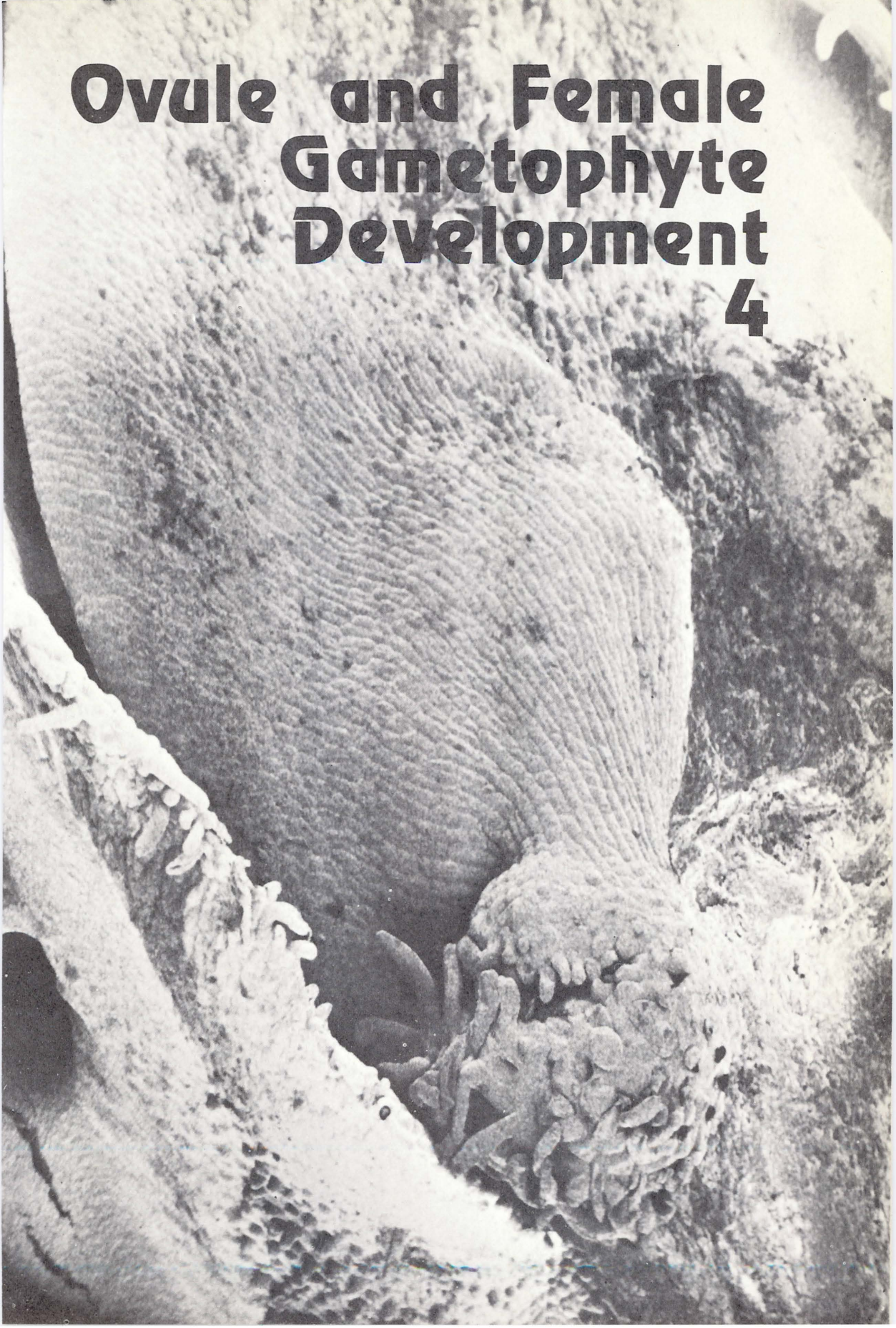
Pollen-Cone Enlargement in Douglas-Fir

The pollen cone enlarges throughout the month of pollen-grain development, usually March. The sporangium enlarges and the inner two or three layers of cells elongate, become spindle-shaped, and the innermost frequently degenerate by the time of pollination. The outer cells enlarge, become vacuolate and form the outer surface layer of the microsporangium. Cells of the tapetum remain unchanged during winter but become separated as the microsporangium enlarges. Tapetal cells separate, become irregular and degenerate as pollen grains mature. In the mature pollen cone, only fragments of a few tapetal cells remain.

Elongation of the pollen cone within its bud scales begins at the end of February, at about the same time as pollen development. Bud scales do not enlarge but the pollen cone does, forcing the bud scales apart. Pollen-cone growth results from elongation of the entire cone axis which causes separation of the microsporophylls and considerable elongation of the stalk at the base of the cone. No apical growth occurs and no new microsporophylls or microsporangia are initiated during this period of pollen-cone growth. Bud burst, resulting in shedding of the pollen, generally occurs early in April.

Scanning electron micrograph of the ovuliferous scale from a Douglas-fir seed cone before pollination showing the developing ovule and the rounded stigmatic tip bearing many hairs.

Ovule and Female Gametophyte Development 4



Chapter 4

Ovule and Female Gametophyte Development

Most accounts of seed-cone development and life histories for conifers describe the ovule after it has developed into a recognizable structure and undergone considerable differentiation. To present a complete story, the early development of the seed cone and the origin of the ovule must be considered as well as the more-familiar female gametophyte development depicted in most elementary texts.

The seed cone has been referred to by many names — some correct, others misleading. Probably the most accurate, from a morphological standpoint, is ovulate strobilus. Pistilate strobilus or cone, female cone or female flower are less accurate but commonly used. The seed cone is a compound strobilus, in that it consists of an axis or stem bearing a series of usually spirally arranged bracts. A single generally flattened, ovuliferous scale or modified branch is found in the axil of each bract and is attached to the cone axis just above the bract. The ovules which, upon fertilization, develop into the seeds, are attached to the upper or adaxial (toward the axis) surface of the ovuliferous scale. In most conifers there are two ovules on each ovuliferous scale. Following fertilization of the egg, the ovule matures to form the seed (Doak, 1935; Chamberlain, 1957; Foster and Gifford, 1959; Bierhorst, 1971).

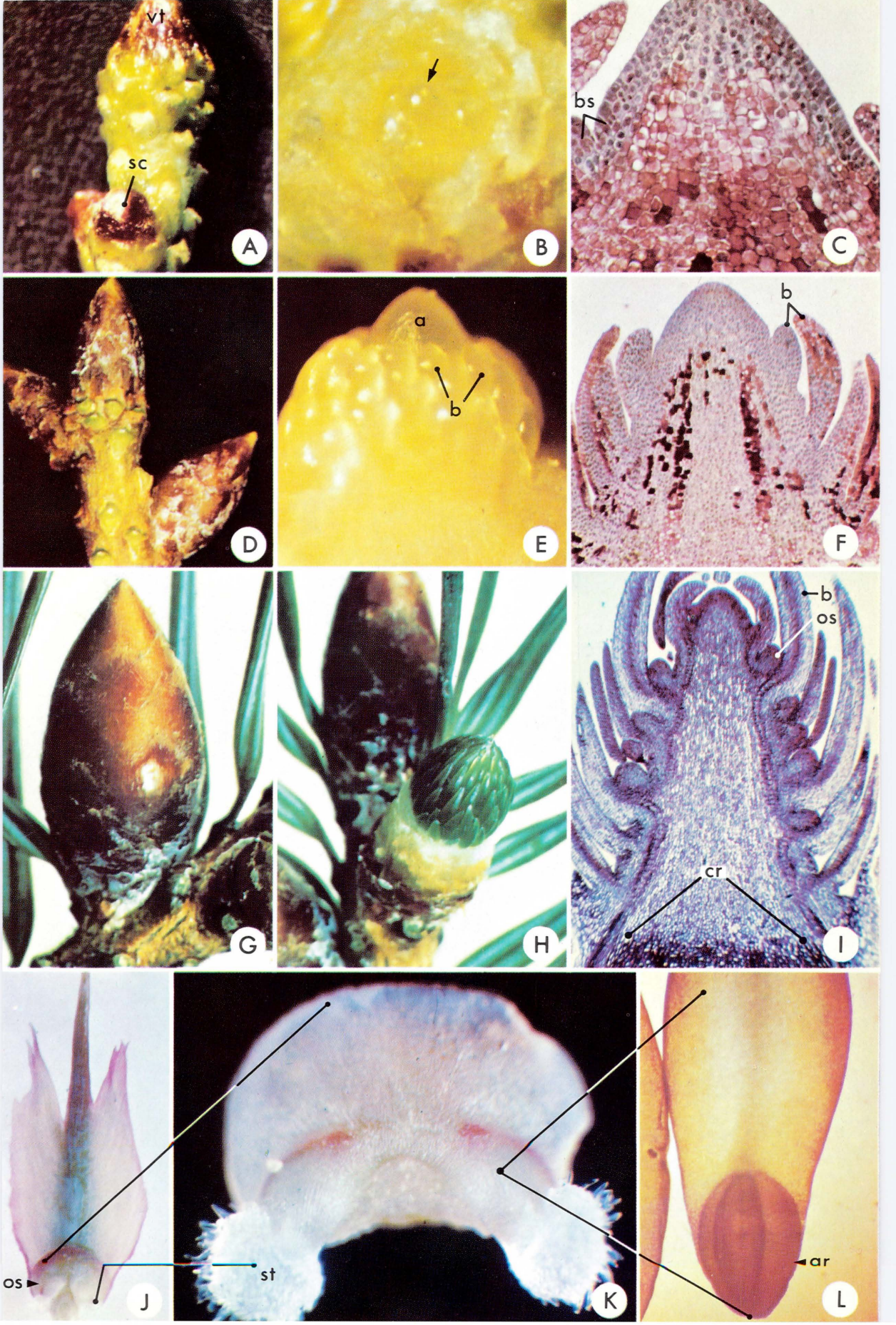
The general phenology of the Douglas-fir seed cone was described in Chapter 1 (Fig. 1.1). The purpose now is to describe in detail early development of the seed cone before dormancy and discuss the origin of bracts, ovuliferous scales and ovules. The female gametophyte, although it undergoes development following dormancy and pollination, will also be described in order to explain the significance of the gametophyte tissue in reproduction.

Early Seed-Cone Development

As stated in Chapter 2, all lateral buds are initiated at the same time, about April 1, and bud types are indistinguishable during the first ten weeks of development (Fig. 2.1). Lateral bud primordia, which potentially can become seed cones, enlarge slightly more than other primordia during this time. By mid-July, all bud scales have been initiated and the seed-cone bud has undergone considerable enlargement and a characteristic pattern of cellular zonation has become evident in the apex. (Fig. 2.3 H) (Owens,

Figure 4.1 Early Development of the Seed Cone.

A-C. Seed-cone bud and apex as they appear in mid-June after most bud scales have been initiated. A. Vegetative shoot showing the vegetative terminal bud (vt) and lateral seed-cone bud (sc). X9. B. Seed-cone bud with all bud scales removed, revealing the tiny apex (arrow). X20. C. Longitudinal section of seed-cone apex as shown in B. Bud-scale (bs) initiation is still evident. X125. D-F. Seed-cone bud and apex collected at the end of July after many bracts have been initiated but before the onset of ovuliferous scale initiation. D. Whole vegetative terminal and seed-cone bud X5. E. Seed-cone bud with all bud scales removed, revealing the apex (a) and bract primordia (b). X80. F. Longitudinal section of the seed-cone apex as shown in E. Several bract primordia are seen along the flanks of the apex. X80. G-I. Dormant, fully differentiated seed-cone bud as it appears from fall until early in March. G. General color and shape of the entire dormant seed-cone bud. X5. H. Dormant seed-cone bud with all bud scales removed, revealing the dark green, pointed bracts. X7. I. Median longitudinal section through a dormant seed cone as shown in H. All bracts and ovuliferous scales (os) have been initiated. The apex of the cone is reduced to a low dome and a distinct crown region (cr) has differentiated at the base of the bud. X25. J. Bract and ovuliferous scale dissected from a seed cone at the time of pollination in April. The trident bract is large and highly differentiated while the axillary, ovuliferous scale is still very small and just beginning rapid marginal growth. X10. K. Enlarged ovuliferous scale as shown in J, showing the position and stage of development of the two lateral ovules with their well-developed stigmatic tips (st) which entrap the pollen grains. X50. L. The fully differentiated female gametophyte dissected from the ovule early in June at the time of fertilization. Three of four darkly stained archegonia (ar) are shown at the lower (micropylar) end of the haploid gametophyte. X 35.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1969). Bud-scale initiation is essentially the same in all bud types except that both seed-cone and pollen-cone buds initiate fewer bud scales than do vegetative apices. Bud scales are borne on a receptacular structure, which develops in the same manner as in vegetative buds but to a lesser extent, and thus attaches to the shoot less firmly than do lateral vegetative buds (Owens and Smith, 1964).

At the end of bud-scale initiation, the apex is approximately 300 μ high and 530 μ in width. Apical enlargement continues but the apex broadens more rapidly than it elongates and by mid-July bract primordia begin to be initiated up the flanks of the apex (Fig. 2.3 E, H).The initiation of a bract is similar to that of a bud scale, leaf or microsporophyll. The bract primordium begins as a rather broad, flattened primordium. Cells on the abaxial portion of the primordium divide and elongate more rapidly than those on the adaxial portion, which results in upward growth of the tip of the bract. This continues with considerable elongation and the primordium soon assumes a pointed form (Figs. 4.1 E, F ; 4.2 A). Unlike leaves and microsporophylls, the bract undergoes considerable marginal growth. As a result, a lamina, or blade as in a leaf, rapidly forms and the bract becomes broad and flattened (Fig. 4.2 B-E). The lamina, in the dormant bud, extends about one-half the length of the bract, and the midrib of the bract distal to the lamina remains elliptical in transection and very similar in structure to the leaf of Douglas-fir (Fig. 4.2). Laminae are reduced or absent on bracts at the very base and tip of the cone (Owens and Smith, 1964).

Bracts are initiated over a period of 2½ months, from mid-July until the end of September, but the rate of bract initiation is not constant during this time. Half the final number of bracts are initiated during the first month, while subsequent bracts are initiated at a slower rate. During bract initiation, apical enlargement does not keep pace and as bracts encroach upon the summit of the apex, the apical dome becomes very low and broad (Fig. 4.1 E,F). Apical zonation gradually diminishes as the apical size decreases and little apical zonation is evident in the dormant seed cone (Fig. 4.1 I) (Owens and Smith, 1964).

Ovuliferous-scale initiation begins about the first of September, 5 months after the seed cone is initiated and after over half the final number of bracts have been initiated. Ovuliferous-scale initiation is delayed in the basal few bracts and begins first in those just above the base of the cone. Initiation then proceeds acropetally and basipetally; however, the lowest bracts never develop ovuliferous scales and the lowest scales formed never become large and seldom develop functional ovules. All functional ovuliferous scales are initiated by the time buds become dormant in the fall (Owens and Smith, 1964).

Although, morphologically, ovuliferous scales are modified lateral shoots (Doak, 1935), their initiation and early development are different from other types of lateral shoots. The ovuliferous scale is more truly axillary in origin instead of arising from cortical cells above the axillary region as do vegetative lateral shoots (Chapter 2). Early development of the bract and ovuliferous

scale are illustrated in Fig. 4.2. An ovuliferous scale is initiated in the axil of a bract when the bract is about 600 μ long and has just begun marginal growth. A slight bulge arises in the median axial region of the bract and extends along the proximal adaxial surface cells of the bract to produce a low, broad swelling of enlarged meristematic cells. The primordium increases in height, extending along the adaxial surface of the bract, and rapidly broadens until it nearly equals the width of the bract. This type of growth has caused some workers to mistakenly interpret the ovuliferous scale as an outgrowth of the bract. When the primordium is viewed from above, it has the appearance of an arc of slightly raised tissue extending the width of the bract. For a time, the ovuliferous-scale primordium grows perpendicular to the bract surface, raising and separating the ovuliferous scale from the bract. Subsequent growth occurs primarily along the arc-shaped margin of the ovuliferous scale and almost parallel to the bract. As a result, the bract and the ovuliferous scale remain unfused except at their very base. Marginal growth of lateral portions of the ovuliferous scale cause it to overgrow the margin of the bract. This growth continues for only a short time before the seed cone becomes dormant (Owens and Smith, 1964). In the latter part of September, megaspore mother cells begin to differentiate, and most have differentiated by mid-October. These form on the two proximal lateral portions of the curved ovuliferous scale (Fig. 4.2 E). The site of the megaspore mother cell, therefore, foreshadows ovule differentiation — whether or not it predetermines it (Allen, 1963). In the dormant seed-cone bud, ovuliferous scales remain as very small, spoon-shaped primordia. They are free from the bract along the curved margin and lack any tissue differentiation (Owens and Smith, 1964).

The seed-cone bud shows mitotic activity in both ovuliferous scales and bracts until early November at lower elevations, when they become dormant. The dormant bud is about 10 mm long and the cone within is about 3.0 mm long (Fig. 4.1 G-I). Seed-cone buds are readily distinguishable externally by their larger size from vegetative lateral buds and pollen-cone buds during dormancy. Unlike vegetative buds, but similar to pollen-cone buds, certain portions (ovuliferous scales) of the seed-cone buds remain metabolically active throughout the winter, at least at lower elevations. This activity is indicated by histochemical tests showing high levels of the enzyme succinic dehydrogenase, a good indication of the potential meristematic activity. Unlike pollen-cone buds, no evidence of early stages of meiosis in the fall or the diffuse diplotene stage has been observed in the megaspore mother cells (Owens and Molder, 1971b).

Development of the Ovule Following Dormancy

Development of the ovule resumes about mid-February and coincides with meiosis of both pollen mother cells and megaspore mother cells. This may be delayed up to a month at higher elevations. The first evidence of ovule differentiation is the development of a rim of tissue around both

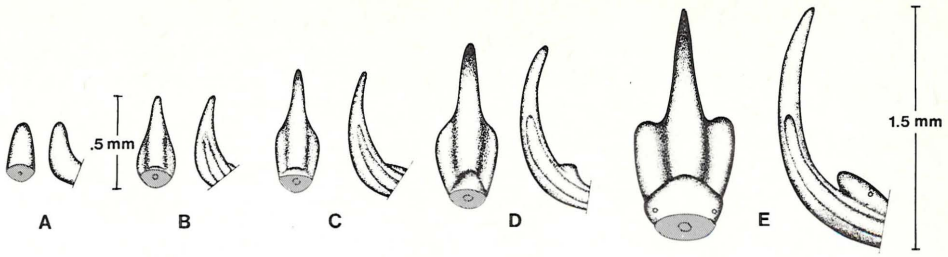


Figure 4.2 Early Development of the Bract and Ovuliferous Scale.

Each stage is shown in face and side view. Bracts, like leaves, are first initiated in mid-July and initiation of new bracts continues until the end of September. Ovuliferous-scale initiation begins in September and continues until dormancy. A. A young bract primordium (about 0.5 mm long) before ovuliferous-scale initiation or formation of a lamina. Unlike leaves, bracts undergo very early upward growth. B. Bract showing the formation of the rudimentary lamina (blade) and the initiation of an ovuliferous scale in the axil of the bract. C. Both lamina and ovuliferous scale broaden rapidly. The latter forms an arc-shaped swelling at the base of the bract to which it is fused. D. The lamina extends about half the length of the bract which is still elongating. The ovuliferous scale begins growth perpendicular to the bract surface, resulting in a more pointed structure slightly higher in the center than along the sides. E. The bract and ovuliferous scale of the dormant seed cone. The lamina has broadened considerably and begun to grow toward the top of the bract, giving it a rudimentary, yet characteristic, trident appearance. The ovuliferous scale has undergone growth all along its margin and has become a flattened structure with a curved margin. It remains fused to the base of the bract but has separate vascular connections to the cone axis. Growth following dormancy occurs along the margin of the ovuliferous scale parallel to but remaining separate from the bract. The circles in E represent the approximate positions of the megaspore mother cells within the dormant seed cone. Bracts in the dormant seed cone are about 1.5 mm long.

of the enlarged megaspore mother cells (Fig. 4.4 B). Two slightly elongated swellings result, which radiate out from the central portion of the spoon-shaped ovuliferous scale (Fig. 4.1 K). Growth of the developing ovules and the scale produces a shift in the orientation of the megaspore mother cells and rudimentary ovules, which begin to face the outer edge and base of the ovuliferous scale, the position assumed at pollination. Rapid cell divisions around the megaspore mother cells continue, forming concentric rings of cells. The inner layers of cells form the poorly defined megasporangium or nucellus, while outer cells constitute the integument or wall of the ovule which encloses the megasporangium. The ovules form a slight swelling on either side of the adaxial surface of the enlarging ovuliferous scale (Allen, 1963; Owens and Smith, 1965).

The adaxial portion of the integument grows much more rapidly than the abaxial portion, producing a one-sided ovule tip. This stigmatic tip is nearly spherical, its surface is well covered with unicellular hairs, and the slit-like opening between the two unequal lips is oriented away from the

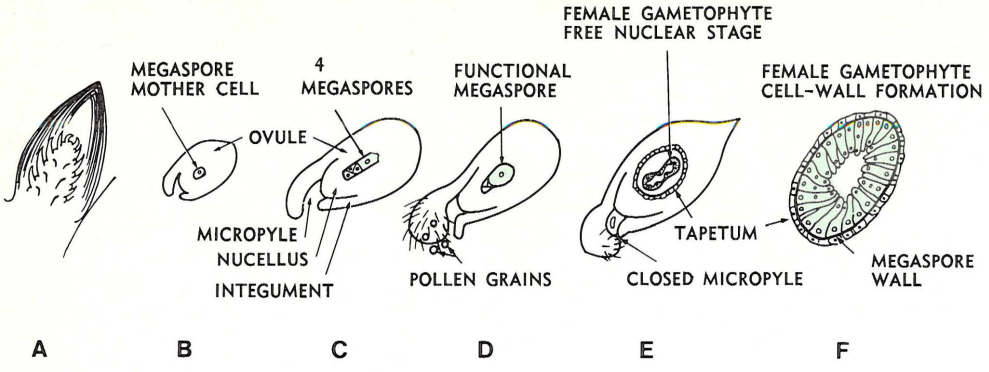
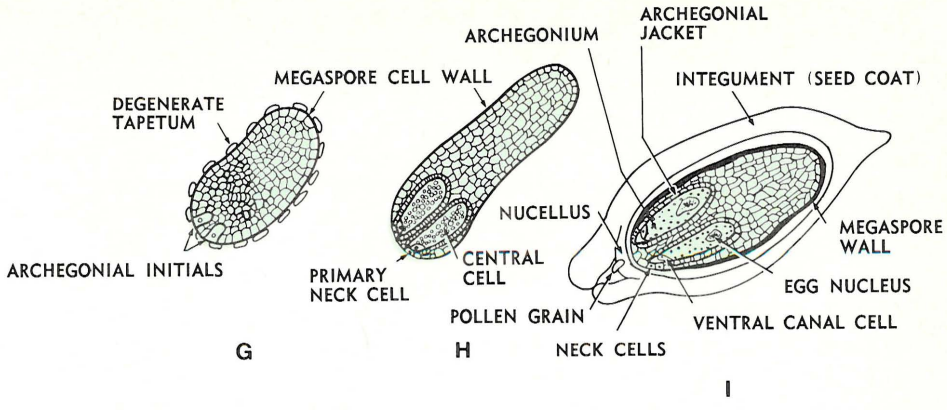


Figure 4.3 Ovule and Female Gametophyte Development Following Dormancy.

The stages shown occur during March, April and May. Gametophyte (haploid) tissue is shown in color. A. The seed-cone bud as it appears when growth resumes in March, showing the position of the ovuliferous scale on which ovules develop. B. An ovule, showing the large megaspore mother cell. C. Meiosis occurs early in March and four haploid megaspores are formed. The wall of the ovule thickens as the ovule enlarges and the integument arms grow outward, leaving an opening between, which is the micropyle. D. Three megaspores degenerate and the fourth enlarges. The integument arms develop unequally, forming a large stigmatic tip which is covered with hairs and serves to entrap pollen. At this stage, early in April, pollination occurs. E. The stigmatic tip grows inward, drawing with it the entrapped pollen. The functional megaspore undergoes several free nuclear divisions (without cell-wall formation) and a nutritive tapetum differentiates. F. Cells of the female gametophyte elongate and undergo cell-wall formation after several hundred free nuclei have formed. The tapetum is distinct at this stage (at the end of April). G. Cell-wall formation is complete and several archegonial initials differentiate at the micropylar end of the female gametophyte. The megaspore cell wall thickens and the tapetum degenerates. H. The nearly mature "frothy stage" of the female gametophyte. Prothallial cells continue to divide and jacket cells differentiate, forming the wall of the archegonium. Each archegonial initial has divided unequally, forming the small primary neck cell and the large central cell. Cytoplasm of the central cell appears "frothy" during formation of the many proteid vacuoles. I. Entire ovule just before fertilization, showing the mature female gametophyte within the thickened megaspore cell wall. Within the archegonium, the central cell has divided unequally, forming the small ventral canal cell and the large egg cell which completely fills the archegonium. The egg nucleus has enlarged and moved to the center of the egg cell. The primary neck cell has divided, forming several neck canal cells. Considerable differentiation of the seed coat occurs before fertilization.

cone axis and upward in the erect and receptive conelet at the time of pollination (Figs. 4.1 K; 5.3 C) (Allen, 1963). The function of this structure in the pollination mechanism is discussed in Chapter 5.

The megaspore mother cell has a hypodermal origin. As a result of many divisions within the surrounding nucellus, it becomes embedded deeper in the nucellar tissue. The megaspore mother cell elongates late in February, and meiosis occurs in March (Fig. 4.4 C). Meiosis is similar to that in



pollen mother cells except that it occurs within a much shorter period of time since there is no diffuse diplotene stage. Four haploid megaspores are formed. They are not formed in a linear series as reported for other conifers (Chamberlain, 1957), but usually appear to be arranged in a tetrahedral group (Figs. 4.3 C; 4.4 D). Three megaspores apparently degenerate while the fourth, functional megaspore enlarges considerably and develops several very large vacuoles and numerous starch grains (Figs. 4.3 D; 4.4 D, F). The megaspore wall at this stage is very thin and difficult to detect (Allen, 1963).

Completely enveloping the growing megaspore is a single or occasionally double layer of large, sporogenous-like cells three or four times the size of other cells of the nucellus. Their cytoplasm is very granular and their nuclei are large and deeply staining, like typical sporogenous cells (Figs. 4.3 E; 4.4 F). They are believed to be nutritive in function (Lawson, 1909) and constitute a "spongy tissue" or tapetum described variously as sporogenous cells (Lang, 1900) and archesporial tissue (Arnoldi, 1900). It has been suggested that cells of the spongy tissue could function as megaspore mother cells but do not go beyond the mother-cell stage (Lawson, 1909). Cells of the tapetum divide slowly and the tapetum becomes several cell layers thick as the female gametophyte develops within. During later stages of female gametophyte development, the tapetal cells become quite irregular and loosely arranged, with numerous intercellular spaces (Figs. 4.3 E-G; 4.5 B) (Lawson, 1909).

Soon after meiosis, the functional megaspore undergoes many reasonably synchronous nuclear divisions without subsequent cell division. These free nuclear divisions occur within the megaspore cell wall, which enlarges and thickens. The large vacuoles already present in the megaspore enlarge even more and form a single, very large central vacuole which keeps the cytoplasm closely pressed against the megaspore wall. The developing female gametophyte, at this stage, appears as a large empty sac bordered by a thin parietal layer of cytoplasm, with a single layer of free nuclei distributed

at regular intervals within the cytoplasm (Figs. 4.3 E; 4.4 G-H) (Lawson, 1909). This early female gametophyte development is slow, occurring over a 4-week period, but it is not interrupted by a resting period as is the case with *Pinus* (Ferguson, 1904).

Cell walls then appear, separating each nucleus from its neighbors. The cells thus formed are bordered on the outside by the thickening megaspore wall and are open on the inside and freely exposed to the central vacuolar sap. These form the primary prothallial cells or alveoli of the female gametophyte. The cells are all haploid, closely packed, usually six-sided when viewed at right angles to the dividing walls and form a regular symmetrical mosaic that appears like a honeycomb. Primary prothallial cells rapidly elongate in an inward direction and encroach upon the central vacuole and close it in (Fig. 4.5 B). The development described by Lawson (1909) for Douglas-fir differs from what is now considered typical for the gymnosperms (Maheshwari and Singh, 1967). In Douglas-fir, development proceeds rapidly and each primary prothallial cell reportedly divides several times, producing many cells to the inside before any crosswalls are formed (Fig. 4.3 F). Walls then form rapidly between all cells of the female gametophyte which, at this point, is an irregular mass of cells enclosed within a distinct megaspore wall (Fig. 4.5 C). The entire female gametophyte appears suspended in a watery fluid within the nucellus (Lawson, 1909).

The current interpretation of these stages (Maheshwari and Singh, 1967) is that following free nuclear divisions, secondary spindles develop and every nucleus becomes connected by spindle fibers to the six adjacent nuclei. Anticlinal walls are laid down centripetally and the gametophyte assumes the honeycomb appearance. These alveoli do not have walls near the large central vacuole but remain open at their inner ends. Each alveolus with its nucleus at the open end grows toward the center of the gametophyte, and the persisting spindles appear to guide the laying down of wall material. Most alveoli, especially those at the ends of the elongated gametophyte, become closed before they extend to the center of the gametophyte and form triangular cells. Only a few alveoli extend to the center of the gametophyte. Some alveoli form archegonial initials while most undergo a series of periclinal but not free nuclear divisions, so that the gametophyte is formed of rows of radiating cells. Consequently, free nuclear divisions probably do not occur in each alveolus, as reported by Lawson (1909).

Throughout this early period of female gametophyte development, tapetal cells separate and begin to show signs of disintegration (Lawson, 1909). Cells of the nucellus divide and form an indistinct, multi-layered tissue, completely enclosing the female gametophyte. A narrow space usually remains between cells of the nucellus and the female gametophyte. The nucellus is continuous with and indistinguishable from the integument which forms the outer wall of the ovule. The integument tip elongates and forms two unequal "lips" appressed together to form a closed slit facing upward (Fig. 5.3 C). The slit between the two lobes is, in effect, the closed mouth of the micropyle. Just within the slit is a short, narrow micropylar canal

that extends inward to the surface of the nucellus (Figs. 4.3 D; 4.5 A) (Allen, 1963).

Early in May, archegonia begin to form within the female gametophyte. Archegonial initials originate as superficial cells at the apex, nearest the micropyle, within the walled female gametophyte, as in other conifers. Some cells,usually four to six, enlarge markedly and function as archegonial initials (Figs. 4.3 G; 4.5 C, D). The nucleus of the archegonial initial moves to the peripheral end of the cell and the cell divides, producing a smaller, outer primary neck cell and an inner central cell (Figs. 4.3 H; 4.5 D, E). The primary neck cell does not increase in size but divides more than once by anticlinal walls. The resulting layer of cells may occasionally undergo division by periclinal walls to form two layers of cells. In most cases, however, only a single layer is formed. These layers form the rudimentary neck cells of the archegonium (Fig. 4.5 E, F) (Allen, 1943). No neck canal cells form and none are known to occur in any gymnosperm. In the evolution of the archegonium, neck canal cells made their final appearance in the pteridophytes (Chamberlain, 1957). The central cell enlarges over the next few weeks until the archegonium reaches nearly mature size. The central cell elongates towards the center of the female gametophyte tissue. At first, it is vacuolate with a large nucleus and granular cytoplasm, but later, the vacuoles become so abundant that the cytoplasm takes on a frothy appearance, as it does in many other conifers (Figs. 4.5 F; 4.6 B) (Allen, 1943). These proteid vacuoles appear similar to nuceli in the mature archegonium (Fig. 4.6 C), and early workers erroneously believed the eggs of gymnosperms differed from all other plants in being multi-nucleate. During early development of the central cell, a single layer of archegonial jacket cells becomes organized around the central cell from the surrounding prothallial cells (Figs. 4.5 F; 4.6 B). Jacket cells have been interpreted as a nourishing sheath and appear much the same here as in many other conifers (Allen, 1946b).

The large nucleus of the central cell remains close to the neck of the archegonium until the central cell divides. Division of the central cell does not occur until late in May, just before fertilization and after the archegonium has reached its mature size (Fig. 4.6 B) (Allen, 1943). Division is unequal and results in a large egg cell and one very small, flattened ventral canal cell at the apex of the archegonium just below the neck cells. A definite membrane separates the ventral canal cell from the egg (Lawson, 1909). This is true in other members of the Pinaceae in which development of the egg has been studied (Chamberlain, 1957). Both the ventral canal cell and cell membrane enclosing it persist up to the time of fertilization (Lawson, 1909). The conifers in which no membrane is formed between the two nuclei are generally those in which the gametes are distinct cells (Chamberlain, 1957). The nucleus of the ventral canal cell shows signs of disintegration and the egg nucleus moves toward the center of the archegonium while becoming greatly enlarged (Figs. 4.3 I; 4.6 C). The drawn-out appearance of the egg-nucleus as it moves away from the ventral canal cell has been suggested as an indication of its rapid movement (Lawson, 1909).

Figure 4.4 Megaspore Formation and Early Development of the Ovule and Female Gametophyte.

The stages shown occur between mid-February, when seed cones resume growth, and late April. A. Median longitudinal section of a dormant seed cone showing the position of the bract (b) and developing ovuliferous scale (square) on which the ovules develop. X23. B. Ovule (o) showing the enlarged megaspore mother cell. X340. C. Results of first meiotic division of the megaspore mother cell. Two daughter cells have been formed. X225. D. Four large haploid megaspores arranged in a tetrahedral group. The largest megaspore is the functional megaspore, while the other three degenerate. Meiosis occurs early in March. X250. E. Longitudinal section of an ovuliferous scale showing the relative size and position of the ovule and the central, developing female gametophyte (f). X50. F. Enlarged, vacuolate, functional megaspore surrounded by large tapetal cells (tc) which, in turn, are enclosed by the nucellus (nl). X330. G. Early free nuclear stage of the female gametophyte within the megaspore cell wall (mcw) showing synchronous nuclear division and the large central vacuole (cv). This stage occurs early in April during pollination. X325. H. Enlarged view of the free nuclear stage of the female gametophyte, showing the thin parietal layer of cytoplasm (cy) in which a single layer of free nuclei (n) are regularly distributed. X600. Stages shown in E-H above occur slowly over a 4-week period from late March to late April.

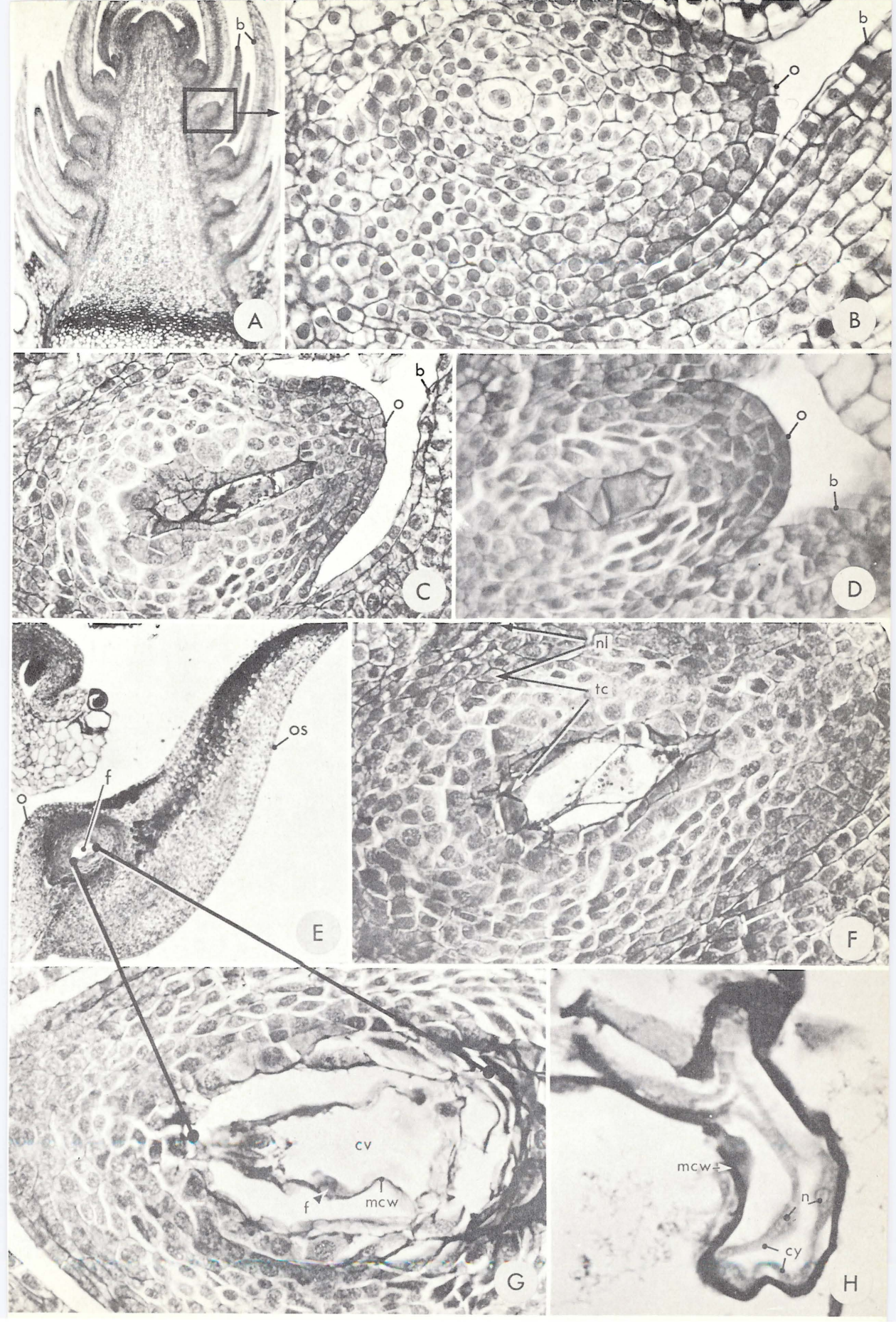

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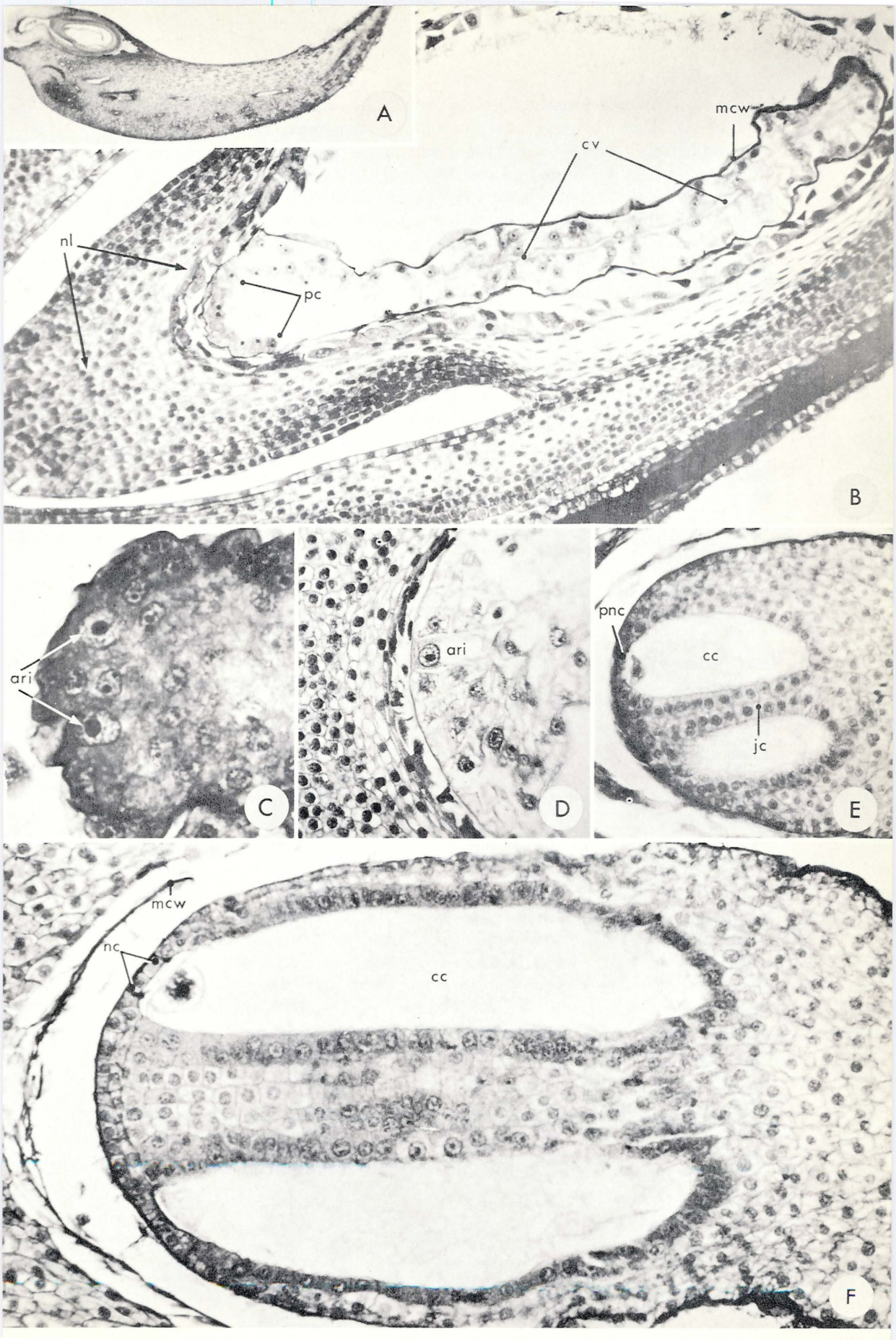
Figure 4.5 Female Gametophyte Development.

A. Longitudinal section of an ovuliferous scale approximately as it appears during May when stages B-F occur. X12. B. Section of a developing ovule collected early in May, several weeks after pollination, showing the nucellus (nl), megaspore cell wall (mcw) and the elongation of the primary prothallial cells (pc) which have encroached upon and nearly filled the central vacuole (cv). X150. C. Enlarged view of apex of the female gametophyte (closest to the micropyle) showing archegonial initials (ari) in the darkly stained, multi-cellular female gametophyte tissue. X470. D. Later stage than in C, showing enlarged superficial archegonial initial. The nucleus has moved to the periphery of the cell in preparation for division. X270. E. Developing archegonium following division of the archegonial initial into an outer, smaller primary neck cell (pnc) and the inner central cell. (cc). Archegonial jacket cells (jc) have formed around the central cell. X140. F. Two enlarged archegonia within the prothallial cells as they appear late in May. Jacket cells are more evident, the cytoplasm of the large central cell has assumed its characteristic frothy appearance and the primary neck cell has divided anticlinally forming a layer of neck cells (nc). X225.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The number of archegonia found in each female gametophyte varies from four to six. Four, however, is the commonest number found. They are situated closely together, with little sterile tissue between them. The necks of the archegonia appear to be separated from one another by a considerable amount of sterile tissue, but the archegonial jackets may come in contact with one another in the middle region where archegonia are widest (Fig. 4.6 C) (Lawson, 1909). This is unlike members of the Cupressaceae and Taxodiaceae, where archegonia are in contact with one another either at the apex or laterally forming an archegonial complex (Chamberlain, 1957).

The Mature Female Gametophyte

The female gametophyte of Douglas-fir is not fully developed until late in May, just before fertilization and almost two months after pollination (Fig. 4.3 I) (Allen, 1943). It is deeply embedded within the ovule and surrounded by a poorly defined nucellus and a thick integument which has begun to show differentiation into the distinct inner, middle and outer layers of the seed coat (Fig. 4.6 A). The micropylar canal has closed as a result of growth of the stigmatic lips, and several elongating pollen grains are usually visible in the micropylar canal (see Chapter 5 for details of pollination). The female gametophyte is bounded on the outside by the thickened original megaspore cell wall. The wall varies in thickness, being thicker at the base than along the sides of the female gametophyte. At the micropylar end, adjacent to the archegonium, the wall appears quite thin. No trace of the wall extends over the apex of the female gametophyte where the neck cells of the archegonia are found. This is a result of female gametophyte growth, which extends the archegonial region forward beyond the limits of the spore wall. The female gametophyte tissue consists of many thin-walled haploid cells that completely enclose the archegonia. A uniform layer of small, female gametophyte cells with dense cytoplasm form the jacket or wall of the archegonium. Each archegonium contains a single, very large egg nucleus which, in turn, is contained within the enormous and highly vacuolate egg cytoplasm. A single, flattened ventral canal cell is present at the tip of the egg cell. The nucleus of the ventral canal cell shows signs of disintegration before fertilization occurs. This is the appearance of the ovule at the time of fertilization early in June, or about two months after pollination (Lawson, 1909; Allen, 1943, 1946b). Many changes have also occurred within the seed cone regarding growth and differentiation of the bracts and ovuliferous scales (Owens and Smith, 1965). Details of this development as it relates to pollination, engulfing of pollen and subsequent cone and seed development are discussed in succeeding chapters.

Female Gametophyte Development in Other Conifers

A complete review of female gametophyte development in conifers is

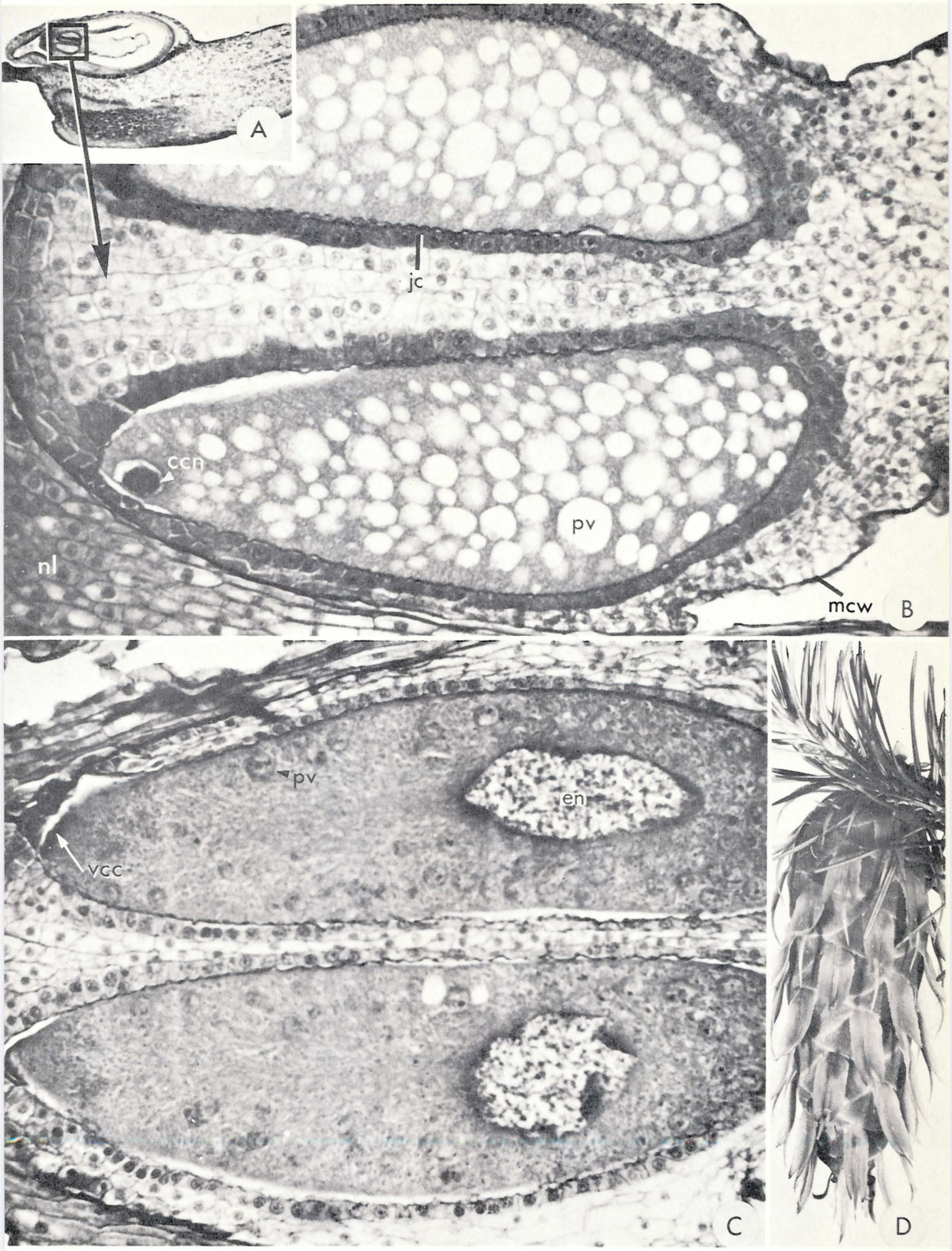
found in Chamberlain's (1957) work, first published in 1935. Many genera are discussed and a complete bibliography is included. Chamberlain's discussion is not as outdated as the year might indicate since most studies of the female gametophyte in conifers were made about the turn of the century when this aspect of plant morphology was at its peak. A more recent review of the literature is provided by Maheshwari and Singh (1967).

Douglas-fir female gametophyte development differs from many other conifers but is similar to other members of the Pinaceae. The conifers studied most in this respect are in the genus *Pinus,* where the principal difference is that female gametophyte development extends over 2 years rather than one. Development reaches only the early free nuclear stage during the first year. Pollination occurs, then both male and female gametophyte development stops. Development resumes the following spring, and the female gametophyte becomes fully developed just before fertilization. *Pinus* is the exception to the general phenological pattern in conifers but is still given as an example of a typical conifer. Douglas-fir, as most other conifers studied, has an uninterrupted period of female gametophyte development. Beyond this major difference, variations in female gametophyte development are more subtle, but important in interpreting evolutionary trends within the conifers. Some of these differences are worth summarizing, the details would have to be obtained from the many research papers available (Chamberlain, 1957; Maheshwari and Singh, 1967).

In Douglas-fir, four megaspores appear to result from meiosis of the megaspore mother cell. These usually have a tetrahedral rather than a linear arrangement, as found in other conifers. Some conifers form only three megaspores; the upper cell from the first division fails to divide and eventually degenerates. Free nuclear division is not restricted to conifers, but it is likely to occur in any plant where the cell is large in proportion to the nucleus. The period of free nuclear division is fairly long in Douglas-fir, but fewer free nuclei form than the 1,000 estimated for *Pinus strobus* (Ferguson, 1904). In general, if the megaspore outline is long and narrow, as in the Douglas-fir, the free nuclear period is shorter than if the megaspore outline is more spherical (Chamberlain, 1957). The reason for this, and the general validity of the statement throughout the conifers, is uncertain. The megaspore cell wall, frequently referred to as a membrane, becomes evident quite early in the female gametophyte development of Douglas-fir. In the mature female gametophyte (prothallium), the megaspore cell wall is absent in the region of the archegonia. This is true in most conifers but not in some, such as *Tsuga* (Lawson, 1909), where the wall is continuous even around the archegonial region. The megaspore wall in Douglas-fir is thicker (4.5 μ) than in most other conifers. The retention of a thickened, highly differentiated megaspore cell wall or coat is a vestige of a characteristic of a more primitive ancestral condition where megaspores were not retained within the megasporangium. Development of more than one megaspore has not been observed in Douglas-fir although this has been noted, though rarely, in some conifers (Chamberlain, 1957). Most, if not all, of the superficial cells at the apex

Figure 4.6 Late Stages of Female Gametophyte Development.

The stages shown occur during the latter part of May, just before fertilization. A. Longitudinal section of an ovuliferous scale and ovule. The outlined region is shown greatly enlarged at successively later stages in B and C. X8. B. Details of a portion of the female gametophyte showing two developing archegonia. The large central cell nucleus (ccn) remains close to the neck of the archegonium, while large proteid vacuoles (pv) give the cytoplasm a frothy appearance. The archegonial jacket cells (jc) are very distinct. Nucellar tissue (nl) is shown on the left. The female gametophyte is bounded on the outside by a thickened megaspore mother cell wall (mcw). X250. C. Portions of two mature archegonia collected about one week after those shown in B (at the end of May). The central cell has divided unequally, forming a very small, flattened ventral canal cell (vcc) at the apex of the archegonium and a large egg cell that completely fills the archegonium. The egg nucleus (en) enlarges as it moves toward the center of the archegonium. The egg cytoplasm is very dense and contains numerous proteid vacuoles. X200. D. A Douglas-fir seed cone shown in natural size (6-7 cm long) at the time of fertilization early in June. The seed cone has fully enlarged by this time but is still green and undergoing tissue differentiation.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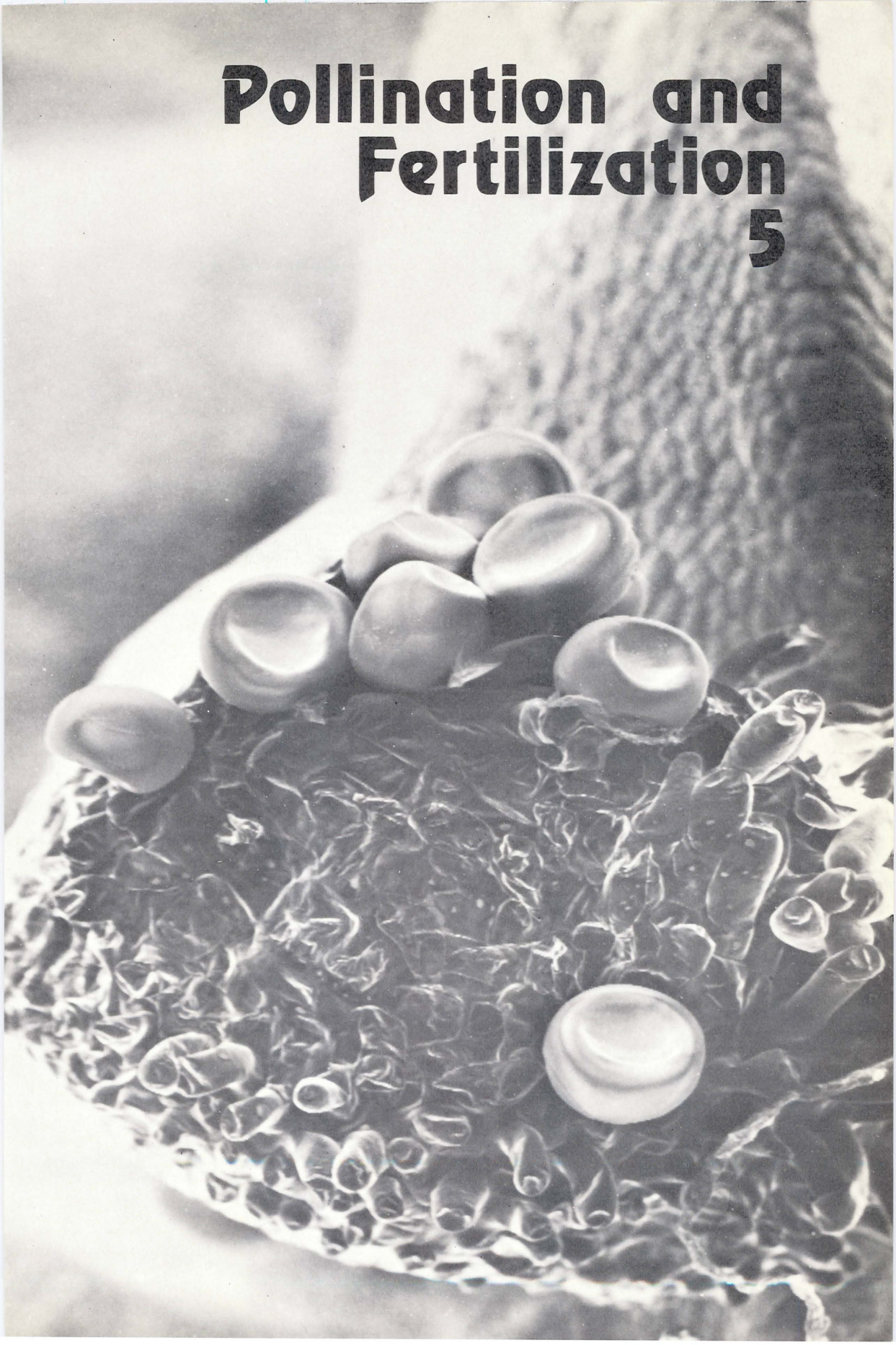
of the archegonium are potentially archegonial initials even though the number that fully develops is usually four to six. The most common number in Douglas-fir is four, while eight is common in other genera. Certain, less-familiar species are reported to have 100-200 archegonia. Forms with fewer archegonia have more vegetative tissue separating the archegonia (Chamberlain, 1957). The archegonia in Douglas-fir are much longer in proportion to their width than is the case in other members of the Pinaceae. Usually only one layer of neck canal cells is present in Douglas-fir but, as in other species, there may be considerable variation in number and layers of cells. The cell membrane of the ventral canal cell persists until the time of fertilization. This cell membrane is also present in *Pinus*, and it has been suggested that it is correlated with the presence of gametes which are reduced to a naked nucleus. However, what appear to be highly differentiated, ciliated sperm with very little cytoplasm have recently been reported in Douglas-fir (Christiansen, 1969a). In the evolution of the archegonium from ancestral pteridophytes, there has been a gradual reduction in the length of the neck and in the number of neck canal cells. Both the neck canal cells and the ventral canal cells have been interpreted as reduced eggs (Chamberlain, 1957).

Scanning electron micrograph of the stigmatic tip on the ovule of a Douglas-fir seed cone at pollination showing many pollen grains near the micropyle. x310.

Pollination and Fertilization

5



Chapter 5

Pollination and Fertilization

Pollination and fertilization are distinct but related events separated in conifers by a considerable amount of time. Pollination is the transfer of pollen from the pollen cone to the seed cone. Unlike flowering plants, it is dispersed in conifers only by wind (Chamberlain, 1957). Fertilization is the fusion of a male gamete, derived from the pollen grain, with the egg of the archegonium. It is preceded by pollen germination, growth and gamete formation. The time interval between pollination and fertilization in most flowering plants is generally short and may be a matter of hours or days. During this time, a pollen tube grows from the stigma of the flower to the ovule which may be a distance of several inches. In conifers, however, even though the distance that the pollen tube must grow through the nucellus is usually only a fraction of an inch, the time interval between pollination and fertilization is several weeks, months or, in some cases, a year or more (Chamberlain, 1957). Douglas-fir is typical of the majority of conifers in this regard; pollination occurs in April and fertilization about 10 weeks later. Several conifers, most notably the pines, have a period of about 14 months between pollination and fertilization. Many factors may adversely affect pollination, as well as development during the long period between pollination and fertilization, thus preventing sexual reproduction and ultimately seed set.

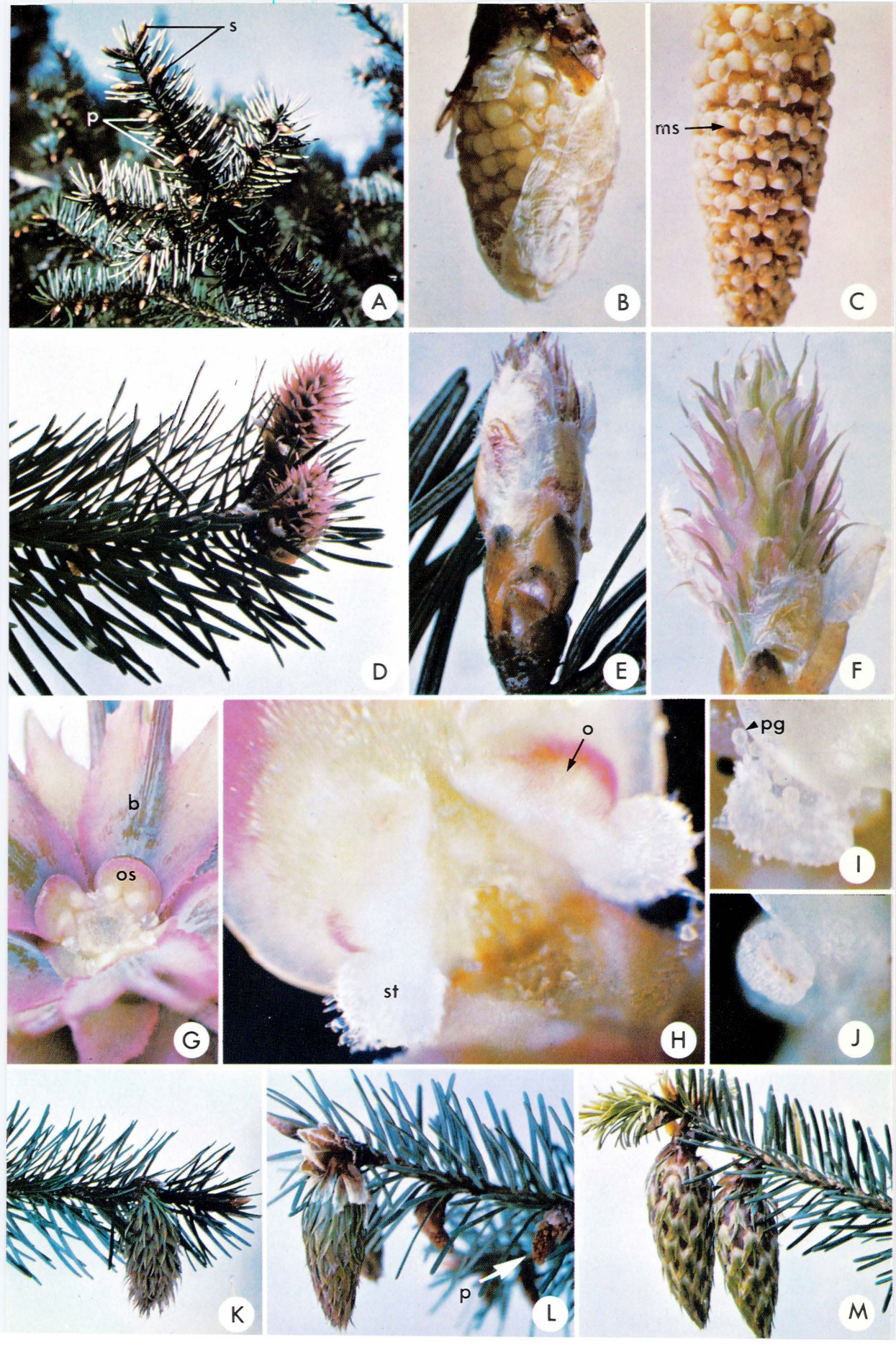
Pollen-Cone Development and Dehiscence

Both pollen and seed cones begin growth and further differentiation during late February, 4 to 6 weeks before pollination or the onset of growth in vegetative buds. Growth is not visible externally during the first few weeks but by mid-March the cones within the bud scales have enlarged enough to cause buds to swell, indicating that pollination is imminent (Fig. 5.1 A) (Owens and Smith, 1964).

No apical growth occurs and no new microsporophylls or microsporangia are initiated following dormancy. All parts of the pollen cone enlarge. Microsporangia swell following meiosis to form two large, rounded pollen sacs on the abaxial surface of the pointed microsporophylls (Fig. 5.1 C). Changes occur in the wall of the microsporangium during pollen maturation. The microsporangial wall of the dormant pollen cone is usually three to five

Figure 5.1 The Pollination Mechanism and the Development of the Pollen and Seed Cones Following Dormancy.

A. A branch, showing pollen (p) and seed cones (s) just before pollen is shed early in April. B. A pollen cone, as shown in A, with all outer bud scales removed, revealing the inner, papery bud scales and the closely packed microsporophylls bearing the rounded microsporangia. X8. C. An elongated pendant pollen cone when pollen is being shed, showing the widely separated microsporophylls and the transversely split microsporangia (ms) or pollen sacs. X8. D. Seed cones at pollination shown about natural size. Abundant anthocyanin pigments in the bracts give the cones of some trees a distinct red color. Cones of other trees may vary from green to yellow. E. Seed cone elongating and pushing through the bud scales. X3. F. Close-up of a seed cone, as in E, just after bud burst early in April. X3. G. Seed cone as shown in F with distal portion removed, revealing the shape of the bracts (b) which function to catch and funnel pollen down to the ovuliferous scale (os). X10. H. Enlarged ovuliferous scale at pollination, as shown in G, showing the position of the ovules (o) and stigmatic tips (st). X50. I. Stigmatic tip at pollination shown from the side adjacent to the bract. Several pollen grains (pg) are shown adhering to the stigmatic tip at the slit-like opening to the micropyle. X50. J. A later stage of the stigmatic tip after the pollen has been engulfed by the inward growth of the two lips. X50. K. By the end of April, the seed cones become about 4 cm long and pendant. L. By mid-May, the bracts have become more closely appressed as a result of rapid ovuliferous scale growth within the cone which has become about 5 cm long. This is just before vegetative bud burst and many pollen cones (p) still remain attached to the shoot. M. By the end of May, vegetative buds have burst, the seed cones have elongated to about 6 cm and ovuliferous scales (shown here as red) have greatly enlarged, altering the appearance of the cone. Fertilization occurs within 1 or 2 weeks.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cells thick. The outer layer which forms the epidermis consists of large, isodiametric, vacuolate cells, while the inner layers are rectangular or spindle-shaped with dense cytoplasm. As the microsporangia enlarge, the innermost layers become stretched and more spindle-shaped. The tapetum and cells of the microsporangial wall adjacent to the tapetum degenerate and all may function as tapetal cells. By the time of dehiscence (when microsporangia split open) the tapetum and the inner layers of the wall have broken down and only occasional fragments of cells are visible adjacent to the epidermis. During microsporangial development, a line of dehiscence differentiates transversely across the abaxial middle portion of both microsporangia and along the outer side of each microsporangium (Fig. 5.1 C).

Increase in length of the pollen cone within the bud scales results from cell elongation within the cone axis, especially the stalk at the base of the cone. Bud scales do not enlarge. The elongating pollen cone within the bud scales pushes the bud scales apart, revealing the more lightly colored, inner bud scales (Figs. 3.1 B; 5.1 B). As a result, the swollen buds become progressively lighter brown toward the tip of the bud. Bud burst or flushing normally occurs over a 2-week period on a given tree. The axis of the pollen cone bends slightly downward after bud burst so that most pollen cones are somewhat pendant. This orients the microsporangia so that the line of dehiscence is directed upward. Dehiscence results from the drying of microsporangia causing the wall to break open and the pollen to be released. Pollen cones elongate considerably following bud burst before dehiscence occurs (Fig. 5.1 B, C). Following bud burst, pollen cones, like seed cones, show a wide range of color from green to yellow to red, which appears uniform on a tree (Griffith, 1968), and is presumably one of the many inherited characteristics in Douglas-fir (Figs. 3.1 A; 5.1 A).

Pollen in Douglas-fir, as in other conifers, is dispersed by wind. Douglas-fir pollen is larger than that of most other conifers and lacks wings or bladders (Figs. 3.5 I; 5.3 D). As a result, it normally has a relatively short dispersal distance. Pollen dispersal studies are difficult in the Pacific Northwest because of the omnipresence of Douglas-fir which loads the air with pollen, resulting in a considerable background level of pollen over vast regions. Silen (1963) reported that in years of heavy pollen-cone production even areas separated from the nearest Douglas-fir by several miles have been shown to average 769 pollen grains per square inch. Areas adjacent to trees averaged 5,116 pollen grains per square inch, while areas 3000 feet from trees averaged 2,000 pollen grains per square inch. The most marked drop in pollen count occurs in the first few hundred feet from the tree and probably only a small fraction of the pollen is dispersed a distance further than 5-10 times the tree height.

Seed-Cone Development and "Flowering"

Seed cones begin growth within the bud scales late in February at the

same time as pollen cones. Bracts and ovuliferous scales enlarge, but most growth results from elongation of the cone axis which produces a marked swelling of the buds and separation of bracts, allowing pollen to pass more readily to the ovules (Owens and Smith, 1965).

The swelling, and resultant change in color, of the seed-cone buds is a result of cone enlargement within the bud scales rather than growth of the bud scales. The bud scales become slightly separated at the distal end of the cone, revealing the light-brown inner bud scales. The seed-cone buds double in length and become light brown at the tip just before bud burst (Fig. 5.1 A, E). Elongation of the axis is greater on the lower surface than on the upper surface of the laterally oriented seed-cone buds. As a result, the proximal portion of the seed-cone axis bends upward and, by the time of bud burst, seed cones are erect (Fig. 5.1 A, D-F) (Owens and Smith, 1965).

Bracts enlarge many times following dormancy and before bud burst. The trident form of the bract is established prior to dormancy. Growth following dormancy is very rapid and bracts reach nearly two-thirds their mature size by the time of pollination. Tremendous intercalary growth and cell elongation cause the increase in length, while marginal growth rapidly increases the width of the lamina or blade portion of the bract. Tissue differentiation is nearly complete within the bract at pollination. By this time the similarity of anatomical structure in the bract and its homologue, the leaf, are quite evident, especially in the narrow, distal portion of the bract where the lamina is absent (Fig. 7.1 C). The rapid, early growth of the bract before pollination has considerable importance in entrapping pollen and funneling it toward the ovules (Owens and Smith, 1965).

Ovules enlarge only slightly before pollination. Each ovule appears as a slight, elongated swelling extending radially from the center of the adaxial surface of the ovuliferous scale (Fig. 5.1 G, H). As described in Chapter 4, female gametophyte development has just begun prior to pollination and is not completed until just before fertilization. The most notable early change in the ovule is the development of the integument into the large stigmatic tip which entraps the pollen. Early development of the stigmatic tip and the ovule as it relates to female gametophyte development is described in Chapter 4. It is suffice to recall that the stigmatic tip results from the unequal growth of that portion of the integument that contains the micropylar canal. The integument tip consists of two unequal lips appressed together to form a closed slit. If the ovuliferous scale is viewed from above (the adaxial surface) at pollination (Figs. 5.1 H, I; 5.2, 5.3 C), the longest lip of each ovule is on the side closest to the center of the ovuliferous scale. This lip and the shorter, outer lip bend outward toward the margin of the ovuliferous scale. As a result, the micropylar canal is curved. The stigmatic tip extends beyond the margin of the ovuliferous scale and occupies much of the space between the scales. The stigmatic tip becomes covered with many unicellular hairs which appear to be quite sticky and pollen grains adhere readily (Fig. 5.3 C, D) (Doyle and O'Leary, 1935; Allen, 1963; Barner

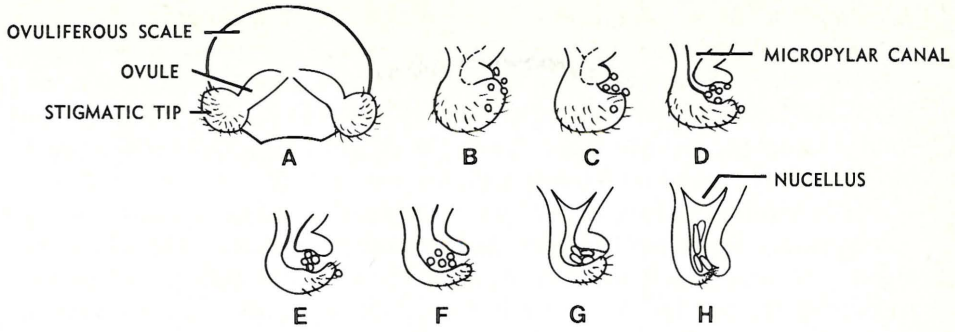

Figure 5.2 Development of the Ovule Tip Following Pollination and the Engulfing of Pollen.

These stages occur during the latter half of April. A. Adaxial view of the ovuliferous scale, ovules and stigmatic tips at the time of pollination. B-H. Ovule tip as seen in side view. B. Pollen adheres to hairs on the stigmatic tip. C-F. Formation of the depression into which the pollen grains sink to become contained within the micropylar canal. G, H. The ends of the stigmatic tip grow inward, closing the micropyle and thereby engulfing the pollen grains which have begun to elongate. Redrawn from Allen (1963).

and Christiansen, 1962).

The seed cone at pollination is upright, about 3 cm long (5.1 D-F), and the base is still enlcosed within bud scales. Its appearance is dominated by the many large trident bracts. The ovuliferous scales are small and not visible unless the bracts are removed (Fig. 5.1 F, G). The development and arrangement of the two structures make them well adapted to effectively capture the available pollen.

The Mechanism of Pollination

The pollen cone at pollination is usually pendant and about 2 cm long. The base of the axis elongates considerably; consequently, nearly all microsporophylls are beyond the bud scales which still enclose the base of the pollen-cone axis. Elongation of the axis has separated the microsporophylls. Microsporangia dry and split, releasing the mature pollen grains. All microsporangia dehisce on an individual cone at about the same time (Fig. 5.1 C), but dehiscence on a given tree may extend over a 2-week period.

At the time of pollination of the seed cones, the broad bracts are reflexed back. Bracts narrow toward their base and the margins curve slightly upward to form a funnel-like structure where bract and ovuliferous scale join. Just above this juncture, the stigmatic tip extends beyond the margin of the bract and fills the space between the bract and the ovuliferous scale (Fig. 5.1 G). As a result, any pollen landing on the bract of the upright cone

would readily pass down the smooth upper surface of the bract to the base where it would land on the sticky, hairy surface of the stigmatic tip approximately where the two lips join (Figs. 5.1 H, I; 5.3 C, D). The lips at this stage are nearly appressed and the pollen does not have an open passage into the micropyle (Figs. 5.2 B; 5.3 C, D) (Allen, 1963). Many pollen grains are commonly found on the stigmatic tip (Fig. 5.3 D).

As pollination comes to an end, a depression begins to form on the surface of the larger lip adjacent to the closed slit or entrance to the micropylar canal. The depression appears to result from the collapse of surface and subsurface cells (Figs. 5.1 I; 5.2 B, C; 5.3 D). The pollen grains gradually sink into this cavity. Growth of the two lips inward slowly encloses the pollen grains except for the actual mouth of the micropyle which becomes lightly sealed, possibly by the remains of hairs, collapsed cells and exudations (Figs. 5.1 J; 5.2 D-H; 5.3 E). Light pressure on the ovule neck liberates the enclosed pollen grains. Although the general pattern of stigmatic tip development in Douglas-fir is similar, individual ovules show considerable variation which is reflected in the appearance of the tip of the micropyle. In some, the larger lobe continues to dominate and a markedly bent tip results, while in others, the two lips become more or less equal to form a straight tip (Fig. 5.2 H). The mouth of the micropyle varies from slit-like to saucer-like or even flat, depending upon the growth of the stigmatic or integument tips (Allen, 1963). Interior Douglas-fir from dry regions has blunt tips resulting from two short, equal lips unlike those described above. This may account for differences observed in mature seed from coastal and inland sources (Fig. 7.1 K, L) (Allen, 1960).

Within a week or so after pollination is complete, the pollen grains have been effectively trapped and engulfed as a result of growth of the integument tip (Figs. 5.2, 5.3 E, F). When pollen is abundant, some pollen grains may not be engulfed and may germinate on the surface of the integument along with those that adhered to the bracts and ovuliferous scales. The pollen, once engulfed, is contained within a chamber that is continuous with the narrower micropylar canal which, in turn, leads to the larger nucellar chamber (Figs. 5.3 F; 5.5 B). The pollen grains usually remain attached to the stigmatic hairs within this outer chamber and become free only when they germinate (Allen, 1963).

The pollen-collecting mechanism has not been described for many conifers. The method of collecting and engulfing pollen in Douglas-fir is similar to that described for *Larix* (Doyle, 1926; Doyle and O'Leary, 1935; Barner and Christiansen, 1960). However, it differs strikingly from that of *Pinus*, where many, if not all, species have a long, two-pronged integument. A pollination drop is reported to be present in the micropyle of *Pinus* to which the pollen adheres. The pollen then floats or is drawn into the micropyle, passes down the canal and eventually comes to rest on the surface of the nucellus (Chamberlain, 1957). Pollen in *Larix* is engulfed in a manner similar to that in Douglas-fir, but fluid is reportedly present within the micropylar chamber and pollen moves to the nucellus in a manner similar to that in

Pinus (Barner and Christiansen, 1960). In Douglas-fir, no fluid is present. Pollen is moved part of the distance to the nucellus by the enfolding of the stigmatic tip, where it germinates and eventually elongates enough to come into contact with the nucellus (Allen, 1946b, 1963).

Silen and Krueger (1962) gave evidence that rainy weather is rarely, if ever, a complete deterrent to successful pollination in Douglas-fir. Artificial pollination, using pollen suspended in water, was effective in Douglas-fir (Allen and Sziklai, 1962) which suggests that the pollen-collecting mechanism, at least in the coastal form, is able to screen pollen from water. Coastal Douglas-fir is probably well adapted to a climate in which pollination may be preceded or followed by rain.

Pollen Germination

Pollen germination and development of the male gametophyte have been studied in Douglas-fir by preparing sections of the ovule for microscopic study (Allen, 1943, 1946b) and by carefully dissecting pollen from the micropyle and observing it microscopically during development (Barner and Christiansen, 1962; Christiansen, 1969a, b). More recent work on the culture and development of pollen in vitro has clarified many earlier observations (Allen, unpublished).

Pollen need not be in the micropyle to germinate but full normal development would probably not occur on the surface of the bract or ovuliferous scale. Pollen germinates within the micropyle about three weeks after it is engulfed by the stigmatic tip of the integument. Pollen grains are not in contact with the nucellus when they germinate; rather, germination occurs when pollen is still adhering to the stigmatic tip just inside the micropylar canal and a considerable distance (several hundred microns) from the nucellus (Fig. 5.3 F). The absence of a pollination drop means that the pollen grain has to grow (elongate) inward to the tip of the nucellus (Allen, 1946b, 1963).

At germination, the pollen grain swells and the exine of the spore wall splits open. There is no apparent pore or line along which the exine splits. The intine remains intact and forms the very plastic wall as the pollen grain elongates. Normally, the distal end of the pollen grain (opposite the prothallial cells) forms the advancing tip of the elongating pollen grain. The proximal end usually remains within the broken exine, or the exine is shed entirely. In either case, no distinct pollen tube forms at this time but the entire pollen grain forms a long tubular structure (Fig. 5.3 F, G). The intine is very plastic and capable of considerable extension (Allen, unpublished). This could be attributed to the nature of the intine which, in Douglas-fir, consists of cellulose and pectin (Owens and Molder, 1971a) and possibly callose, as in other plants (Clowes and Juniper, 1968), although tests for the latter were not made in Douglas-fir (Chapter 3). The thick intine becomes very thin during pollen elongation (Fig. 5.3 G-I), which continues for several weeks.

Figure 5.3 Engulfing of the Pollen and Pollen Growth.

A. Seed cone at the time of pollination. X. 2.5. B. Median longitudinal section of a seed cone at the time of pollination, as in A, showing bracts (b), ovuliferous scales (os), and ovules (o). X 2.5. C. Scanning electron micrograph of the tip of the ovule fixed at the time of pollination, showing the stigmatic tip, stigmatic hairs, and mouth of the micropyle (mm). X 40. D. Scanning electron micrograph of a fresh stigmatic tip collected during pollination, showing several pollen grains attached to the stigmatic hairs. Collapsed hairs indicate the location of the mouth of the micropyle and the depression into which the pollen grains sink. X 80. E. Scanning electron micrograph of a stigmatic tip fixed following pollination, showing the mouth of the micropyle and the result of inward growth of the two lips which engulf the pollen grains. X 100. F. Longitudinal section through a portion of the ovule in late May, showing elongated pollen. The stigmatic tip has grown inward, as in E, bringing the pollen closer to the nucellus (nl). The archegonium (ar) is at the "frothy" stage. X 60. G-M. Various stages of pollen development as seen with the phase microscope. Pollen was carefully dissected from the micropyle during May. G. Elongated pollen grain, showing broken exine (e) and distinct intine (i). Large vacuoles and the prothallial cells (p) are evident. The stalk cell (sc) is attached to the body-cell complex (bcc) by a thin strand of cytoplasm (arrow). The dense cytoplasm indicates the position of pollen-tube (pt) formation. X 125. H. A somewhat later stage of pollen-tube formation. Pollen tubes may form from the sides or either end of the elongated pollen grain. X 300. I. Elongated pollen grain, showing the close association that exists between the body-cell complex and the formation of the pollen tube. X 125. J. A pollen grain, showing the penetration of the pollen tube into the nucellus. X 125. K. Enlarged view of the body-cell complex during its lobed phase when the body cell divides to form the two male gametes. X 300. L. Division of the dense body-cell nucleus forming one large and one small male gamete (mg). X 600. M. During the later stages of pollen-tube formation, the dense cytoplasm containing the tube nucleus, the two male gametes and often the stalk cell form a long narrow cylinder which passes down the pollen tube and into the egg cell. X 200.

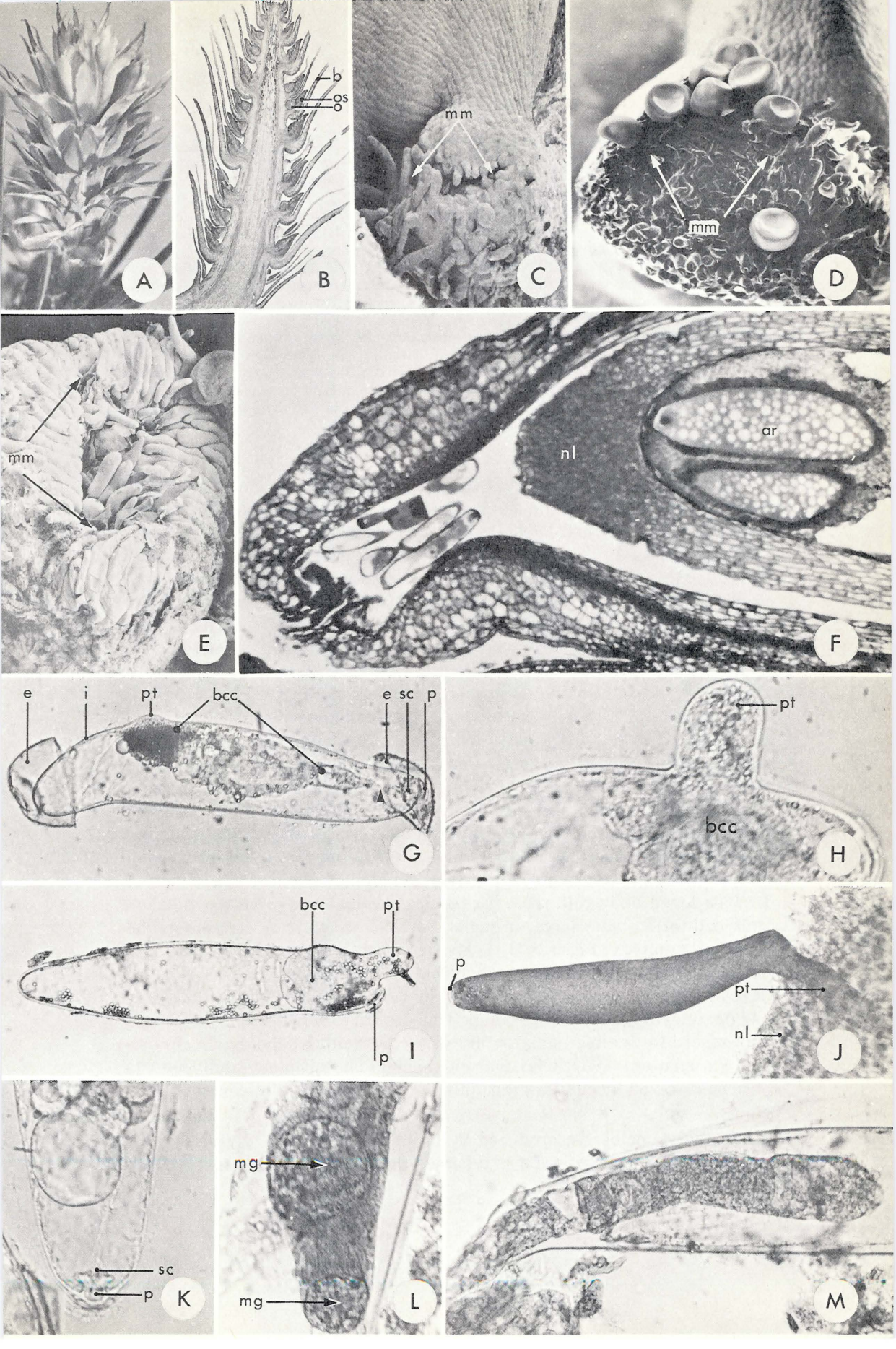
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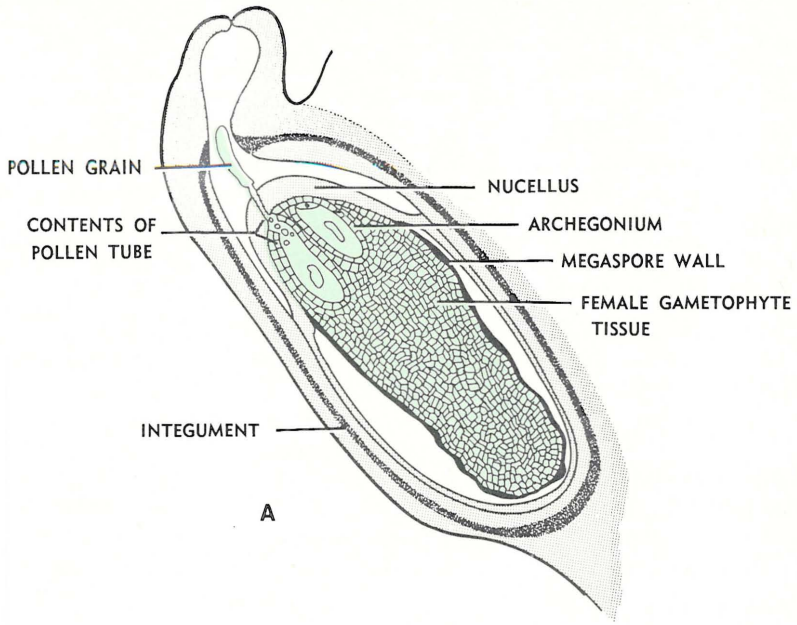
Figure 5.4 The Ovule and Female Gametophyte at Fertilization.

Gametophyte (haploid) tissue is shown in color. A. The integument, making up the wall of the ovule, has undergone considerable differentiation to form the seed coat. The pollen tube has penetrated through the nucellus and released its contents (tube nucleus, two gametes, and stalk cell) into the egg cytoplasm. The nucellus has become quite thin as a result of ovule growth. A space usually exists between the nucellus and the female gametophyte tissue. B. The larger male gamete fuses with the egg nucleus, while the supernumerary nuclei (those of the ventral canal cell, stalk cell, tube cell and the smaller male gamete) remain near the neck of the archegonium. C. The membranes of the egg nucleus and male gamete disappear and the 13 chromosomes from each are arranged on separate spindles. One of several possible fates of the supernumerary nuclei is shown. Here, three of the four nuclei are undergoing divisions (perhaps after fusion). D. The two spindles, each with 13 chromosomes, have come together and are arranged at metaphase for the first division of the diploid zygote and formation of the embryo. Another of the many possible fates of the supernumerary nuclei is shown. Here, cell walls have formed after fusion and division of some nuclei.

Following germination, several changes occur within the elongating, five-celled pollen grain or young male gametophyte. The cytoplasm of the tube cell enlarges greatly and becomes extremely vacuolate so that there exists only a thin parietal layer of cytoplasm and several cytoplasmic strands traversing the large vacuoles. The body cell also enlarges and usually moves slowly from the stalk cell, which remains attached to the second prothallial cell. A thin strand of cytoplasm links the stalk and body cell for a time but, as the body cell moves closer to the distal end of the elongating pollen grain, the cytoplasmic strand usually breaks (Fig. 5.3 G). The tube nucleus usually precedes the body cell in its movement through the pollen grain. Occasionally the stalk cell separates from the second prothallial cell and moves with the body cell and tube nucleus, all within the cytoplasm of the tube cell. No particular means of locomotion is visible but cytoplasmic streaming is rapid within the elongating pollen grain. The body cell becomes very large and lobed. The cytoplasm of the body cell becomes so dense that a whole mount of these pollen grains reveals little of the internal structure or changes occurring within the body cell. During this lobed phase, the body-cell nucleus divides to form two gametes of unequal size (Fig. 5.3 L). The lobed body cell, the adjacent tube nucleus and often the attached stalk cell form a very large, irregular, sac-like structure referred to as the body-cell complex (Fig. 5.3 H, I, K). The exact structure of the body-cell complex is unknown (Allen, unpublished). Pollen grains at this stage of development have been studied only in whole mounts, where the intine and dense cytoplasm obscure detail. This has led to considerable conjecture with regard to the internal structure of the elongating pollen grain (Barner and Christiansen, 1962; Christiansen, 1969a). The resulting confusion can only be resolved by careful preparation of thin sections of elongated pollen grains.

The two gametes are produced by an apparent unequal division of the body-cell nucleus (Fig. 5.3 L). Whether the unequal size of the gametes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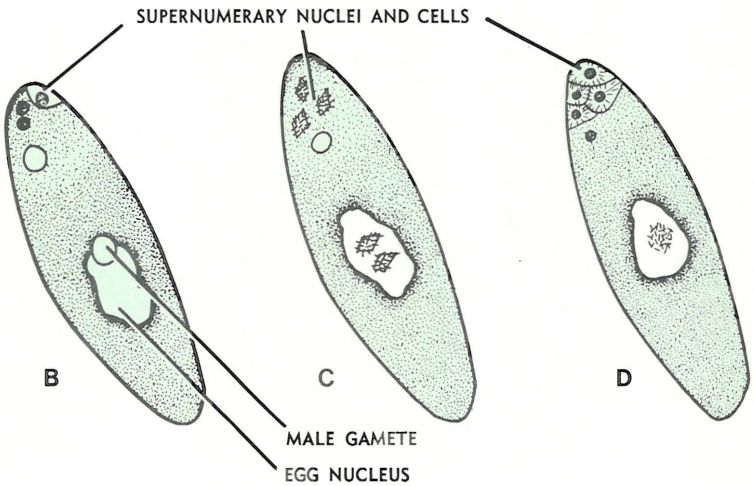

SUPERNUMERARY NUCLEI AND CELLS
B
C
D
MALE GAMETE
EGG NUCLEUS

means that there is an unequal distribution of genetic information, or that one nucleus is just more compact than the other has not been determined. Little, if any, cytoplasm encloses the male gametes and both gametes are retained within the body-cell complex.

The elongated pollen grain has been called a pollen tube but this terminology is incorrect, since a true pollen tube eventually forms when the elongated pollen grain reaches the nucellus. The pollen tube (or germ tube) forms at the point where the pollen grain comes in contact with the nucellus and may be in response to the contact. Although this has not been demonstrated, it is indicated in cases where the elongated pollen grain has come to lie across the surface of the nucellus and two separate pollen tubes have formed from the sides of the pollen grain. Pollen tubes normally form from the distal tip, or adjacent to the tip of the elongated pollen grain, perhaps because that is usually the point of contact with the nucellus. The dense, sac-like body-cell complex appears to be associated with the site of pollen-tube formation (Fig. 5.3 G-I), regardless of whether the pollen tube develops at the proximal or distal end of the elongated pollen grain. A localized swelling occurs in the wall of the elongated pollen grain and becomes extended to form a narrow pollen tube that grows into the nucellus (Fig. 5.3 G-J) (Allen, unpublished). The pollen tube usually elongates in a direct course and penetrates through about 300 μ of nucellar tissue. It then passes along the surface of the female gametophyte to the neck of the archegonium which it penetrates to reach the egg. There does not appear to be a general breakdown of nucellar tissue, as reported by Lawson (1909), and the pollen tube penetrates firm tissue in order to reach the female gametophyte (Allen, 1946b). Toward the end of pollen-tube formation, the dense cytoplasm containing the tube nucleus, the body cell that appears to enclose the two gametes, and often the stalk cell, forms a long, narrow cylinder about equal in diameter to the pollen tube (Fig. 5.3 M). This entire complex passes down the pollen tube (Allen, unpublished).

The male gametes of conifers show considerable variation in organization. They may be a highly organized cell with a cell wall and cytoplasm around the nucleus, or a naked nucleus with little or no surrounding cytoplasm and no cell wall at the time of fertilization. The former is considered more primitive and is found in members of the Cupressaceae, Taxodiaceae, Podocarpaceae and Taxaceae (Chamberlain, 1957). Similar but motile, ciliated sperm occur only in the more primitive gymnosperms — the cycads and *Ginkgo*. In the Pinaceae, no cell wall is visible separating the two gametes within the body cell. The wall, or membrane, of the body cell separates the gametes from the general cytoplasm of the pollen tube. Just before the gametes are discharged from the pollen tube, the body-cell wall disappears, releasing the two gametes in a mass of cytoplasm that does not appear to be marked off from the cytoplasm of the pollen tube (Chamberlain, 1957). A similar pattern occurs in Douglas-fir, except that the gametes are reportedly ciliated and capable of rapid movement and therefore could be referred to as sperm (Christiansen, 1969a). This report, if correct, would

be unique in conifers. The observation, however, must be confirmed in thin sections of pollen taken directly from the micropyle or at least from uncontaminated cultures.

The formation of two gametes within a pollen grain is the established pattern throughout the seed plants. In primitive seed plants, the paired gametes are highly organized cells of equal size. In the more-advanced gymnosperms, including Douglas-fir and most other conifers, the gametes differ in size. In *Taxus, Cephalotaxus* and *Torreya,* division of the body cell is so unequal that one gamete is only a small, lenticular cell cut off from the periphery of the body cell. In these cases, the smaller gamete aborts. The formation of several small gametes has been reported in two species of *Cupressus* (Doak, 1932). This recalls the condition found in certain primitive cycads which produce a dozen or more motile gametes (Chamberlain, 1957).

Fertilization

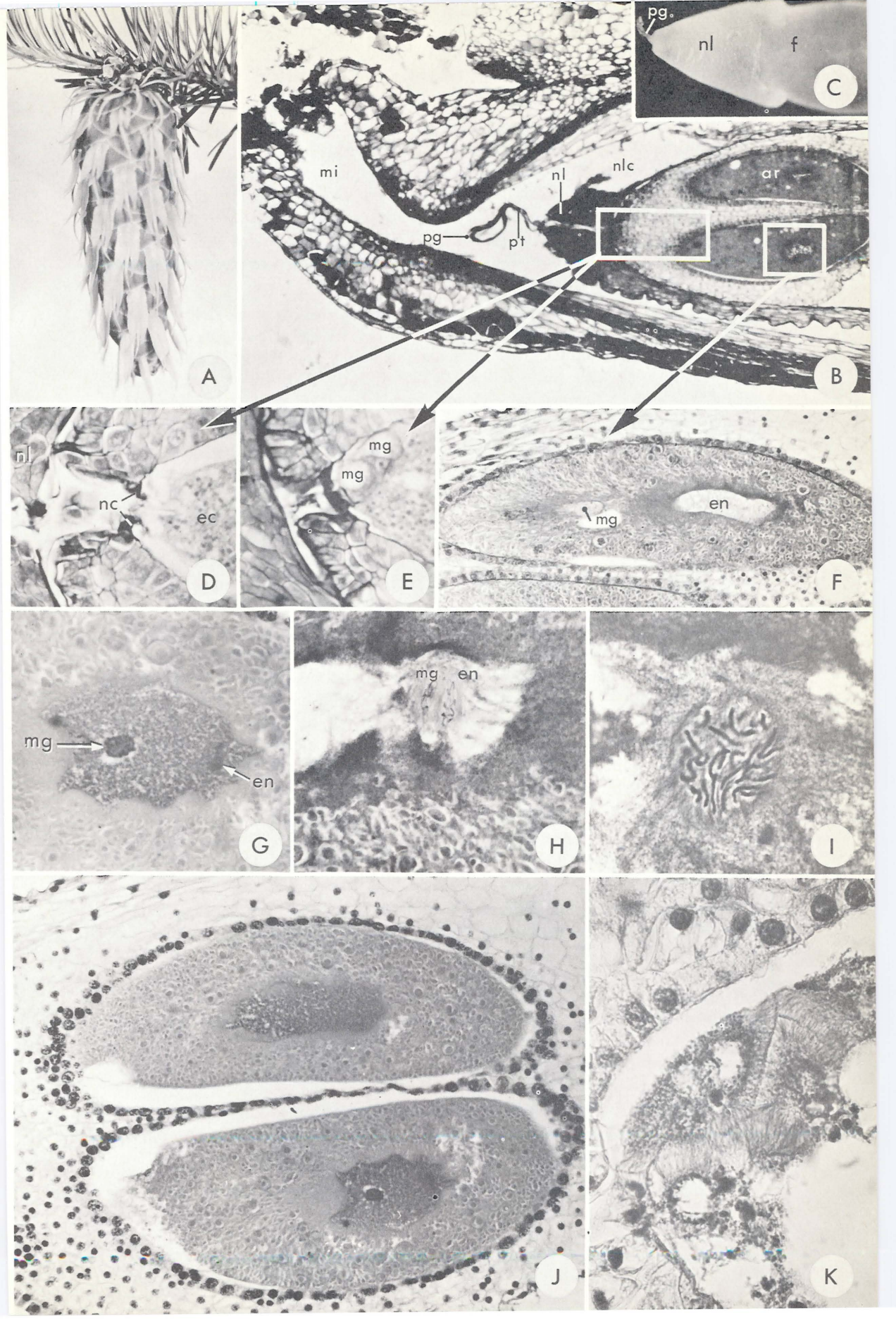
The term fertilization as applied to plants may involve more than the fusion of a male gamete with an egg. In gymnosperms, fertilization may involve several other and often unusual events from the time the pollen tube reaches the neck of the archegonium until the first division of the zygote. The fate of all nuclei and cells passing through the pollen tube of Douglas-fir must be considered, since they do not all disappear after fusion of the male gamete and the egg. The misinterpretation of these structures once they are within the egg has led to erroneous conclusions regarding the normal pattern of fertilization and early embryo development (Allen, 1943, 1946b).

Fertilization in Douglas-fir normally occurs between June 1 and June 20 at lower elevations in the Pacific Northwest and British Columbia. In any one year there is a variation of several days between ovules on an individual tree. Certain trees tend to be much earlier or later than the average and exhibit this behavior year after year (Allen, 1946b).

The female gametophyte is fully developed at the time of fertilization, as described in Chapter 4 (Figs. 4.6 C; 5.4 A). This is not true of some conifers where the pollen tube reaches the female gametophyte when the latter is still only at the free nuclear stage or at least before archegonia are recognizable. In these cases, the male gametes are not discharged from the pollen until archegonia are fully developed (Chamberlain, 1957). Archegonia in Douglas-fir are separated from each other by sterile prothallial tissue. Neck cells for the individual archegonia are also separated by sterile prothallial tissue so that it is impossible for one pollen tube to enter more than a single archegonium. The egg nucleus enlarges considerably and moves to the center of the egg before fertilization (Fig. 4.6 C). The egg takes on an extraordinary appearance. The cytoplasm surrounding the egg nucleus becomes more dense. These dense regions become separated

Figure 5.5 Fertilization.

A. The seed cone as it appears at fertilization, which usually occurs sometime between June 1 and June 20. X 3/4. B. Longitudinal section of the ovule at fertilization, showing mature archegonia (ar), the closed micropyle (mi), the nucellar chamber (nlc), the elongated pollen grain (pg) and the pollen tube (pt) penetrating the nucellus (nl). X 50. C. Female gametophyte (f) with the attached nucellus and pollen grain, as shown in B. This structure was dissected from the ovule early in June. X 25. D. Pollen-tube penetration of the nucellus,neck cells (nc) and egg cell (ec). X 325. E. Entrance of two male gametes (mg) into the egg cell. X 325. F. Movement of the male gamete through the egg cytoplasm to the egg nucleus (en). X 120. G. Chromosomes from both the male gamete and the egg nucleus become visible as they gradually approach one another. The deep depression in the egg nucleus was caused by the entrance of the male gamete. X 240. H. The 13 chromosomes of the egg and the 13 chromosomes of the male gamete, arranged on separate spindles, have come together laterally to form a common spindle at the center of the egg cell. X 320. I. Male and female chromosomes intermingled at the center of zygote. Together, the two sets of chromosomes (13 male plus 13 female) have reconstituted the diploid (2n or 26) sporophyte number of chromosomes and from this point on the cells of the zygote divide equationally to retain this number. X 650. J. Two archegonia at fertilization, as shown in H above. X 120. K. A greatly enlarged view of the micropylar end of the egg, showing formation of cell walls between the supernumerary nuclei. X 440.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from one another and soon show a fine fibrous structure. They appear to offer resistance to the expansion of the nuclear membrane; consequently, the contour of the nucleus becomes interrupted by several, dense fibrous pockets (Lawson, 1909). Similar observations have been made in the egg nucleus of *Pinus* (Ferguson, 1904) and *Tsuga* (Murrill, 1900). The dense cytoplasmic pockets around the egg nucleus of Douglas-fir have been observed to form the spindle fibers for the first division of the fusion nucleus or zygote (Lawson, 1909). The spindle fibers are believed to have a nuclear origin in *Pinus* and *Tsuga*.

The pollen tube penetrates the nucellar tissue and the neck cells of the archegonium and then releases, through its tip, its entire contents into the egg cell; the tube nucleus, the two male gametes and the stalk cell (Fig. 5.5 B, D-E). Details of this process are difficult to observe because of the disruption of tissues during pollen-tube penetration. The larger male gamete moves rapidly toward the egg nucleus where nuclear fusion occurs (Figs. 5.4 B; 5.5 F). The remaining supernumerary nuclei are often left close to the neck or somewhere between the neck and the fusion nucleus (Fig. 5.4 B). Several fates are possible for the supernumerary nuclei: (l) they may disorganize during a period of several days; (2) they may fragment and thus increase the number of apparent free nuclei in the neck region; (3) one or more of them may divide mitotically and cell walls may form in the neck region (Figs. 5.4 B-D; 5.5 K), or (4) the nuclei may fuse to form larger nuclei which gradually degenerate (Allen, 1943, 1946b).

The ventral canal nucleus may fuse with supernumerary nuclei or undergo independent division within its own cell wall (Allen, 1943). This may appear similar to triple fusion in angiosperms, where the endosperm tissue results. The significance of fusion, division or fragmentation of supernumerary and ventral canal nuclei is not known but their presence can result in the misinterpretation of the normal course of fertilization and early embryo development (Allen, 1943, 1946b). It should be recognized that these do not represent either the endosperm of angiosperms or a second embryo.

Fusion of male and female nuclei in Douglas-fir is similar to that described for other members of the Pinaceae (Chamberlain, 1957). The egg nucleus flattens somewhat on the side nearest the approaching male gamete, as in *Pinus* (Ferguson, 1940). The male gamete meets and gradually sinks into the egg nucleus but retains its identity for some time (Fig. 5.4 B). The membranes around both the male and female nuclei soon disappear, the two groups of chromosomes become evident (Fig. 5.5 G) and spindle fibers become visibly associated with each group of chromosomes (Figs. 5.4 C; 5.5 H). The two spindle figures come together laterally to form a common, multi-polar spindle with its main axis usually perpendicular to the long axis of the egg. The multi-polar spindle figure appears to contract and become bipolar by metaphase of the first division of the zygote (Figs. 5.4 D; 5.5 I) (Allen, 1946b).

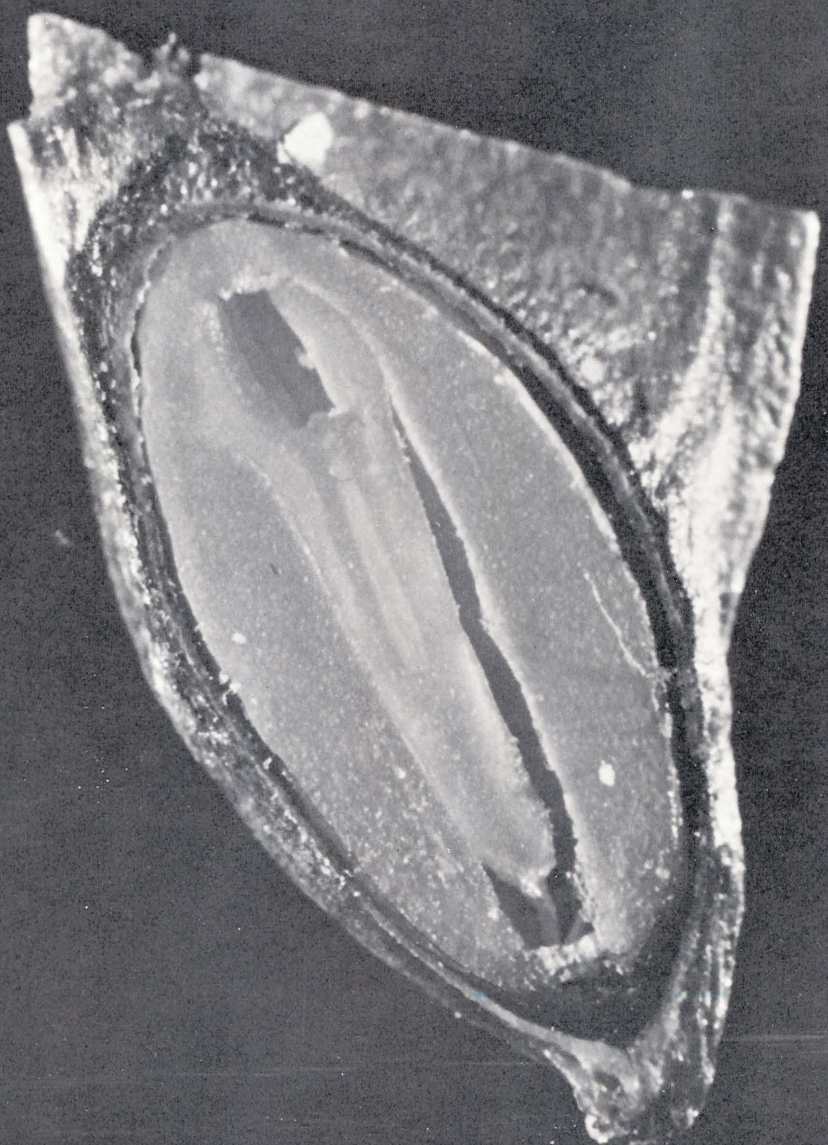
Little is known of the behavior of gymnosperm chromosomes at fertilization. A description of chromosome behavior in *Abies* was given by Hutchin-

son (1924), but efforts to confirm this in other conifers have failed. After the two spindles unite in *Abies,* homologous chromosomes from maternal and paternal nuclei pair. Pairing, however, has not been confirmed in other conifers. In Douglas-fir, when chromosomes become arranged at metaphase, there is a tendency for homologous chromosomes to lie loosely together (Fig. 5.5 I). If pairing actually occurs and separation of chromosomes is non-random at the first division, very distinct genetic polar differences could occur in the developing embryo. Studies of later stages of embryogeny in some conifers suggest such differences do occur (Chapter 6).

Future work on fertilization in conifers may have more of a theoretical and morphogenetic than a practical value. Few generalizations can be made since the only genus extensively studied has been *Pinus*. Other studies represent only one or two species of a genus. Of some significance is the possibility that male gametes of Douglas-fir may be ciliated and motile (Christiansen, 1969a). The varied behavior of the supernumerary nuclei in Douglas-fir should also be kept in mind lest they are mistaken for endosperm or additional embryos. These nuclei ultimately degenerate, as they do in other conifers. Fertilization of the egg nucleus by the ventral canal cell, a form of parthenogenesis, has been reported in other conifers (Chamberlain, 1957), but has not been observed in Douglas-fir. Ultra-structural studies with the electron microscope might provide some very basic information concerning both male and female gametophyte development. Most of the information available is based on very early work which, though carefully done, did not have the benefit of our modern, refined equipment and techniques.

Dissected mature seed of Douglas-fir. x30.

Embryo and Seed Development 6



Chapter 6

Embryo and Seed Development

The term "embryo" is difficult to define precisely when applied to plants. It broadly denotes the initial developmental phase of all classes of plants, but is more commonly used to denote the early developmental stages of plants which possess archegonia and/or seeds — the embryophytes. Embryogeny or embryogenesis usually designates the progressive development by growth and differentiation of a fertilized egg (zygote) into a young sporophyte (Foster and Gifford, 1959). Some authors, with some justification, might extend the term to include the development of the perennially embryonic shoot or root apex. Although developing apices and embryos consist of numerous meristematic cells and can be studied in similar ways, they represent quite different systems not comparable in environmental, histological or physiological relationships. It is because of this apparent similarity that apices have frequently been substituted for the less-accessible embryos in studies of plant morphogenesis.

Embryogeny in about 40 of the 53 living conifer genera has been investigated and much of this has been summarized by Buchholz (1950), Johansen (1950) and Wardlaw (1955). The same general pattern of embryogeny is usually found in all species of a genus, and closely related genera may also show similar patterns. The literature, however, is primarily descriptive because most studies were made during the early part of this century. Relatively few studies have been made since 1950. As a result, few physiological, biochemical or histochemical studies have been made. Many new possibilities for both descriptive and experimental studies are now available and a new look at conifer embryogenesis is needed.

Embryogeny in conifers begins with the fertilized egg and continues over several months to the formation of the complete dormant embryo. It is a continuous process that can be divided into three stages: development of the proembryo, the early embryo, and the late embryo. Proembryo development consists of stages from the first division of the fertilized egg forming the two-nucleate stage to the 12-celled stage when primary suspensor cells elongate, forcing the developing embryo through the base of the egg into the female gametophyte. The early embryo is represented by the rapid enlargement of the embryo and the appearance of the generative meristem of the root. The late embryo stages show the establishment of distinct meristems and the cotyledons. A fourth stage can be added, the dormant embryo, which allows for a complete description of the fully developed embryo. Any separation of a continuous developmental process is artificial, but often

justified by facilitating the task of description. Excessive subdivision, however, leads to complexity, and embryogeny is complex enough without splitting it into too many arbitrary units. Studies of embryogeny in other conifers, notably *Larix* (Schopf, 1943), have unnecessarily subdivided the early and late embryo stages. Although this may allow for minute comparisons of species and genera, it is not advantageous for a description and comparison of conifers in general.

Douglas-fir embryogeny is generally similar to that of *Pinus*, as described in nearly all elementary texts. The description, diagrams and photographs provided in this chapter represent Douglas-fir embryogeny. Important differences from other conifers will be discussed, as well as the possible evolutionary or adaptive significance of these differences.

The Proembryo

As noted in Chapter 5, there is no actual fusion of the two gametes during syngamy in Douglas-fir. Chromosomes of the egg and sperm come together on separate but parallel spindles, then immediately arrange themselves on the equatorial plate in preparation for the first division. The zygote stage is very brief and does not include a stage when maternal and paternal chromosomes are freely intermingled in a single nucleus. Possible pairing, or at least an association of homologous chromosomes, however, appears to occur on the spindle just before the first division.

As in most gymnosperms the proembryo passes through a free-nucleate stage during which nuclear divisions occur without cell-wall formation. The first two divisions of the zygote result in the formation of four free nuclei near the center of the archegonium (Figs. 6.1 A, B; 6.2 C, D). At first these nuclei are small and consist mainly of chromatin, but they enlarge quickly as they descend toward the basal end of the archegonium (Figs. 6.1 C; 6.2 E). During early development, the nuclei of the proembryo are surrounded by very granular cytoplasm. This may represent a region of digestion lying between the heterogenous food reserve of the egg cytoplasm and the growing nuclei of the new sporophyte. After the four free nuclei reach the base of the archegonium, they again divide to form eight nuclei. Cell walls form to separate these nuclei and produce two tiers of cells with four cells in each tier. The first cell wall formed is always transverse to the long axis of the archegonium and is followed by the formation of two axial walls. This results in a two-tiered proembryo, the upper cells of which are open to the egg cytoplasm (Figs. 6.1 C-E; 6.2 E, F). Cells of the lower tier then divide by transverse walls to interpose a third tier of cells between the upper (open) and lower tiers. The proembryo then consists of 12 cells in three tiers; an open tier at the top, a middle suspensor tier, and a lower embryo tier (Figs. 6.1 F; 6.2 G) (Allen, 1943, 1946b). A rosette tier, formed by the division of the cells of the open tier, was described for Douglas-fir by Lawson (1909) but has not been observed in subsequent studies (Buchholz,

1926, 1931; Allen, 1943, 1946b). Four tiers of cells were found in all Pinaceae studied, with Douglas-fir being a possible exception. Three tiers were common in other families of conifers (Chamberlain, 1957; Wardlaw, 1955).

In the proembryo, the proximal tier has the distal ends of its cells open to the general mass of the egg cytoplasm (Figs. 6.1 F; 6.2 G). This open tier possibly serves for a short time to transmit reserve food to the lower portion of the proembryo. The next tier commonly present in the Pinaceae comprises the rosette cells, which are absent in Douglas-fir. These were thought to be functionless by early workers, but Buchholz (1918, 1931) has demonstrated that several, if not all four rosette cells may give rise during early embryogeny to small embryos. These embryos usually become twisted and abort when they consist of about a dozen cells. The middle tier of cells (the suspensor cells) elongate to hundreds of times their original length to form the primary suspensor, which pushes the distal tier of cells out into the female gametophyte tissue. Each cell of the distal tier of four cells may develop into an embryo, or in the case of Douglas-fir, all four may form into one embryo. None of the other tiers take part in the formation of root, shoot, leaves or cotyledons of the embryo (Allen, 1943, 1946b). A differentiation of the proembryo into tiers is characteristic of most conifers but is less distinct in other gymnosperms where more massive, many-celled proembryos occur. The precision with which the tiered proembryo originates is in marked contrast to the comparatively unstratified arrangement characteristic of the early embryogeny of what are believed to be more primitive gymnosperms (Chamberlain, 1957; Wardlaw, 1955).

The free nuclear stage prior to cell-wall formation is characteristic of the majority of gymnosperms. This phenomenon has been attributed to the large size of the egg cytoplasm and resultant failure of early nuclear divisions to segment the cytoplasm (Chamberlain, 1957). In extant conifers, the eggs are comparatively small, and most form four free nuclei. There are exceptions such as *Sequoia*, which has a very small egg, and no free nuclear stage (Wardlaw, 1955). In conifers, it is possible that cytokinesis is induced in the proembryo by factors independent of the ratio of nuclear to cytoplasmic volume, and the nearly universal occurrence of free nuclear division in the formation of the proembryo of conifers may have evolutionary if not developmental significance.

During the formation of the 12-celled proembryo in Douglas-fir, a variety of changes occur at the micropylar end of the fertilized egg. As described in Chapter 5, the ventral canal cell, stalk cell, remaining male gamete and tube nucleus may undergo a variety of fusions and/or divisions (Allen, 1943, 1946b). Divisions of the supernumerary nuclei generally occur simultaneously with the second division of the zygote. As a result, the supernumerary nuclei may give the superficial appearance of a second embryo forming near the neck of the archegonium. This, however, is not the case. These cells have never been observed to develop into embryos, but rather degenerate during later stages of embryo development.

The proembryo stage ends when the tier of primary suspensor cells elon-

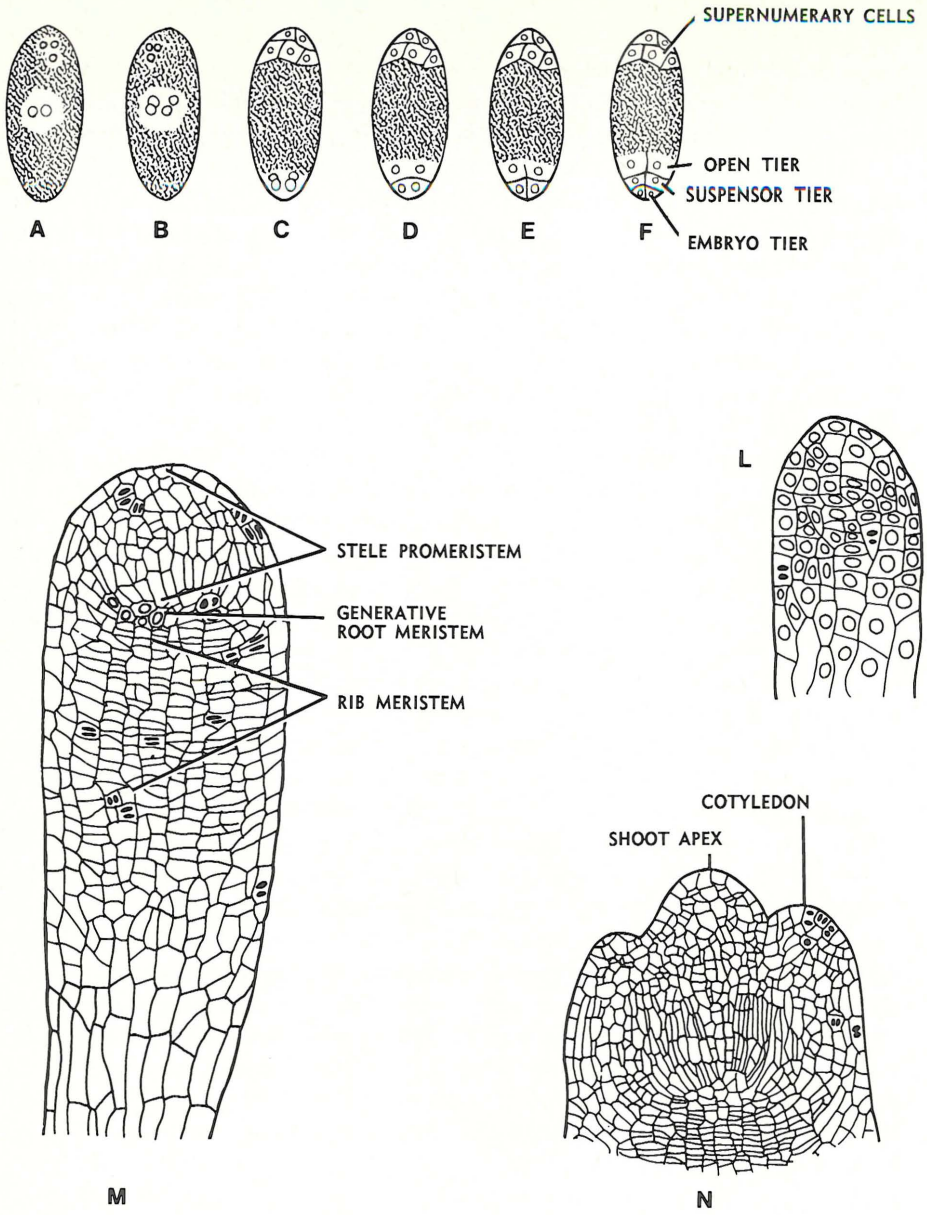
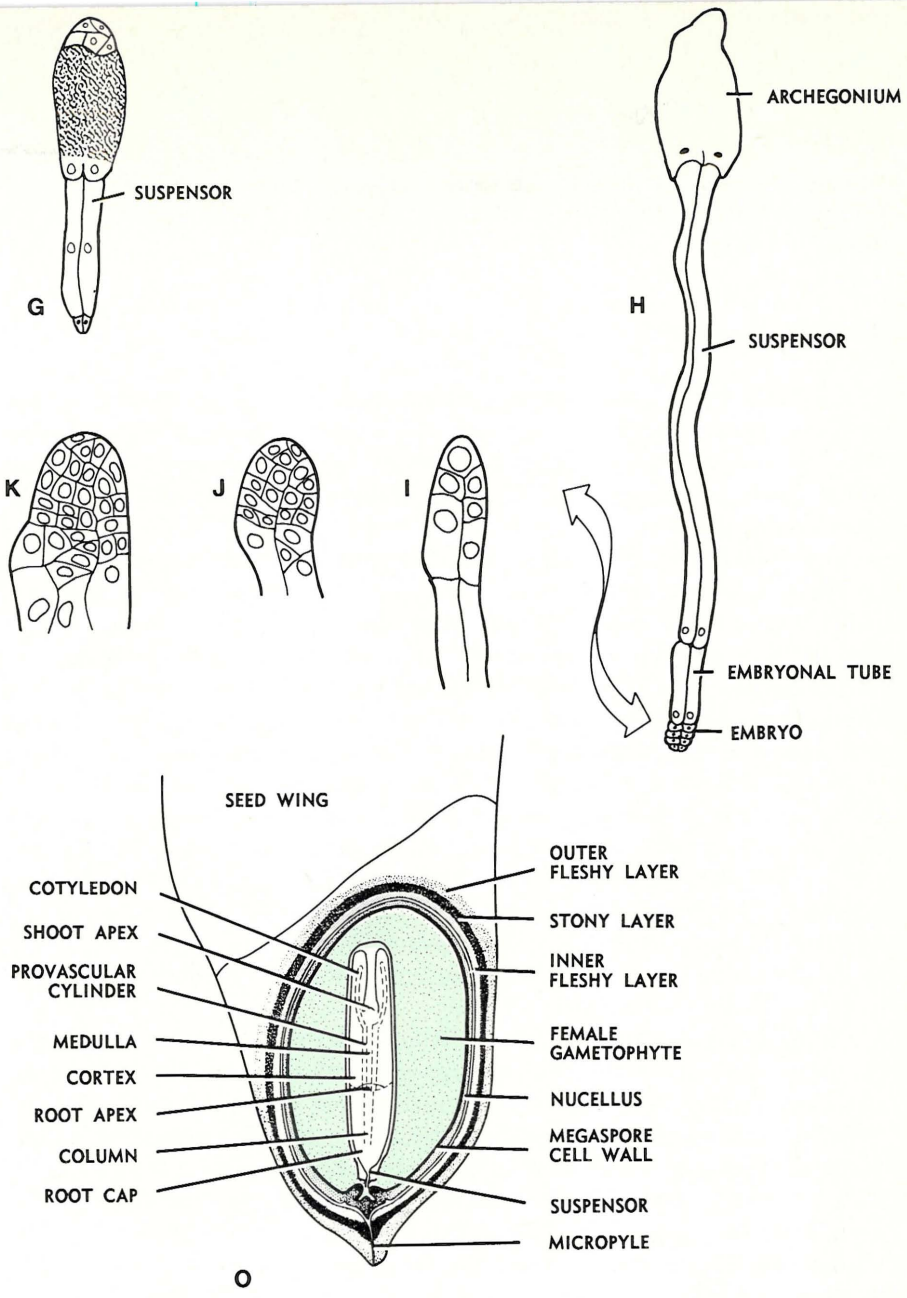


Figure 6.1 Embryo Development.

A-G. The proembryo, which includes all stages from the first division in the zygote to the 12-celled stage when primary suspensor cells elongate. Gametophyte (haploid) tissue is shown in color. A. The two-nucleate stage following the first division of the zygote, showing the two free nuclei in the center of the archegonium. Three supernumerary nuclei are at the top (neck) of the archegonium. B. The four-nucleate stage, showing the four free nuclei in the center of the archegonium which migrate to the base of the archegonium. C. The four free nuclei become arranged in one tier at the base of the archegonium. Supernumerary nuclei have divided and cell walls have formed between them. Division of supernumerary nuclei generally occurs simultaneously with the second division of the zygote. D. The four free nuclei divide to form eight nuclei in two tiers. E. Two axial cell walls form after the transverse walls, resulting in two tiers consisting of four cells each. Cells of the upper tier are open to the egg cytoplasm. F. Cells of the lower tier divide transversely, interposing a third tier of cells. The proembryo now consists of 12 cells in three tiers, an open tier at the top, a middle suspensor tier and a lower embryo tier. G. Primary suspensor cells elongate, forcing the lower (embryo) tier out through the wall of the archegonium. H-M. The early



embryo is represented by rapid enlargement of the embryo and the appearance of the generative meristem of the root. H. Elongation of the suspensor cells and embryonal tube cells pushes the early embryo deeply into the female gametophyte tissue. The archegonium appears empty and begins to collapse, while the nuclei of the open tier degenerate. I. Early embryo development (Type B), showing one of the two more vigorous terminal cells which have attained dominance over the other two apical cells. J-K. Later stages of Type B development, showing the dominant group of terminal cells overtopping the less-vigorous group of cells. L. Longitudinal section through the club-shaped (massive) embryo. Cells undergo many transverse divisions just below the apical dome, forming a rather long rib meristem. M. Longitudinal section through the early embryo. A distinct rib meristem extends to within about eight cells of the apical surface cells. At the top of the rib meristem, a generative root meristem becomes evident. Above the generative root meristem arises the stele promeristem. N. Longitudinal section through a larger embryo in which the root apex has formed but is inactive. O. A median section through the seed as seen from the upper surface, showing the seed coat and the dormant embryo embedded within the female gametophyte. Redrawn in part from Allen (1943, 1946b).

gates and the embryo tier is pushed through the base of the egg into the female gametophyte tissue. The proembryo stage in Douglas-fir is usually complete within a week of fertilization, by the end of the second week in June.

The Early Embryo

The early development of more than one embryo per ovule is common in gymnosperms, if not characteristic of the group. Although more than one and often many embryos begin to develop, the mature seed seldom yields more than a single seedling. All other embryos cease development somewhere along the way.

More than one embryo may originate in conifers in different ways. Several archegonia are present in each ovule and all the eggs may be fertilized. A separate embryo can develop to a considerable extent from each zygote, but only one embryo usually reaches maturity. This simple polyembryony occurs in all conifers, with a few exceptions like *Torreya* which has only one archegonium. A second form, cleavage polyembryony, also occurs where distal tiers of the proembryo separate into four filamentous embryos. It occurs in varying degrees in nearly all families of conifers including the Pinaceae; it is a constant feature of *Pinus, Cedrus, Tsuga, Pseudolarix* and *Larix* but has not been observed in *Picea* and *Pseudotsuga* (Chamberlain, 1957). *Larix* shows a delayed form of cleavage polyembryony where separation of the individual units occurs much later in embryo development than in *Pinus* (Schopf, 1943). Cleavage polyembryony occurs only in rare cases in *Abies*. Its occurrence in the Pinaceae and other families is discussed in more detail by Chamberlain (1957).

Simple polyembryony occurs in Douglas-fir, which usually has four to six archegonia per ovule, but cleavage polyembryony is absent. Behavior, however, is not stereotyped and considerable variation occurs in early embryo development. Some of these variations superficially resemble cleavage polyembryony. Three general types of development have been described (Allen, 1946b), two of which occur more frequently than the other.

In Type A, each of the four cells of the apical tier in the proembryo, all derived from the same fertilized egg, contribute more or less equally to the developing embryo. At no time does one of the four cells of the apical tier dominate. The terminal cells divide longitudinally or obliquely to establish a group of apical initials such as occur at the shoot apex of many gymnosperms. These initials contribute to the entire embryo and eventually give rise to the shoot apex of the embryo. This type of development is relatively common in Douglas-fir.

A second pattern of development, Type B, is the most common in Douglas-fir. Two terminal cells attain dominance, apparently by more vigorous growth than is exhibited by the other two. Cells of the dominant pair divide in two planes, adding cells to all four embryo units for a limited time. The terminal cells eventually give rise to a group of apical initials

which develop similarly to those of the first type (Figs. 6.1 J, K; 6.3 B-D).

A third variation, Type C, also occurs. In a very few instances, some evidence indicates that one terminal cell of the proembryo may dominate the other three by gradually overtopping them and acquiring three or four cutting faces. The terminal cell is then truly an apical cell, such as occurs in the embryo of *Pinus* (Buchholz, 1918).

The most common type of development (type B), in which two units overtop the other two (Fig. 6.3 B-D), might be explained on the basis of the unequal position of the four original free nuclei at the base of the egg (Schopf, 1943). Schopf's hypothesis was based on his intensive study of the embryogeny of *Larix*. He concluded that the position each proembryo cell occupies at the base of the archegonium, which is correlated with positions of the nuclei, more or less predetermines its fate. Cells which occupy the broader diameter of the archegonium are favored over the other two. Any slight advantage for the larger cells would, therefore, become increasingly important in selection of the most successful embryonic unit.

Whatever the nature of the factors deciding the part which a given embryonic unit will play in cleavage polyembryony, whether genetic or fortuitous, there is only a short period for competition before the units are freed to continue development at their own individual rates. In simple polyembryony, at least in Douglas-fir, the embryonic units remain together and presumably their growth is retarded until the dominant units gain the ascendency: if the embryo units are more or less equipotential, the entire embryo forges ahead at its maximum rate (Allen, 1946b).

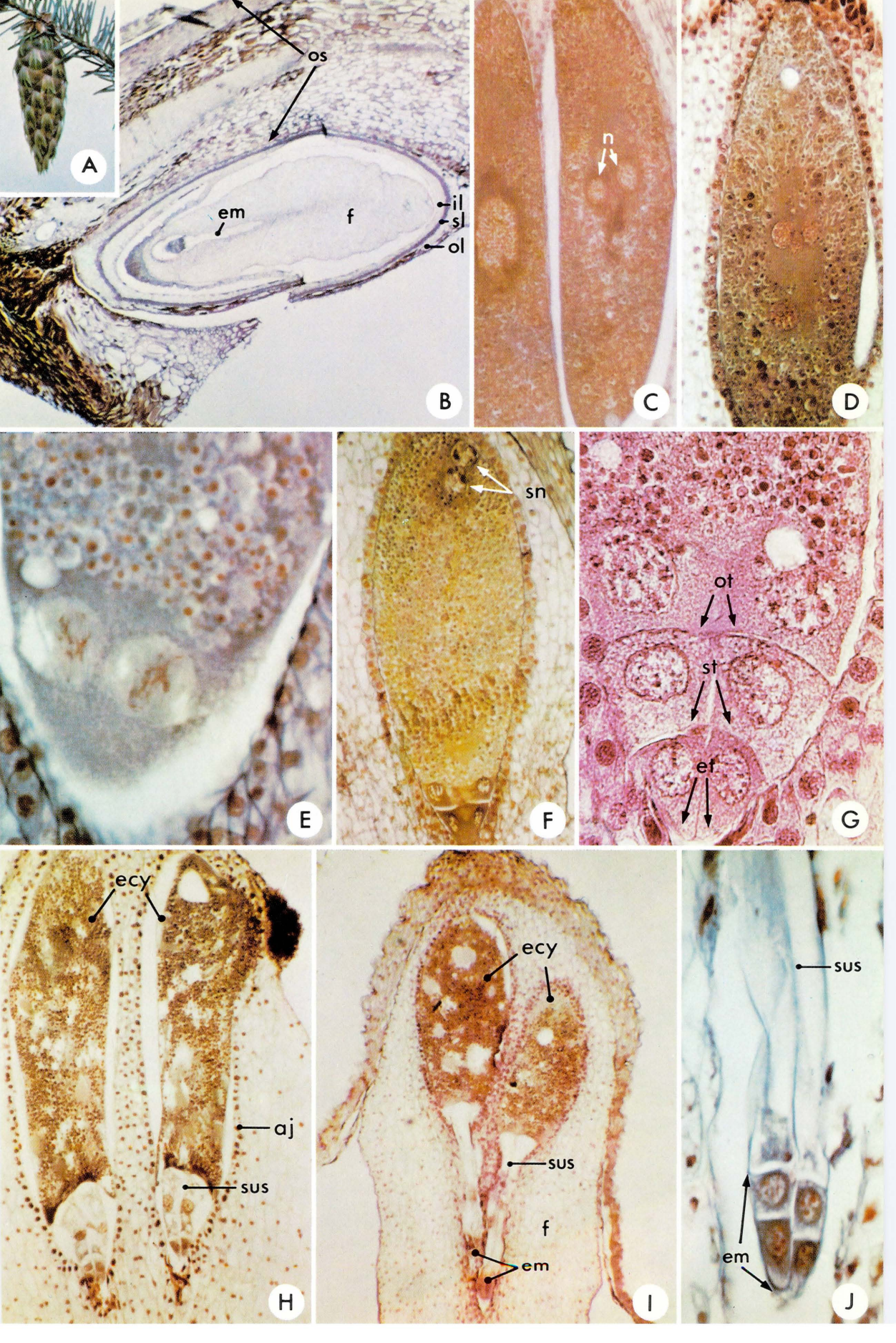
It is apparent that while species exhibiting both simple and cleavage polyembryony are capable of producing vast numbers of embryos per seed, usually only one embryo matures. Rosette embryos which occur in some species seldom develop beyond a filamentous stage and usually only one of the four embryos resulting from cleavage polyembryony, generally the most terminal, continues to develop. The embryo from only one archegonium, again the most vigorous embryo, usually develops within the ovule. All other embryos abort and cannot be detected in the mature seed (Chamberlain, 1957; Wardlaw, 1955). Whatever the cause of repression, this development appears to be a remarkable example of early natural selection of a more vigorous embryonic unit or one more favorably situated.

The evolutionary relationship between simple and cleavage polyembryony is unsettled. Superficially, the complexity and precision of cleavage polyembryony suggests that it is a specialization derived from simple polyembryony; however, Buchholz (1926, 1950) believes that cleavage polyembryony is a primitive character and that simple polyembryony has been derived from it. Whether simple polyembryony, as found in Douglas-fir, actually represents a primitive condition is still speculative.

As mentioned earlier, extension of the apical cells into the female gametophyte tissue results from elongation of suspensor cells and formation and elongation of embryonal tubes (Figs. 6.1 G, H; 6.2 H-J). Details of

Figure 6.2 Proembryo and Early Embryo Development.

A. The seed cone during June when the proembryo and early embryo stages are present. X 2/5. B. Longitudinal section of an ovuliferous scale (os) and ovule, showing the position of the early embryo (em) within the female gametophyte (f). The seed coat has begun to differentiate an inner layer (il), a middle or stony layer (sl) and an outer layer (ol). X 15. C. The two-nucleate stage of the proembryo. Two nuclei (n) are in the center of the archegonium on the right while fertilization has not occurred in the archegonium on the left. X 100. D. The four-nucleate stage, showing all four free nuclei as they begin to migrate to the base of the archegonium. X 100. E. Enlarged view, showing two of the four free nuclei at the base of the archegonium. The nuclei greatly enlarge then divide. X 500. F. The eight-celled proembryo stage during the formation of the transverse cell walls but before axial cell walls have formed. X 100. Two supernumerary nuclei (sn) are visible at the top of the archegonium. X100. G. Enlarged view of the base of the archegonium, showing the 12-celled proembryo consisting of three tiers of cells: an open, upper tier of cells (ot); a middle, suspensor tier (st), and a lower, embryo tier (et). The egg cytoplasm still contains abundant proteid vacuoles. X 300. H. The suspensor tier of cells (sus) elongate, forcing the embryo tier through the archegonial jacket (aj). The egg cytoplasm (ecy) becomes vacuolate and begins to break down. X 90. I. Low magnification of the micropylar half of the female gametophyte, showing suspensor elongation forcing the embryo into the female gametophyte tissue. X 50. J. Enlarged view of the early embryo at the end of the suspensor. X 300.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this development have not been fully studied in Douglas-fir, but the sequence appears to be similar to that of other conifers. The rather thick and perhaps more rigid suspensor in Douglas-fir, consisting of four cells, may result in the less-tortuous pathway made by the embryo as it is thrust deeper and deeper into the female gametophyte tissue. The abundant starch surrounding the embryo disappears, especially for a long distance in front of the embryo. Apparently more-soluble foodstuffs take the place of starch and are rapidly absorbed by the growing embryos. Cell walls in the female gametophyte tissue adjacent to the embryo break down, leaving a cavity behind the advancing embryo. At the same time, cells at the periphery of the female gametophyte divide and grow rapidly (Fig. 6.3A) (Allen, 1946b).

Once a group of apical cells has appeared at the tip of the embryonal tubes, no matter what type of earlier development (A, B or C), the young embryo of Douglas-fir develops much like that of *Larix* (Schopf, 1943). A large apical cell, appearing much like that of certain lower vascular plants, may be present in embryos formed by one (Type C) or two (Type B) terminal cells of the proembryo. An apical cell, if present, appears for only a short time. The cell pattern varies considerably, but continued cell division in all planes produces a club-shaped embryo similar to that in many other gymnosperms (Figs. 6.1 J-L; 6.3 B-E). At no time is the embryo or any part of it invested with a distinct surface layer or dermatogen. Surface cells exhibit numerous periclinal divisions throughout embryogeny (Allen, 1946b).

The club-shaped embryo elongates as a result of many transverse cell divisions in the region below the domed apical portion. Derivatives elongate in a longitudinal direction. This serves to separate the actively dividing apical region from the suspensor system by a rather long, distinct rib meristem. It extends from the suspensor system to a point some eight or ten cells away from the free apex of the embryo. At the base of the latter region there exists a small group of cells which behave independently of the rib meristem of the root (Figs. 6.1 M; 6.3 E, F). The small, spherical apical region between the rib meristem and the apex has been called the "pleromic centrum" by Schopf (1943) and represents the earliest recognizable stage of the embryo stele which eventually gives rise to the vascular tissue. This is perhaps more appropriately termed the "stele promeristem"; however, no exact predestination of tissues is implied since it is realized that cells of this region may give rise in varying degrees to cells or tissues other than those of the stele. The embryonic stele of the dormant embryo does arise primarily from the stele promeristem in Douglas-fir (Fig. 6.1 O).

At the end of the early embryo stage (by early July, or about a month after fertilization), the young embryo of Douglas-fir consists of the stele promeristem, delimited at one end by the dome-shaped free surface which will give rise to the shoot apex of the mature embryo, and at the other end by a generative root meristem in its youngest recognizable stage. Behind the latter is a massive zone of rib meristem which is continuous with the suspensor system (Figs. 6.1 M; 6.3 E). The cell pattern now evident foreshadows to a great extent the future course of embryo development

(Allen, 1946b).

The Late Embryo

The late embryo stage shows the establishment of distinct meristems and the cotyledons. The promeristems referred to below represent meristematic cells that may give rise to specific organs within the embryo. However, there are seldom clear-cut promeristems that give rise to specific tissues or organs and, as a result, the histogen terminology which suggests a predestination may be wisely avoided in conifer embryos.

The stele promeristem is delimited at its ends by the free embryo apex and by the root initials and is surrounded by a poorly defined cortex promeristem (Figs. 6.1 M; 6.3 E). These promeristems correspond to the terms plerome and periblem, respectively, used by many investigators. The stele and cortex promeristems enlarge mainly by intercalary growth to form the embryonic stele and the embryonic cortex of the dormant embryo. There is no evidence that the embryonic stele and cortex can be rigidly separated at any stage of development. The rib meristem, lying between the root initials and the suspensor system, adds peripherally to the embryonic cortex and to the suspensor. The rib meristem (Schopf's columnar tissue) shows early differentiation into a central core or column and an investing tissue, the pericolumn. Cells of the column elongate little, while cells of the pericolumn gradually change orientation and come to lie more or less parallel to the surface of the stele and tend to divide in a plane perpendicular to that surface (Figs. 6.1 M, N; 6.3 E-G). At the suspensor end of the elongating embryo, cells are added continually to the massive suspensor and are the only cells of the young embryo that differentiate and attain maturity (Allen, 1947a).

The shoot apex contributes nothing to the growth of the embryo as a whole and remains in an inactive state after its differentiation (Figs. 6.1 N; 6.3 F-H). It is entirely superficial, a structure set upon the developing embryo, and results from the activity of surface cells at the embryo apex (Allen, 1947a). Similar observations have been made for *Larix* (Schopf, 1943) and *Abies* (Hutchinson, 1924).

The root apical meristem is internally situated and intimately associated with the surrounding meristematic tissue (Figs. 6.1 M, N; 6.3 E-H). It forms before the shoot apex and is affected more by growth of adjacent tissues. The root initials contribute only in a small way to the embryonic stele, but considerably to the column and the embryonic cortex. The greater part of embryo enlargement during the late stage, however, results not from apical but rather intercalary growth. The pattern of cellular arrangement in the embryo delimiting particular primary meristematic regions is essentially the result of very active growth of the primary stele, which is set off from the rest by the relatively inactive root initials (Allen, 1947a).

The cotyledons form during the latter part of July, two or three weeks

later than the initial zone (Fig. 6.3 F-I). Surface cells of the shoulder of tissue adjacent to the shoot apex contribute to the development of the cotyledon primordia. Cotyledons, unlike true leaves, arise quite independently of the shoot apex, although in a very similar manner. Six or usually seven cotyledons occur in a whorl around the apex in Douglas-fir (Allen, 1947a). Cotyledon number varies considerably in conifers (Bierhorst, 1971). The Pinaceae generally have large numbers (*Pinus* averages about eight), while many other families have only two or three. In general, polycotyledony is considered more primitive, and the lower numbers in some other species are believed to have resulted from fusions (Chamberlain, 1957). Provascular differentiation occurs very early in the meristematic stele and progresses acropetally into the cotyledons and basipetally into the developing stele. The embryo of Douglas-fir is well developed by early August and few changes can be observed between that stage and the mature, dormant embryo (Figs. 6.1 O; 6.3 I) (Allen, 1947a).

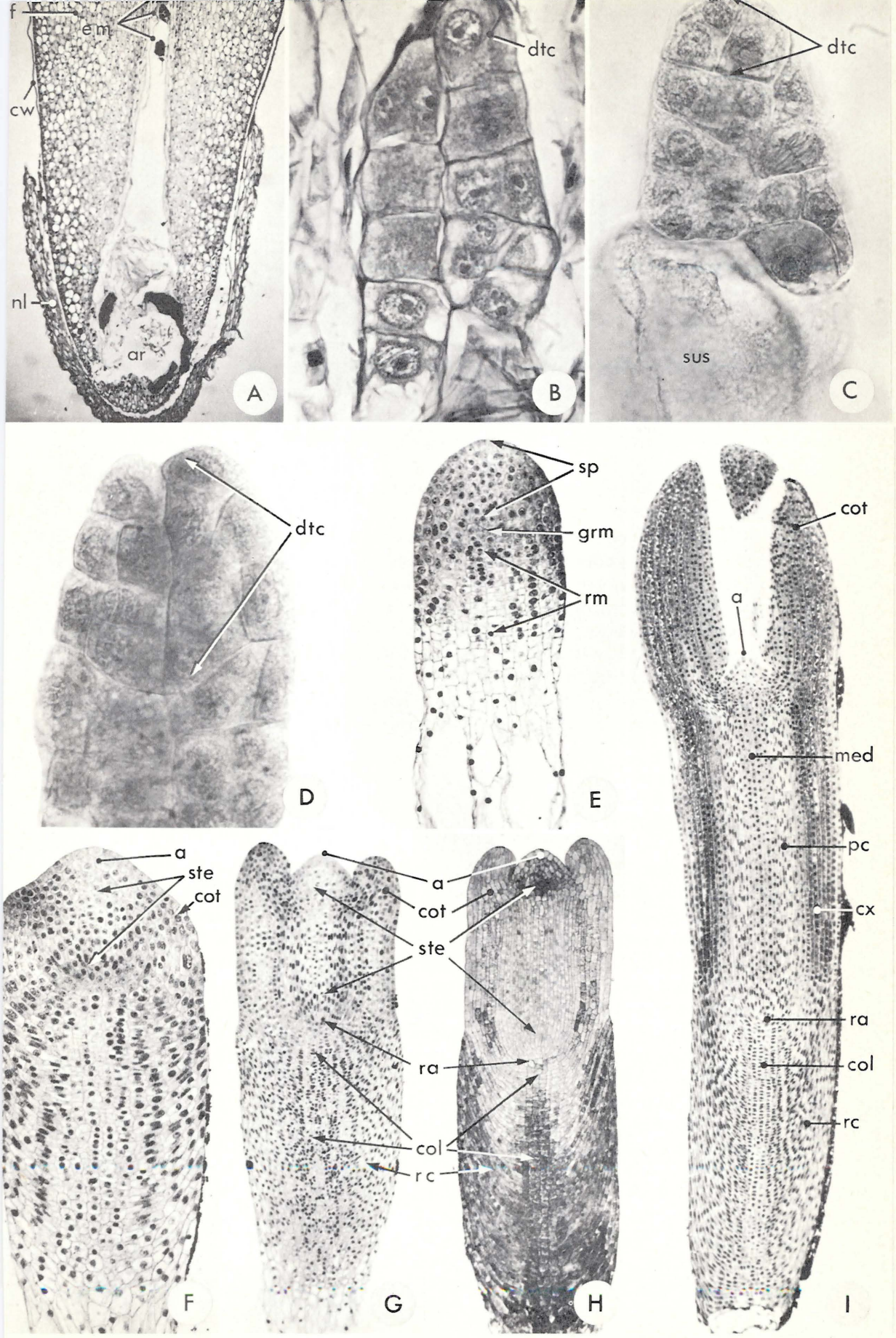
The Dormant Embryo

The mature embryos of several gymnosperms have been described by many investigators and have been completely reviewed recently by Guttenberg (1961). Most students of conifer embryogeny have retained the histogen terminology and have augmented it with new terms. Hanstein's (1868, 1870) histogen concept assigns specific histogens or primary meristems and exact destinies to regions of the meristem from their very origin; that is, specific meristems give rise to specific tissues. The histogen concept is not universally applicable in angiosperms, nor does it have any place in the description of gymnosperm embryos and apical meristems. Therefore, its use by many has made the literature more confusing. A compromise terminology for meristematic regions of the embryo shoot and root apices has been proposed (Allen, 1947a, b).

The generative root meristem refers to the entire formative region of the root, including several differentiated zones comparable to those recognized in gymnosperm shoot apices (Allen, 1947b). The root initial zone consists of a monolayered shell of initials that gives rise to several zones of the root generative meristem. These give rise, above, to the embryonic stele and embryonic cortex and, below, to the column. These zones are analogous to zones recognized in certain gymnosperm shoot apices (Foster, 1939, 1941) and have been termed mother-cell zones (Allen, 1947a). Exact predetermination for the derivatives of any mother-cell zone is not implied, since it is impossible to determine the exact fate of any given cell and its derivatives. The stelar mother-cell zone gives rise to the embryonic stele. Certain of the cells formed will develop into provascular tissue of the primary root. The column mother-cell zone lies distal to the root-initial shell and gives rise to the column which, in turn, adds to the peripheral tissue of the rootcap. Divisions within these cells are mainly transverse, giving

Figure 6.3 Early, Late, and Dormant Stages of Embryo Development

A-E. Early embryo development. A. Longitudinal section through the micropylar half of the female gametophyte (f) enclosed by remnants of the nucellus (nl) and megaspore wall (mcw), showing the collapsed archegonia (ar), three early embryos (em) and the cavity left by the advancing embryos. Suspensors have torn away during sectioning. X 25. B. Early embryos of the Type B, showing the overtopping by the dominant, more vigorous terminal cells (dtc). X 420. C,D. Later stages of the Type B early embryo, showing the dominant group of terminal cells overtopping the less vigorous terminal cells and the suspensors (sus). X 420. E. Longitudinal section through the early embryo, showing the generative root meristem (grm) at the top of the rib meristem (rm) and above that, the stele promeristem (sp). X 100. F. Longitudinal section through a late embryo at the time of cotyledon (cot) initiation. The rib meristem and stele promeristem rapidly divide below the lightly stained inactive shoot apex (a). X 80. G, H. Longitudinal sections through later embryos, showing the development of the stele (ste), cotyledons, shoot apex, root apex (ra) and the column (col) of the root cap (rc). X 36. I. Longitudinal section through a dormant embryo, showing the well-developed stele consisting of the provascular cylinder (pc), medulla (med) of the stele, cortex (cx), root apex, column and root cap. X 40.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rise to a typical rib meristem. The cortical mother-cell zone produces the embryonic cortex and lies on the periphery of the column mother-cell zone. Derivatives of this zone may add to the rootcap (Figs. 6.1 O; 6.3 I).

The embryonic stele consists of a provascular cylinder surrounding a medullary core. The provascular tissue and the medulla become distinguishable within 50 μ of the root initials. The medullary core enlarges nearer the shoot apex at the cotyledonary node, then ends abruptly at the level where the cotyledons are separate and the shoot apex begins (Figs. 6.1 O; 6.3 I).

The shoot apex of the dormant embryo is cone-shaped and quite small, about 130 μ in height (Figs. 6.1 O; 6.3 I). The outer, peripheral region of the shoot apex can be distinguished from an inner cone of tissue, which contains more abundant ergastic materials and displays greater stainability of its nuclei. At germination, cells of the inner cone commence meristematic activity earlier than the more peripheral cells.

A provascular strand diverges from the embryonic stele and extends nearly to the tip of each cotyledon (Figs. 6.1 O; 6.3 I). There are indications of the early differentiation of precursory phloem tissue near the base of each cotyledon in the dormant embryo.

The dormant embryo of Douglas-fir has a well-developed provascular system within the cotyledons and embryonic stele to within a short distance of the generative root meristem. The shoot apex, however, is poorly developed and contains no provascular system. This is true of the embryos of many seed plants. The embryo is, in a sense, a complete plant with an elementary vascular system, an axis with cotyledons and a root apex which is a coordinated part of it. The shoot apex, however, consists of only a small mound of inactive cells superimposed upon the embryonic plant with no provascular connection with the rest of the embryo (Allen, 1947a).

Seed and Wing Development

Even before fertilization, the seed coat or testa begins to differentiate from the integument (Figs. 5.3 F; 6.2 B). The integument differentiates into three layers characteristic of most conifer seeds (Fig. 6.2 B). (1) The outer layer is continuous with the adaxial surface of the ovuliferous scale. The cells become vacuolated and many accumulate ergastic materials. This layer changes little during ovule and seed development and forms the thin outer covering of the seed which attaches to the seed wing. (2) Cells of the middle layer remain smaller, isodiametric, and divide frequently, forming a distinct layer up to six cells thick. This layer differentiates into a stony layer in which the cells are small, and have thick, lignified, secondary cell walls with numerous simple pits. (3) The inner, fleshy layer adjacent to the nucellus consists of a few layers of small, undifferentiated cells. Cells of this layer become slightly elongated and vacuolated during ovule

development. In the mature seed (Fig. 6.1 O), cells of both the inner and outer layers are elongated and flattened against the stony layer. Some cells of the inner and outer layers may also become slightly lignified (Owens and Smith, 1965).

In a mature seed, the embryo is loosely packed within the rather firm, cream colored, nutritive female gametophyte tissue. This tissue, enlarged during embryo and seed development and containing abundant stored food, apparently functions as a food reserve for the embryo and very young seedling upon germination. A thin white megaspore cell wall encloses the entire female gametophyte. The grey-colored megasporangium or nucellus, attached to the outside of the megaspore cell wall, is thick and conspicuous at the micropylar end but becomes as thin as the megaspore cell wall at the opposite end. The megaspore cell wall becomes infolded at the micropylar end and the nucellus at that point becomes rather hard, forming a distinct brown tip where the micropylar canal and nucellus meet. The female gametophyte tissue and fibrous suspensor are firmly attached to this portion of the nucellus. No nucellar chamber persists in the mature seed (Fig. 6.1 O).

Seed wings develop from the ovuliferous scale and not from the seed coat. Seed wings begin to differentiate at about the time of pollination, when ovuliferous scales are only about 2 mm long (Fig. 4.4 E). Cell divisions occur just beneath the epidermis of the ovuliferous scale at the distal end of each ovule. Three hypodermal layers of small, meristematic cells result and slowly extend beneath the ovule and along the ovuliferous scale, forming the outline of the seed wing. Cells of the outer hypodermal layer accumulate considerable ergastic materials and elongate much more than the overlying epidermal cells. As a result, epidermal cells become stretched and flattened. The fully developed seed wing, therefore, consists of two cell layers: an outer epidermis and a layer of greatly enlarged hypodermal cells, both being derived from the ovuliferous scale and not from the ovule (Owens and Smith, 1965).

The seed wing differentiates by early July, about two months before the cones open and the seeds are released. However, complete separation of the seed wing from the ovuliferous scale generally occurs during the latter part of August. Separation of both seed and wing from the ovuliferous scale is apparently a result of dissolution of the middle lamella between the two layers of undifferentiated cells found beneath the ovule and the seed wing. The cells separate along the middle lamella with little breakage of cell walls. The separated seed wing, therefore, usually has a poorly defined third layer of undifferentiated cells attached to its under surface (Owens and Smith, 1965).

Mature seeds of Douglas-fir are externally variable in appearance (Sziklai, 1963) (Fig. 7.1 K, L). The fully developed seed without the wing is ovate, about 8 mm long, 3 mm wide and 2 mm thick. The seed wing is about one-half of an inch long and one-quarter of an inch wide. When the seed wing is broken off, that portion of the seed wing which remains attached

to the seed gives the seed a triangular appearance (Fig. 6.1 O). Once the seed is separated from the ovuliferous scale, the side previously adhering to the ovuliferous scale is light in color, while the upper exposed surface is quite dark. The ovule tip containing the micropyle is usually still distinct in the mature seed and forms a neck of variable length at that end of the seed. The micropyle forms a weak spot in the seed coat or testa through which the root of the seedling emerges at germination (Allen, 1942a).

Coastal and interior Douglas-fir seed can be distinguished by careful observation of several external features (Fig. 7.1 K, L). For a discussion of all the criteria upon which the distinction can be made, the reader is referred to two articles by Allen (1960, 1961). Such a distinction is of considerable importance since either seed type grown in the other locality yields an inferior tree. More recent work (El-Lakany and Sziklai, 1970) has suggested that the origin of Douglas-fir seed can be determined by nuclear volume and amount of DNA per nucleus in cells of the embryo.

Empty seeds that appear to be otherwise normal are common and usually result from failure of pollination or from self-pollination. Pollination and fertilization have no apparent effect upon the normal growth of the cone. Normal-appearing but empty seeds can be produced in the absence of either process. This development of empty seed is a common occurrence in Douglas-fir. Parthenogenesis, or the development of an embryo without fertilization, has been suggested but never conclusively demonstrated in Douglas-fir or any other conifer (Allen, 1942b,c). Low yields of viable seed from isolated Douglas-fir trees indicate that this species has a high incidence of self-incompatibility. A comprehensive cytological study was made on self-incompatibility in Douglas-fir as it affects low yields of viable seed (Orr-Ewing, 1956, 1957). Detailed cytological comparisons were made of developing ovules from trees which had been both self-pollinated and cross-pollinated. Neither pollen germination nor pollen development were in any way inhibited by self-pollination, and fertilization and proembryo development proceeded normally. However, almost all embryos from self-pollination collapsed at an early stage of development. It appeared that embryo collapse was caused by some failure in the relationship between the young embryos and their surrounding gametophytes. The conclusion made from this work was that embryo-collapse was caused by the increased homozygosity of recessive deleterious genes. As a result of these findings, it is apparent that cross-pollination is essential for the production of large quantitites of viable seed. Therefore, isolated trees or stands of young trees that produce few pollen cones are not good sources of viable seed.

The seed cones show little external change during the period of embryo and seed development from early June until late August. At the time of fertilization in June, the seed cone is at least three-fourths of its final size. Bracts are completely developed and ovuliferous scales are nearly fully enlarged and appear as distinct, spoon-shaped structures in the axils of the bracts. Cone enlargement is complete by early July and mature cones are usually 7 to 8 cm in length (Fig. 7.1 A, J). From July to September,

differentiation and maturation of tissues occur throughout the cone (Owens and Smith, 1965).

Mature open Douglas-fir cone. x2.5.

Cone Maturation and Seed Release

7

Chapter 7

Cone Maturation and Seed Release

Seventeen months elapse between initiation and maturity of the seed cone of Douglas-fir (Fig. 1.1). The cones are fully elongated by early July of the second season. The months of July and August show little external change in the appearance of the seed cone. Internally, however, seeds develop and considerable differentiation and maturation of tissues of the cone occur. This short period represents the culmination of 17 months of complex development and differentiation of tissues and organs of the seed cone. The final phase in cone maturation involves the drying and death of the vegetative tissues and the resultant opening of the ovuliferous scales and release of the mature seed.

Seed-Cone Maturation

After cones are fully elongated in early July, the seed-cone axis undergoes considerable secondary growth and becomes quite thick and woody. Secondary xylem and phloem of the cone axis are similar to vascular tissues formed in the vegetative shoot, except that the xylem consists primarily of very thick-walled fiber-tracheids which make the axis extremely hard (Owens and Smith, 1965).

Numerous resin canals with thick-walled epithelial cells are scattered throughout the secondary xylem and cortex of the mature cone. The cortical resin canals of the cone axis are continuous with those of the ovuliferous scale. Within the ovuliferous scale, the two large basal resin canals branch repeatedly and form up to 60 small resin canals. Resin canals in the distal portion of the ovuliferous scale are found between the many, small vascular bundles and, together, they form a broad band across the ovuliferous scale. The numerous resin canals in the seed cone usually make themselves apparent by the abundant amount of resin on the surface of many mature seed cones, especially if the seed cones have been damaged by insects. Excessive resin can frequently prevent ovuliferous scales from opening fully and can cause seeds and seed wings to adhere to the ovuliferous scales rather than be released (Owens and Smith, 1965).

The change in appearance of the seed cone following pollination is primarily a result of elongation of the cone axis and growth of the ovuliferous

scales, rather than bract growth. As a result, the appearance of the seed cone changes from a structure dominated by a series of long trident-tipped bracts and inconspicuous ovuliferous scales (Fig. 5.3 A) to a seed cone with a prominant series of large, tightly appressed spoon-shaped ovuliferous scales (Fig. 7.1 A, B). The bract is homologous to a leaf and the mature bract is structurally similar to the leaf of Douglas-fir, with the exception of a broad lamina or blade in the proximal portion of the bract. A single leaf trace supplies the bract and passes unbranched along the center of the midrib to just below the tip of the bract. The vascular strand in that portion of the bract extending beyond the ovuliferous scale is similar to that in many conifer leaves, in that both endodermis and transfusion tissue are present (Fig. 7.1 C) (Owens and Smith, 1965; Owens 1968). In the covered portion of the bract, however, neither endodermis nor transfusion tissue differentiate (Fig. 7.1 D). Bracts vary in appearance in different portions of the cone. The lowermost bracts, which lack ovuliferous scales, are needle-like and have no laminae. These appear quite intermediate between bracts and true leaves of Douglas-fir. Bracts subtending basal sterile ovuliferous scales have a very rudimentary lamina which extends three-fourths the length of the bract. Bracts in the distal half of the cone are somewhat shorter, narrower, and the lamina extends almost to the tip of the bract (Fig. 7.1 B). Bracts appear to function in catching pollen and funnelling it down to the stigmatic tip of the ovule (Chapter 5). This appears to be their primary function since, in the mature cone, they are not involved in cone opening or seed release (Owens and Smith, 1965).

The ultimate shape of the ovuliferous scale is already evident in the dormant seed-cone bud. Subsequent growth of the ovuliferous scale is essentially equal all along the curved margin. Marginal growth and thickening of the ovuliferous scale is continuous from early March until early July. During this time, it increases in size many times but still retains essentially the spoon-shape established in the ovuliferous scale primordium the previous fall. Like bracts, ovuliferous scales vary in shape and size in different parts of the cone (Fig. 7.1 B). The basal five to ten ovuliferous scales are small, sterile and slightly wider than their length. Scales from the middle of the cone are about 2.5 cm long and enclose one-third of the circumference of the cone. Ovuliferous scales become very small and sterile at the tip of the cone. Their exposed portion is green and that portion covered by adjacent scales is white during enlargement of the cone. They become brown throughout before cone opening (Owens and Smith, 1965).

Two vascular bundles extend from the cone axis into the base of each ovuliferous scale. These are branch traces, which indicates that the ovuliferous scale is a highly modified, flattened, lateral, fertile shoot. The two bundles fuse at the base of the ovuliferous scale, then branch dichotomously. Vascular bundles within the ovuliferous scale enlarge by secondary growth and form a broad band of vascular tissue at the base (Fig. 7.1 G)(Owens and Smith, 1965).

Cone Opening and Seed Release

Cone opening is caused by drying and not by growth. During August, ovuliferous scales lose their green color and turn brown (Fig. 7.1 A). As drying continues, the ovuliferous scales gradually begin to separate. Dry summers cause rapid drying and earlier opening of the cone. As a result, the time that the seed is shed may vary by at least a month, from year to year. The large zone of sclereids at the abaxial base of the ovuliferous scale is responsible, upon drying, for the opening of the ovuliferous scales in the mature cone. Thin, dehydrated sections of this tissue, when observed under the dissecting microscope, swell with the addition of water and shrink again when dehydrated. The highly lignified secondary walls of the sclereids are evidently responsible for their very hygroscopic nature (Owens and Smith, 1965).

An early study on the amount of moisture which must be lost before Douglas-fir cones will open was done by Willis (1917). No opening was observed until 19 to 34% of the wet weight of the cone was lost. Further drying increased the degree of opening until, when 51% of the wet weight had been lost, all cones were fully opened. Drying and degree of opening was shown to be a complex result of temperature, humidity and air circulation.

Under natural conditions cones may repeatedly open and close to some extent in response to changes in moisture content (Fig. 7.1 J). Repeated opening and closing apparently results in progressively more shrinkage of the sclereids and wider opening of the cones. This fact has been utilized in commercial processing of cones, where partially dried cones taken from storage may be soaked in water, then kiln-dried to obtain more fully open cones and a higher yield of seed. The repeated opening and closing of seed cones with changes in moisture content of the environment results in progressively wider opening of seed cones on the trees in subsequent dry spells. Consequently, seed not released during the major seedfall may be released later in the season. About 70 to 90% of Douglas-fir seeds fall during September and October, and most of the remainder between November and March (Allen, 1942a; Isaac, 1943).

The amount of seedfall is difficult to estimate and to predict from year to year. An average mature Douglas-fir tree may produce about one pound or 40,000 seeds per crop, but since many trees in forest stands produce little or no seed even in good seed years, the average seed production is considerably less than one pound per tree (Isaac, 1943). An individual mature tree in an excellent seed year might produce 2,000 to 3,000 seed cones and disseminate up to 2 or 3 pounds of seeds (Allen, 1942a). Such figures give only a general idea of quantity of seeds produced, and the original references should be consulted for a more complete discussion. In a poor or even fair seed year, nearly all seeds produced may be lost to insects, birds and rodents (Isaac, 1943).

Seed dissemination over an area is extremely variable, primarily as a

Figure 7.1 Seed-Cone Maturation.

A. Mature seed cones early in August. Ovuliferous scales are beginning to turn brown. X 1/2. B. Ovuliferous scales and bracts dissected from the top (left) to the base (right) of a cone, late in August. X 3/4. C. Transverse section through the distal portion of the bract which lacks a broad lamina or blade. Its structure is similar to that of a leaf in that a distinct endodermis (end) surrounds the single vascular bundle (vt) and the two are separated by transfusion tissue. X 80. D. Transverse section through the basal portion of the bract which possesses a broad lamina and is covered by the subjacent ovuliferous scale. The structure at this point is much less leaf-like and lacks an endodermis. X100. E. Longitudinal section through an ovuliferous scale at the time of pollination, showing the position of the cells which are beginning to elongate and will differentiate into the mechanical sclerenchyma tissue (mt) responsible for opening of the seed cone. X 25. F. Longitudinal section of a mature ovuliferous scale, showing the position of the bract (b), the seed (se) which is empty as a result of the sectioning process, the seed wing (sw), the abscission layer (al) beneath the seed, and the mechanical tissue composed of sclereids at the base of the mature ovuliferous scale. G. Transverse section through the base of bract and mature ovuliferous scale, showing the seed, the band of vascular tissue, and the large area of mechanical tissue. X 5. H. Transverse section through the mechanical tissue of the ovuliferous scale, showing the very thick-walled sclereids. Outer sclereids (lower) are smaller in diameter than are the inner sclereids. X 75. I. Longitudinal section through the mechanical tissue of the ovuliferous scale, showing the sclereids. X 75. J. Mature seed cones early in September as they are drying and beginning to open and shed the seed. The chlorotic appearance of the new shoots is common when they are subtended by many developing seed cones. K. Coastal Douglas-fir seed. The darker, outer surface is shown on the two seeds on the right and the lower surface on the left. X 3. L. Interior Douglas-fir seed shown as in K, with characteristically shorter seed tip. X 3.


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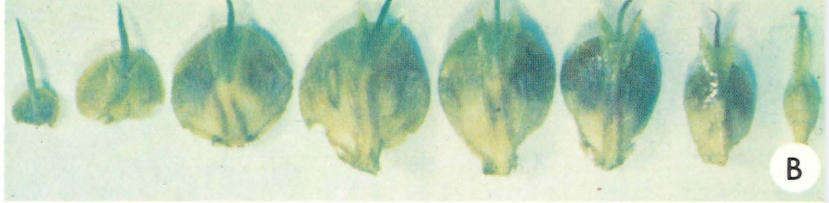
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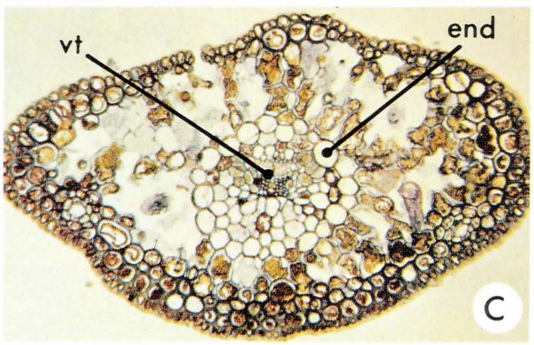
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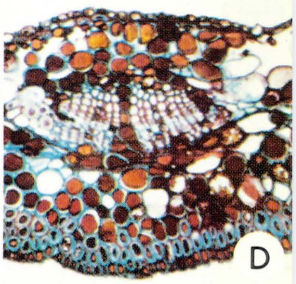
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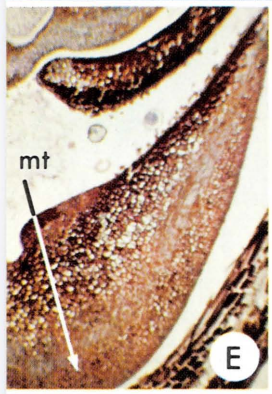
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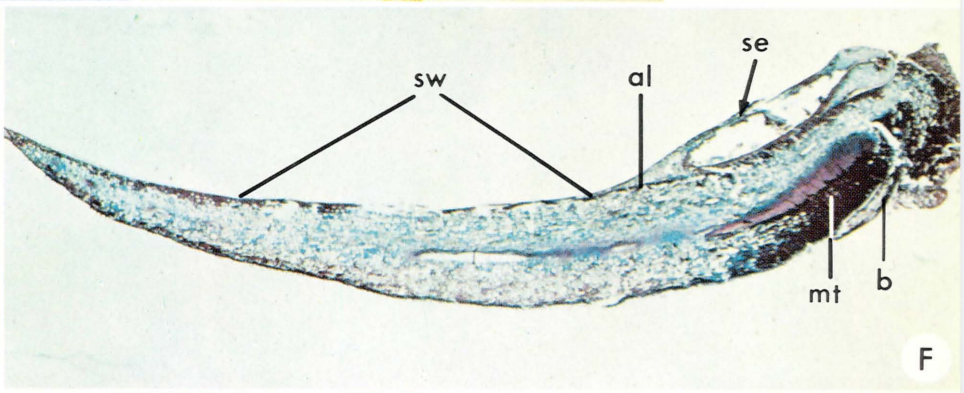
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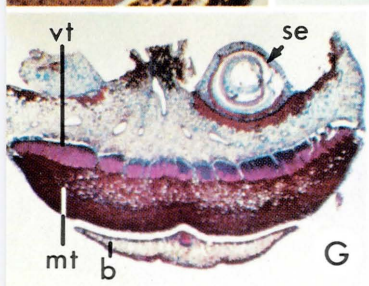
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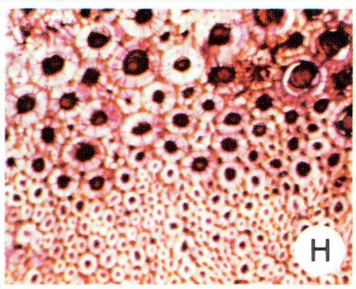
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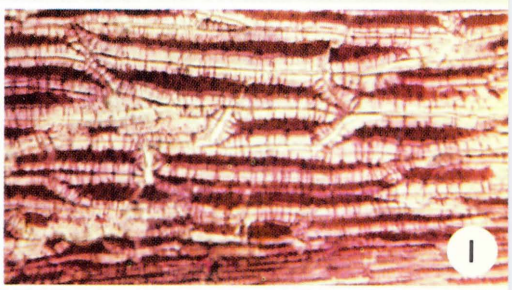
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result of variation in air movement. Whirling seeds of Douglas-fir fall at a rate of 175 to 250 feet per minute, so a very gentle wind can laterally carry them several hundred feet from the crown of the tree (Isaac, 1943). Most seeds fall within 1,000 feet of the parent tree, but in some instances upward air currents and strong winds have carried Douglas-fir seeds as far as a mile (Allen, 1942a; Isaac, 1943). Most of the seeds borne by trees in the interior of a dense forest are screened by neighboring trees and do not travel great distances, while seeds from marginal trees escape this screening effect and are dispersed widely over open areas (Isaac, 1943).

Seed Production

Various aspects of seed production have been dealt with in previous chapters and a brief summary is given here. The quantity of seeds produced in Douglas-fir, as in most other conifers, varies from tree to tree and from year to year. In Douglas-fir, some seeds are produced annually in a region, except for about 1 year in any 4- or 5-year period. Abundant or medium cone crops occur every 2 to 7 years, and commonly about every 5 years in a region, but the cycle is unpredictable (Isaac, 1943; Lowry, 1966). Several attempts have been made to correlate cone crops with environmental factors but with little success. The 17-month cycle from the time of axillary bud initiation in April to seed release in September of the following year encompasses a wide range of environmental conditions, and certain stages of the cycle are influenced more by environment than others.

Since essentially equal numbers of axillary primordia are initiated from year to year on a tree, the potential number of cones produced is determined by the percentage of these primordia that develop into vegetative, seed- or pollen-cone buds. The early period of axillary primordial development is of major importance in determining the cone crop produced, since absence of a cone crop is more frequently due to failure of axillary primordia to differentiate into cone buds than failure at any later stage of development. Once axillary primordia have differentiated into distinct seed- and pollen-cone buds, there appears to be little abortion of buds during the rest of the first year's development. Loss of seed cones by abortion is most common for the few weeks during and following pollination. Low temperatures are known to cause much of the abortion at this time but other unknown factors are also involved. Some trees show a tendency for seed cones to abort year after year.

The ovule and female gametophyte show few irregularities in development that would affect seed production. In spite of this, the formation of empty seeds is very common in Douglas-fir and may result from the formation of non-viable pollen, a lack of pollen when seed cones are open and receptive, or self-incompatibility. Once fertilization has occurred and early embryonic stages have developed, environmental factors, excluding insects, do not appear to affect adversely later stages of cone and seed development.

Bibliography

Allen, G. S. 1941. A basis of forecasting seed crops of some coniferous trees. J. Forest. 39: 1014-1016.

. 1942(a). Douglas fir (*Pseudotsuga taxifolia* (Lamb.) Britt.): A summary of its life history. B.C. Forest Service, Research Note No. 9. 27p.

. 1942(b). Parthenocarpy, parthenogenesis, and self-sterility of Douglas fir, J. Forest. 40: 642-644.

. 1942(c). Douglas-fir seed from young trees. J. Forest. 40: 722-723.

. 1943. The embryogeny of *Pseudotsuga taxifolia* (Lamb.) Britt. Amer. J. Bot. 30: 655-661.

. 1945. Embryogeny and the development of the apical meristems of *Pseudotsuga taxifolia* (Lamb.) Britt. Ph.D. dissertation. Berkeley, University of California. 165p.

. 1946(a). The origin of the microsporangium of *Pseudotsuga*. Bull. Torrey Bot. Club 73: 547-556.

. 1946(b). Embryogeny and development of the apical meristems of *Pseudotsuga*. I. Fertilization and early embryogeny. Amer. J. Bot. 33: 666-677.

. 1947(a). Embryogeny and the development of the apical meristems of *Pseudotsuga*. II. Late embryogeny. Amer. J. Bot. 34: 73-80.

. 1947(b). Embryogeny and the development of the apical meristems of *Pseudotsuga*. III. Development of the apical meristems. Amer. J. Bot. 34: 204-211.

. 1960. A method of distinguishing coastal from interior Douglas-fir seed. B.C. Lumberman 44(8): 26,28,30.

. 1961. Testing Douglas-fir seed for provenance. Proc. Int. Seed Test. Ass. 26: 388-403.

. 1962. Factors affecting the viability and germination behavior of coniferous seed. VI. Stratification and subsequent treatment, *Pseudotsuga menziesii* (Mirb.) Franco. Forest. Chron. 38: 485-496.

. 1963. Origin and development of the ovule in Douglas-fir. Forest Sci. 9: 386-393.

. 1967. Stratification of tree seed. Combined proc., Int. Plant Prop. Soc. 17: 99-106.

Allen, G. S. and W. Bientjes. 1954. Studies on coniferous tree seed at the University of British Columbia. Forest Chron. 30: 183-196.

Allen, G. S. and O. Sziklai. 1962. Pollination of Douglas-fir with water suspensions of pollen. Forest. Sci. 8: 64-65.

Arnoldi, W. 1900. Beiträge zur Morphologie der Gymnospermen. III. Embryogenie von *Cephalotaxus fortunei*. Flora 87: 46-63.

Barner, H. and H. Christiansen. 1960. The formation of pollen, the pollination mechanism, and the determination of the most favourable time for controlled pollination in *Larix*. Silvae Genet. 9: 1-11.

. 1962. The formation of pollen, the pollination mechanism, and the determination of the most favourable time for controlled pollination in *Pseudotsuga menziesii*. Silvae Genet. 11: 89-102.

Bierhorst, D. W. 1971. Morphology of vascular plants. New York, Macmillan. 560p.

Buchholz, J. T. 1918. Suspensor and early embryo of *Pinus*. Bot. Gaz. 66: 185-228.

. 1926. Origin of cleavage polyembryony in conifers. Bot. Gaz. 81: 55-71.

. 1931. The pine embryo and the embryos of related genera. Trans. Ill. State Acad. Sci. 23: 117-125.

. 1933. The classification of coniferales. Trans. Ill. State Acad. Sci. 25: 112-113.

. 1950. Embryology of gymnosperms. Proc. Int. Congr. Bot. 7: 374-375.

Chamberlain, C. J. 1935. Gymnosperms; structure and evolution. Chicago, University of Chicago Press [1935] New York, Johnson Reprint Corporation, 1957, 484p.

Ching, T. M. and K. K. Ching. 1962. Physical and physiological changes in maturing Douglas-fir cones and seed. Forest Sci. 8: 21-31.

Ching, T. M. and S. C. Fang. 1963. Utilization of labeled glucose in developing Douglas fir seed cones. Plant Physiol. 38: 551-554.

Christiansen, H. 1969(a). On the pollen grain and the fertilization mechanism of *Pseudotsuga menziesii* (Mirb.) Franco. var. viridis Schwer. Silvae Genet. 18: 97-104.

. 1969(b). On the germination of pollen of *Larix* and *Pseudotsuga* on artificial substrate, and on viability tests of pollen of coniferous forest trees. Silvae Genet. 18: 104-107.

Clowes, F. A. L. and B. E. Juniper. 1968. Plant cells. Oxford, Blackwell Scientific Publications Ltd. 546p.

Courtot, Y. and L. Baillaud. 1955. Sur la répartition des sexes chez un *Chamaecyparis*. Ann. Sci. Univ. Besancon. Bot. Ser. II. 6: 17-81.

Crozier, A., H. Aoki, R. P. Pharis and R. C. Durley. 1970. Endogenous gibberellins of Douglas fir. Phytochemistry 9: 2453-2459.

Dinus, R. J. 1964. Chromatographic studies of the growth hormones present in the buds of different-aged Douglas fir *(Pseudotsuga menziesii* (Mirb.) Franco.) trees. Master's thesis. University of Washington, Seattle, Washington. 82p.

Doak, C. C. 1932. Multiple male cells in *Cupressus arizonica*. Bot. Gaz. 94: 168-182.

. 1935. Evolution of foliar types, dwarf shoots and cone scales of *Pinus* , with remarks concerning similar structures in related forms. Ill. Biol. Monogr. 13(3): 1-106.

Doyle, J. 1926. The ovule of *Larux* and *Pseudotsuga*. Proc. Roy. Ir. Acad. B, 37: 170-180.

Doyle, J. and M. O'Leary. 1935. Pollination in *Tsuga, Cedrus, Pseudotsuga,* and *Larix*. Sci. Proc. Roy. Dublin Soc. 21: 191-204.

Ebell, L. F. 1962. Growth and cone production responses of Douglas fir to chemical fertilization. Canada Department of Forestry Res. Branch Memo. B.C. 62-8. 44p.

. 1967. Cone production induced by drought in potted Douglas-fir. Canada Department of Forestry. Bi-Mon. Res. Notes. 23: 26-27.

. 1970. Physiology and Biochemistry of flowering of Douglas fir. Proc. IUFRO. Sect. 22. Working Group Meeting on Sexual Reproduction of Forest Trees, Varparanta, Finland. 10p.

. 1971. Girdling: its effect on carbohydrate status and on reproductive bud and cone development of Douglas fir. Can. J. Bot. 49: 453-466.

Ebell, L. F. and E. E. McMullan. 1970. Nitrogenous substances associated with differential cone production responses of Douglas fir to ammonium and nitrate fertilization. Can. J. Bot. 48: 2169-2177.

Eis, S. 1970. Reproduction and reproductive irregularities of *Abies lasiocarpa* and *A. grandis*. Can. J. Bot. 48: 141-143.

Eis, S., E. H. Garman and L. F. Ebell. 1965. Relation between cone production and diameter increment of Douglas fir *(Pseudotsuga menziesii* (Mirb.) Franco), grand fir *(Abies grandis* (Dougl.) Lindl.) and western white pine *(Pinus monticola* (Dougl.)). Can. J. Bot. 43: 1553-1559.

Eklund, B. 1954. Årsringsbreddens klimatiskt betingade variation hos tall och gran inom norra Sverige åren 1900-1944. Medd. Skogsforskn Inst., Stockh. 44(8) 150p.

El-Lakany, M. H. and O. Sziklai. 1970. Variation in nuclear characteristics in selected western conifers and its relation to radiosensitivity. Radiat. Bot. 10: 421-427.

Engler, A. 1926. Angiospermae. Bd. 14a of Die natürlichen Pflanzenfamilien hrsg. A. Engler und K. Prantl. 2 aufl. Leipzig, Englemann. 167p.

Eriksson, G. 1968. Temperature response of pollen mother cells in *Larix* and its importance for pollen formation. Stud. Forest. Suecica 63: 1-131.

Esau, K. 1953. Plant anatomy. New York, Wiley. 735p.

Ferguson, M. C. 1904. Contributions to the knowledge of the life history of *Pinus*, with special reference to sporogenesis, the development of the gametophytes, and fertilization. Proc. Wash. Acad. Sci. 6: 1-202.

Foster, A. S. 1939. Structure and growth of the shoot apex of *Cycas revoluta*. Amer. J. Bot. 26: 372-385.

. 1941. Zonal structure of the shoot apex of *Dioon edule* Lindl. Amer. J. Bot. 28:

557-564.

Foster, A. S. and E. M. Gifford. 1959. Comparative morphology of vascular plants. San Francisco, W.H. Freeman. 554p.

Fowells, H. A., Comp. 1965. Silvics of forest trees of the United States. Washington (U.S. Department of Agriculture, Agriculture Handbook No. 271) p.546-556.

Galun, E. 1959. Effects of gibberellic acid and naphthaleneacetic acid on sex expression and some morphological characters in the cucumber plant. Phyton. 13: 1-8.

Giertych, M. M. 1967. Analogy of the differences between male and female strobiles in *Pinus* to the differences between long- and short-day plants. Can. J. Bot. 45: 1907-1910.

Griffith, B. G. 1968. Phenology, growth, and flower and cone production of 154 Douglas fir trees on the University Research Forest as influenced by climate and fertilizer, 1957-1967. Vancouver, B.C., University of British Columbia, Fac. Forest. Bull. 6. 70p.

Guttenberg, H. von. 1961. Grundzüge der Histogenese Höherer Pflanzen. II. Die Gymnospermen. Bd. 8, heft 4 of Handbuch der Pflanzenanatomie, 2 aufl. Berlin, Gebrüder Borntraeger. 172p.

Hanstein, J. L. E. R. von. 1869. Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen. Festschr. Niederrhein. Ges. Natur. Heilkunde. 109-145.

. 1870. Die Entwicklung des Keimes der Monokotylen und Dikotylen. Bot. Abhand. Gebiete Morphol. Physiol. 1: 1-112.

Hashizume, H. 1959. [The effect of gibberellin upon flower formation in *Cryptomeria japonica*.] J. Jap. Forest. Soc. 41: 375-381.

. 1960. [The effect of gibberellin upon sex differentiation in *Cryptomeria japonica* strobiles.] J. Jap. Forest. Soc. 42: 176-180.

. 1961. [The effect of gibberellin on sex differentiation in *Cryptomeria japonica* strobiles. II. Effects of auxin and urea on gibberellin-induced sex transition to female in strobiles.] J. Jap. Forest. Soc. 43: 47-49.

. 1969. [Auxins and gibberellin-like substances existing in the shoots of conifers and their roles in flower bud formation and flower sex differentiation.] Bull. Tottori. Univ. Forests 4. 46p.

Henry, A. and M. G. Flood. 1920. The Douglas firs: A botanical and silvicultural description of the various species of *Pseudotsuga*. Proc. Roy. Ir. Acad. 35(B): 67-92.

Heslop-Harrison, J. 1957. The experimental modification of sex expression in flowering plants. Biol. Rev. 32: 38-90.

Hillman, W. S. 1962,. The physiology of flowering. New York, Holt Rinehart and Winston. 164p.

Hutchinson, A. H. 1915. On the male gametophyte of *Picea canadensis*. Bot. Gaz. 59: 287-300.

. 1924. Embryogeny of *Abies*. Bot. Gaz. 77: 280-289.

Irgens-Moller, H. 1967. Patterns of height growth initiation and cessation in Douglas fir. Silvae Genet. 16: 56-58.

Isaac, L. A. 1943. Reproductive habits of Douglas fir. Washington, D.C., Charles Lathrop Park Forestry Foundation. 107p.

Jensen, W. A. 1962. Botanical histochemistry; principles and practice. San Francisco, W.H. Freeman and Co. 408p.

Johansen, D. A. 1940. Plant microtechnique. New York, McGraw-Hill. 523p.

. 1950. Plant embryology, embryogeny of the spermatophyta. Waltham, Mass., Chronica Botanica Co. 305p.

Kato, J., W. K. Purves and B. O. Phinney. 1962. Gibberellin-like substances in plants. Nature 196: 687-688.

Khoshoo, T. N. 1961. Chromosome numbers in gymnosperms. Silvae Genet. 10: 1-9.

Kozlowski, T. T. 1964. Shoot growth in woody plants. Bot. Rev. 30: 335-392.

Krueger, K. W. 1967. Nitrogen, phosphorus, and carbohydrate in expanding and year-old Douglas-fir shoots. Forest Sci. 13: 352-356.

Krugman, S. L. 1967. A gibberellin-like substance in immature pine seed. Forest Sci. 13: 29-37.

Lang, W. H. 1900. Studies in the development and morphology of cycadean sporangia, II. The ovule of *Stangeria paradoxa*. Ann. Bot. 14: 281-306.

Lawson, A. A. 1909. The gametophytes and embryo of *Pseudotsuga Douglasii*. Ann. Bot. 23: 163-180.

Livingston, G. K. 1969. The meiotic chromosomes of Douglas fir. Int. Bot. Congr. II: 130 (abstr.).

Lowry, W. P. 1966. Apparent meteorolgical requirements for abundant cone crop in Douglas fir. Forest Sci. 12: 185-192.

Maheshwari, P. and H. Singh. 1967. The female gametophyte of gymnosperms. Biol. Rev. (Cambridge) 42: 88-130.

Martinez, M. 1963. Las pináceas mexicanas. 3 ed. Mexico, Universidad Nacional Autónoma de Mexico.

Matthews, J. D. 1963. Factors affecting the production of seed by forest trees. Forest. Abstr. 24(1): i-xiii.

Mergen, F. and L. E. Koerting. 1957. Initiation and development of flower primordia in slash pine. Forest Sci. 3: 145-155.

Michalski, L. 1967. Growth regulators in the pollen of Pine *(Pinus sylvestris L.)* Acta Soc. Bot. Pol. 36: 475-481.

Michniewicz, M. 1967. The dynamics of gibberellin-like substances and growth inhibitor in ontogeny of conifers. Wiss. Z. Univ. Rostock, Math-Naturwiss. Reihe, 4/5, 577-583.

Moens, P. B. 1964. A new interpretation of meiotic prophase in *Lycopersicon esculentum* (tomato). Chromosoma 15: 231-242.

Morris, R. F. 1951. The effects of flowering on the foliage production and growth of balsam fir. Forest. Chron. 27: 40-57.

Murneek, A. E. 1925. Is fruiting of the apple and exhaustive procees? Proc. Amer. Soc. Hort. Sci. 22: 196-200.

Murrill, W. A. D. 1900. The development of the archegonium and fertilization in the hemlock spruce *(Tsuga canadensis,* Carr.). Ann. Bot. 14: 583-607.

Nitsch, J. P., E. B. Kurtz, Jr., J. L. Liverman and F. W. Went. 1952. The development of sex expression in cucurbit flowers. Amer. J. Bot. 39: 32-43.

Orr-Ewing, A. L. 1956. An investigation into the effects of self-pollination of *Pseudotsuga menziesii* (Mirb.) Franco. Ph.D. dissertation. Vancouver, B.C. University of British Columbia. 110p.

. 1957. A cytological study of the effects of self-pollination on *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genet. 6: 179-185.

. 1966. Inter- and intraspecific crossing in Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genet. 15: 121-126.

Owens, J. N. 1963. Origin and differentiation of the seed cone of *Pseudotsuga menziesii* (Mirb.) Franco. Ph.D. dissertation Corvallis, Oregon State University. 120p.

. 1968. Initiation and development of leaves in Douglas fir. Can. J. Bot. 46: 271-278.

. 1969. The relative importance of initiation and early development on cone production in Douglas fir. Can. J. Bot. 47: 1039-1049.

Owens, J. N. and Marje Molder. 1971(a). Pollen development in Douglas fir *(Pseudotsuga menziesii).* Can. J. Bot. 49: 1263-1266.

. 1971(b). Meiosis in conifers; prolonged pachytene and diffuse diplotene stages. Can. J. Bot. 49: 2061-2064.

Owens, J. N. and R. P. Pharis. 1967. Initiation and ontogeny of the microsporangiate cone in *Cupressus arizonica* in response to gibberellin. Amer. J. Bot. 54: 1260-1272.

. 1971. Initiation and development of western red cedar cones in response to gibberellin induction and under natural conditions. Can. J. Bot. 49: 1165-1175.

Owens, J. N. and F. H. Smith. 1964. The initiation and early development of the seed cone of Douglas fir. Can. J. Bot. 42: 1031-1047.

. 1965. Development of the seed cone of Douglas fir following dormancy. Can. J. Bot. 43: 317-332.

Owston, P.W. 1969. The shoot apex in eastern white pine: its structure, seasonal development and variation within the crown. Can. J. Bot. 47: 1181-1188.

Pharis, R. P. and W. Morf. 1968. Physiology of gibberellin-induced flowering in conifers. In: Biochemistry and physiology of plant growth substances, ed. By F. Wightman and G. Setterfield. Ottawa, Runge Press, 1968. (International conference on plant growth substances, 6th, Ottawa, 1967). p.1341-1356.

Pharis, R. P. W. Morf. and J. N. Owens. 1969. Development of the gibberellin-induced ovulate strobilus of western red cedar: quantitative requirement for long-day short-day long-day. Can. J. Bot. 47: 415-420.

Pharis, R. P., M. D. E. Ruddat, C. C. Phillips and E. Heftmann. 1965. Precocious flowering of Arizona cypress with gibberellin. Can. J. Bot. 43: 923-927.

Pilger, R. 1926. Ubersicht über der Coniferen. In: aufl. 2, bd. 13 of Pflanzenfamilien, hrsg A. Engler und K. Prantl. Berlin, Duncker und Humblot. p. 164-166.

Rehfuess, K. E. 1970. Needle analyses on cone-bearing and non-cone-bearing silver firs. PflErnahr. Bodenk. 127: 75-84.

Ruddat, M. , R. P. Pharis, H. Aoki and A. Crozier. 1968. Gibberellin-like substances from vegetative tissue of a conifer, Arizona cypress. Plant Physiol. 43: 2049-2053.

Saito, Y. 1957. Artificial control of sex differentiation of Japanese red pine and black pine strobiles. J. Fac. Agr. Tottori Univ. 3: 1-29.

Sax, K. and H. J. Sax. 1933. Chromosome number and morphology in the conifers. J. Arnold Arboretum, Harvard University 14: 356-375.

Schopf, J. M. 1943. The embryology of *Larix*. Ill. Biol. Monogr. 19: 1-97.

Silen, R. R. 1962. Pollen dispersal considerations for Douglas fir. J. Forest. 60: 790-795.

. 1963. Effects of altitude on factors of pollen contamination of Douglas-fir seed orchards. J. Forest. 61: 281-283.

. 1967. Earlier forecasting of Douglas-fir cone crop using male buds. J. Forest. 65: 888-892.

Silen, R. R. and D. L. Copes. 1972. Douglas-fir seed orchard problems — a progress report. J. Forest (In press).

Silen. R. R. and G. Keane. 1969. Cooling a Douglas-fir seed orchard to avoid pollen contamination. U.S. Forest Serv. Res. Note PNW 101. 10p.

Silen, R. R. and K. W. Krueger. 1962. Does rainy weather influence seed set of Douglas fir? J. Forest. 60: 242-244.

Sterling, C. 1946. Organization of the shoot of *Pseudotsuga taxifolia* (Lamb.) Britt. I. Structure of the shoot apex. Amer. J. Bot. 33: 742-750.

Stoate, T. N., I. Mahood and E. C. Crossin. 1961. Cone production in Douglas-fir *(Pseudotsuga menziesii)*. Empire Forest. Rev. 40: 105-110.

Sudworth, G. B. 1908. Forest trees of the Pacific slope. U.S. Gov't Print. Off., Washington, D.C. 441p.

Swanson, C. P. 1957. Cytology and cytogenetics. Englewood Cliffs, Prentice Hall. 596p.

Sziklai, O. 1963. Variation and inheritance of some physiological and morphological traits in *Pseudotsuga menziesii* (Mirb.). Ph.D. dissertation. Vancouver, B.C., University of British Columbia, 137p.

Thomas, G. and K. K. Ching. 1968. A comparative karyotype analysis of *Pseudotsuga menziesii* (Mirb.) Franco. and *Pseudotsuga wilsoniana* (Hayata). Silvae Genet. 17: 138-143.

van Vredenburch, C. L. H. and J. G. A. la Bastide. 1969. The influence of meteorological factors on the cone crop of Douglas-fir in the Netherlands. Silvae Genet. 18: 182-186.

Wardlaw, C. W. 1955. Embryogenesis in plants. New York, Wiley, 381p.

Wareing, P. F. 1958. Reproductive development in *Pinus sylvestris*. In: Physiology of forest trees; a symposium, ed. by Kenneth V. Thimann. New York, Ronald Press. (International Symposium on forest tree physiology, lst, Harvard University, 1957). p.643-654.

Willis, C. P. 1917. Incidental results of a study of Douglas-fir seed in the Pacific northwest. J. Forest. 15: 991-1002.

Zenke, U. 1953. Untersuchungen über den Ablauf der Meiosis bei *Pseudotsuga taxifolia* Britton. Silvae Genet. 2: 96-102.

Appendix

Source of Illustrations

The illustrations were selected from published and unpublished work by Dr. George S. Allen (G.S.A.) and Dr. John N. Owens (J.N.O.). Published illustrations (indicated here by the year of publication) were previously presented in black and white; some of them are now reproduced from the original material, in color. The source of each illustration is listed below.

Figure 1.1	J.N.O.
Figure 2.1	Owens, 1969.
Figure 2.2	A-C J.N.O.; D Owens, 1968; E-G Owens, 1969.
Figure 2.3	A, B J.N.O.; C Owens, 1969; D J.N.O.; E-I Owens,1969.
Figure 2.4	Owens, 1969.
Figure 2.5	A, B J.N.O.; C Owens, 1969;D-J J.N.O.
Figure 3.1	A-C G.S.A.; D, E J.N.O.; F Owens, 1963; G, H J.N.O.; I Owens, 1963; J J.N.O.; K Owens and Molder, 1971b; L J.N.O.
Figure 3.2	J.N.O.
Figure 3.3	A Owens and Molder, 1971b; B J.N.O.; C-D Owens and Molder, 1971b; E J.N.O.; F Owens and Molder, 1971b; G J.N.O.; H-K Owens and Molder, 1971b.
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Figure 6.1	A-N Allen, 1943, 1946b, 1947a; O G.S.A., J.N.O.
Figure 6.2	A J.N.O.; B-D G.S.A.*; E G.S.A.; F G.S.A.*; G G.S.A.; H, I G.S.A.*; J G.S.A.
Figure 6.3	A G.S.A.*; B-D Allen, 1943; E-H G.S.A.*; I Allen, 1945.
Figure 7.1	A J.N.O.; B-I Owens, 1965; J J.N.O.; K-L G.S.A.

Source of illustrations for chapter headings:

Chapter 1	A. Craigmyle
Chapter 2-5,7	J. Owens
Chapter 6	E. Chatelle

* Photographs retaken from G.S. Allen's microscope slides.

** Drawings based on studies by G.S.A.

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