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The Reproductive Biology of Lodgepole Pine

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About the Forest Genetics Council of British Columbia

The Forest Genetics Council of BC (FGC) is a multi-stakeholder group representing the forest industry, Ministry of Forests and Range, Canadian Forest Service, and universities. Council's mandate is to champion forest gene resource management in British Columbia, to oversee strategic and business planning for a cooperative provincial forest gene resource management program, and to advise the Chief Forester on forest gene resource management policies.

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Gratitude is extended to the many provincial and federal agencies, private industries and the University of Victoria that have supported over many years the research upon which this publication is based, and the research assistants and associates, technicians, graduate students and post-doctoral fellows who carried out or assisted in much of the research. Special thanks to Dr. Derek Harrison who did much of the early coastal and interior pollination studies and to Jordan Bennett who carried out much of the more recent field research on pollination and cone and seed production in interior seed orchards. A special thanks to the referees who offered many useful suggestions that have been incorporated.

Preface

British Columbia has 25 native conifer species, of which 15 are commercially important and used in reforestation. Genetic tree improvement or selective breeding programs started in BC about 50 years ago and are now underway for 10 conifers. Seed orchards are at various stages of development, expansion and replacement. It is important for reforestation with genetically improved seed from superior seed orchard trees to have an understanding of the reproductive biology of each species. This may allow us to maximize seed production in seed orchards.

Until the mid-1960s, Douglas-fir was the only native species for which the reproductive biology was well studied (Allen and Owens 1972). Since then extensive research has been done on the reproductive biology of 13 of the other commercially important conifers native to British Columbia. These studies include vegetative bud and shoot development as they relate to cone initiation; time and method of cone initiation; cone induction; pre- and post-dormant cone-bud development; pollen and ovule development; pollination; pollen physiology, storage, handling and testing; embryo, seed and cone development; seed physiology; seed extraction, handling, storage and testing; and, many aspects of seedling physiology, growth, development and quality testing. Although these studies are numerous and found in published papers and reports, there is a need for a simplified general description of the reproductive biology of each species or closely related species.

In response to this need, five short manuals describing the reproductive cycles of 10 species were written and published nearly 20 years ago, from 1984 through 1986. These are now out of date and most are out of print. Some of these will be rewritten and new information added by this author, as has been done for lodgepole pine, but they may be published in various sources. In addition, some species not covered in the earlier manuals, such as western white pine (Owens 2004) and western larch (Owens 2006, in press) have been written in a similar format but the latter has been published by other sources. All of the manuals are intended to serve as brief, readable, informative and well-illustrated references for foresters, silviculturists, physiologists, breeders, seed orchard personnel, cone collectors and others involved in seed procurement, production and handling. Each manual will contain a glossary of terms commonly used in conifer reproduction plus specific terms that are unique to the particular genus or species. The manuals will explain the complete reproductive cycle, provide useful guidelines for cone induction, assist in forecasting pollen, cone and seed production, enable more effective pollination and pollen management for breeding and cone and seed production, and explain many of the causes for cone and seed loss in natural stands and seed orchards. The manuals are intended for use by students and teachers of forestry and biology at introductory levels, but they also provide some detailed information that will be needed over the next 20 years by researchers and others working in forest genetics, molecular biology and biotechnology.

This book, *The Reproductive Biology of Lodgepole Pine*, is a complete revision of the 1984 publication *The Reproductive Cycle of Lodgepole Pine* (Owens and Molder 1984). It is not meant to be a complete literature review. Only the most pertinent and accessible references are cited and listed. These provide a source for numerous related articles.

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Introduction

The reproductive biology of a species involves more than just knowing the reproductive cycle. Each species has a reproductive potential (RP) determined by the number of cones per tree and seeds per cone that can be produced at a particular age and size of the tree. The tree also has a reproductive success (RS) determined by the proportion of cones and filled seeds that survive to maturity.

The reproductive biology has been well studied for lodgepole pine growing in seed orchards and natural stands. This information makes it possible to determine such things as RP and RS and some of the causes for losses, plus provide methods to increase cone and filled-seed production. Understanding the causes of poor cone and seed production is essential if we are to correct the problems.

In a seed orchard, the number of cones initiated, cone survival and percentage of filled seed may be increased through cultural treatments and through proper pollen and orchard management techniques. These techniques may vary with the tree species and must be applied at the correct time and in a suitable manner for the species. In natural stands, control is usually not possible.

Lodgepole pine begins to form seed cones at 10 to 15 years old, followed a few years later by pollen cones. Mature seed cones are small, only about 3.5–5 cm long and weigh 5–10 g. They have about 100 (ovuliferous) scales, about 80% of which are sterile. Most sterile scales are in the basal half of the cone. Sterile scales bear no or only rudimentary unfertile ovules. The 20 to 25 fertile scales bear two ovules, giving each cone a seed potential of 40 to 50 fertile ovules per cone. Young trees may bear no or few cones, whereas mature trees may bear over 500. The RP of a tree is the number of cones (C) times the average number of fertile ovules per cone (O/C). In lodgepole pine, the RP is commonly several thousand seeds per tree. However, the RS of a tree at the end of the reproductive cycle may be much less and is determined by the filled seeds per cone (FS/C) times the number of mature cones. In lodgepole pine, filled seeds are commonly only about 50% of potential seeds, or 20 to 25 filled seeds per cone. This is referred to as the Seed Efficiency (SEF). Cone survival is extremely variable but is commonly only about 50–70%. Therefore, the RS for lodgpole pine may be only 25–35% of the potential (Owens et al. 2005). (For more detailed methods see Appendix 1.)

The goal of this book is to provide information about the reproductive biology of lodgepole pine in natural stands and in seed orchards. This information should make it possible to determine RP and RS, some of the causes for cone and seed loss, and provide methods to increase cone survival and filled seeds.

Taxonomy and Distribution

Lodgepole pine (*Pinus contorta* Dougl.) (Figs. 1, 2) is in the Pinaceae, the largest family of conifers. The Pinaceae has 11 genera and over 200 species.

Figure 2. *Interior lodgepole pine.*

The genus *Pinus* has about 108 species but the actual number is uncertain and the taxonomy of the family unsettled (Farjon 1998). *Pinus* is the largest genus of conifers and shows considerable variation but possesses so many uniquely derived characters that attempts to split the genus have failed. The genus is generally separated into three subgenera: *Pinus*, the hard pines; *Strobus*, the soft or white pines; and *Ducampopinus*, containing a single species found in Vietnam. The subgenus *Pinus* contains about 77 species and is often called Diploxylon, because of the double vascular strands in the leaves, or they are called hard pines, based on wood structure. The subgenus is further separated into several Groups, Sections or Subsections depending on which of the classification systems proposed over the past 90 years is being used. The system used here follows the one proposed by Little and Critchfield (1969) which is now widely accepted. According to this system, lodgepole pine is placed in Subsection 14, Contortae with three other species including *P. banksiana*.

Lodgepole pine is one of the most familiar, widespread and important pine species in North America. It extends from the Mackenzie district of the Yukon in Canada, south to southern Colorado in the Rocky Mountains and northern Baja, California in the west (Fig. 3). Within this distribution it grows at a wider range of elevations than any other conifer, occurring just above sea level along the Pacific Coast, up to about 3400 m in the southern Sierra Nevada and Rocky mountains (Fowells 1965, Wheeler and Critchfield 1985). Because of its wide distribution, two to four geographical varieties or subspecies are commonly recognized (Pfister and Daubinmire 1975). *Pinus contorta* var. *contorta*, or shore pine, occurs along the Pacific coast and is the most distinct in form, being generally short and crooked with a flattened crown and non-serotinous cones. *Pinus contorta* var. *latifolia*, to which the common name of lodgepole pine is usually applied, is distributed throughout the interior regions. Both *Pinus contorta* var. *murrayany*, a tree of the Cascade and Sierra Nevada mountains, and *Pinus contorta* spp. *bolanderi*, from the Mendocino white plains in California, may be considered as separate interior varieties or subspecies, or may be combined with *Pinus contorta* var. *latifolia* (Little and Critchfield 1969).

The distinction between the latter three subspecies is based primarily on quantitative morphological traits and behavioural traits. Where the ranges of the subspecies overlap, they freely interbreed. Along the northeastern limits of lodgepole pine distribution, its range overlaps with that of its closest relative, *Pinus banksiana* Lamb, and they hybridize when the phenology (the timing of annual development) is similar (Illingworth 1971).

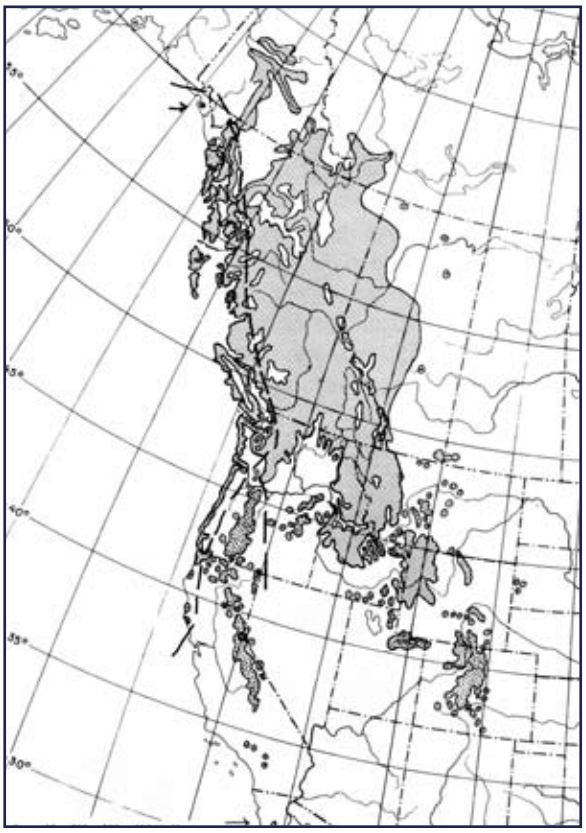


Figure 3. *Distribution of lodgepole pine. (Redrawn from Critchfield and Little 1966.)*

Economic Importance

The wide distribution of lodgepole pine, the predominance of the species in many regions, and its value as a source of timber products make it one of the most extensively harvested trees in the west. Its uses include interior paneling, exterior trim, particleboard, posts, framing material for light construction and many specialty items. Most importantly, it is extensively used for pulp and paper (see references in Baumgartner 1975). In British Columbia, it is the most widely distributed, harvested and planted species and one of the most commercially valuable.

Its value is immeasurable as protective cover for watersheds, as wildlife habitat, as a major and aesthetically pleasing component of the environment for recreation, and as an essential element in the long-term maintenance of the local environment (see references in Baumgartner 1975).

Lodgepole pine is considered to be a fire, or "pioneer" species, quick to occupy a site where natural or human-caused disturbances have created a condition of full sunlight. It is characterized by low shade tolerance, rapid growth in young trees, slow growth in older trees, and the ability to grow on almost any forest soil. Lodgepole pine produces large quantities of seed, and good cone crops occur every one to three years. Serotinous cones in some interior provenences require high temperatures to release the seeds. Consequently, seeds from many years of cone production may be "stored" in the unopened cones on the trees. Often, as a result of fire, this great storehouse of seeds is released all at one time leading to rapid establishment of uniform and often very dense stands that may stagnate in a few years when trees are still quite small.

This type of seed release and regeneration is closely linked to the ecology of the species in terms of fire, fungal pathogens and insect pests. Serious diseases may include (in decreasing order of damage) dwarf mistletoes, stem rusts, and root diseases, but there are few cone and seed diseases (van der Kamp and Hawksworth 1985). Insect pests include over 35 species that feed on lodgepole pine and these include (in decreasing order of damage) the mountain pine (bark) beetle, defoliating insects such as needle miners and sawflies, and seed, cone and seedling insects (Amman and Safranyik 1985).

A scenario has been developed to explain the complex relationship between fire, certain pathogens and insect pests and the regeneration of virtually pure stands forming large climax communities of lodgepole pine. Fire spreads into lodgepole pine forests through corridors of partially decayed logs. The resulting fires cause the regeneration of very uniform stands that, over time—perhaps 60 years—result in the death of many suppressed trees that form low-decay-type fuels. A subsequent fire may thin much of the stand but leave many damaged trees. The damaged trees are very susceptible to a host of fungal pathogens, especially the brown-rot complex including *Porea asiatica* that invades fire wounds. In time, advanced decay develops in the butts and stems of infected trees. The mountain pine beetle (*Dendroctonus ponderosae* Hopk.) preferentially attacks the larger damaged trees often over wide areas usually killing them (Fig. 4). This again sets the stage for subsequent fires as needles and snags fall and logs decay (Gara et al. 1985).

The wide distribution, diversity, utility and economic importance of lodgepole pine make it a valuable species for reforestation. Because reforestation requires a constant supply of high-quality, economical seeds, a tree improvement program was establish in British Columbia and seed orchards for several forest regions were established in the Okanagan Valley near Vernon and near Prince George. These orchards are coming into full production but these cannot meet the current demand for Class A (genetically selected and orchard-produced) seeds. There have been problems in some orchards with low cone production and in others with few seeds per cone. Effective management of the breeding program and the seed orchards requires knowledge of not only the reproductive cycle and pollination, but

also as much as possible about many aspects of the reproductive biology of each species and variation among clones in seed orchards from cone initiation through cone and seed maturity.

Figure 4. *High elevation Rocky Mountain stand of lodgepole pine in central British Columbia infested with the mountain pine beetle (brown area in centre).*

Reproductive Cycle

The wide distribution of lodgepole pine makes it difficult to describe the phenology of the reproductive cycle. The occurrence of a particular event may vary by more than a month in individuals at the extremes of its distribution or elevation. Consequently, the phenology given (Fig. 5) is that of *Pinus contorta* var. *latifolia* growing at moderate elevations in approximately the centre of its range (Fig. 3) in central British Columbia. Even though the phenology will vary with the geographical distribution, the sequence and details of reproductive development remain the same and a single general description of the reproductive cycle is valid.

The reproductive cycle of lodgepole pine is similar to most pines in that it extends over about 26 months from pollen-cone and seed-cone bud initiation to seed and cone maturity (Fig. 5). Pollen cones are initiated late in the summer, become dormant and over-winter, and pollen forms the next spring. Lateral buds that may form either vegetative long-shoots or seed cones are initiated late in the summer and begin to differentiate in the fall. They over-winter as undifferentiated vegetative buds or partly differentiated seed-cone buds. Both bud types complete development the next spring. Pollination occurs late in May or in June. Pollen grains enter the cone and are taken into the ovules where they germinate and the pollen tubes grow into the nucellus. In the year of pollination, the female tissues begin to develop in the ovules but development stops within a few weeks and cones become dormant in July or August. Seed cones remain dormant until the following spring. Growth resumes in April and the sperms and eggs form by June, when fertilization occurs. Embryo, seed and cone development are completed by late summer. In coastal areas, cones dry and open in late summer or fall, but in many interior regions, the cones are serotinous and may remain unopened for several years (Owens et al. 1981, 1982).

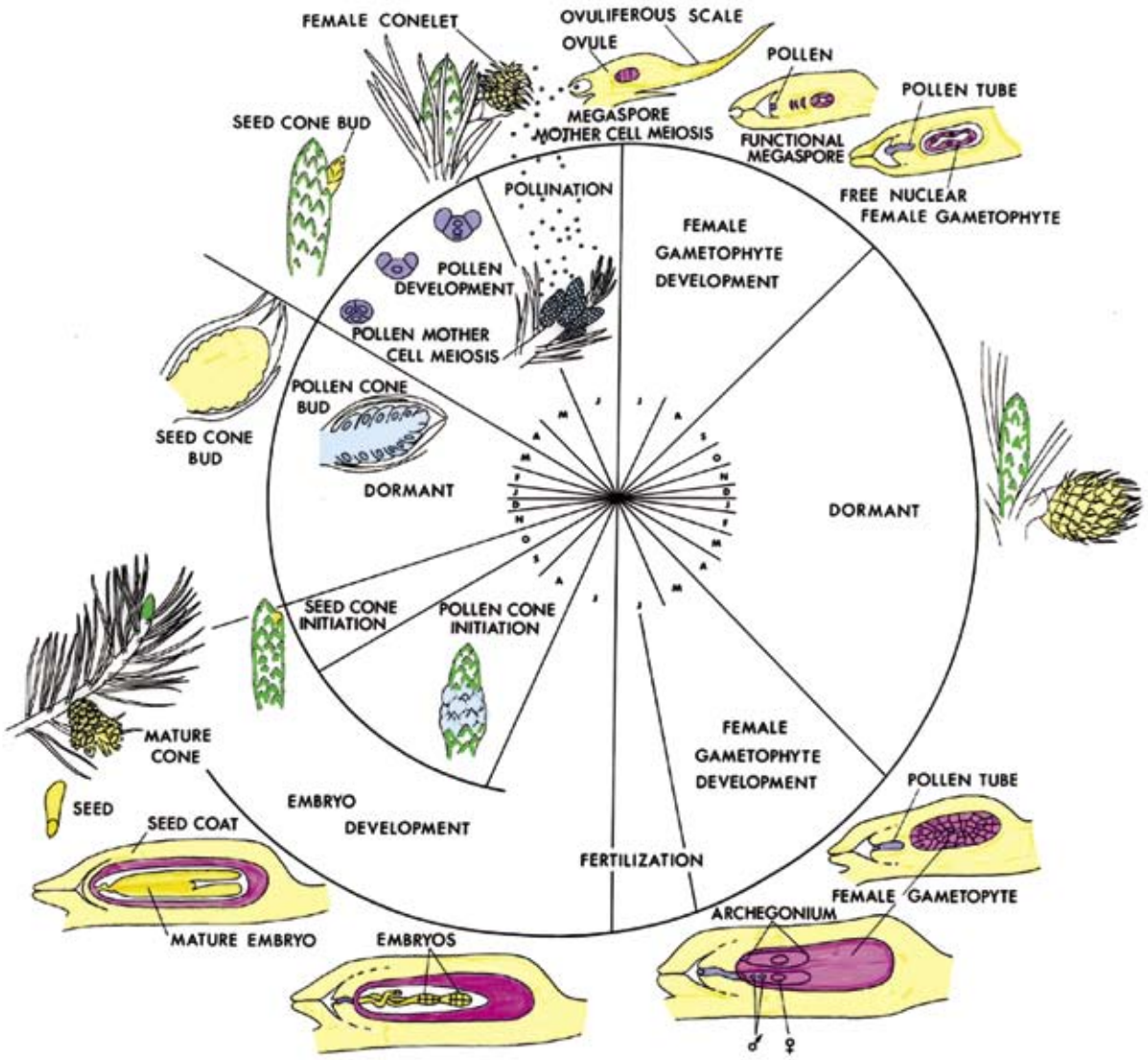


Figure 5. *The reproductive cycle of lodgepole pine. Male development is highlighted in blue and shown within the spiral. Seed-cone, seed and embryo tissues are highlighted in yellow and shown outside the spiral. Female reproductive tissues (female gametophyte or megagametophyte) are shown in pink outside the spiral. Vegetative portions of buds are shown in green. The spokes in the centre of the spiral represent months of the year.*

Long-shoot Bud Development

Compared to other conifers, pines have very different types of vegetative buds, called long-shoot buds (LSB). During the spring, summer and early autumn lodgepole pine forms LSB (telescoped shoots) at the tips of the branches (Fig. 6). These contain all the structures that will be found on the shoots when they elongate the following year. In this discussion, initiation refers to the origin or the earliest stages in development of a structure and differentiation refers to the subsequent process of cells, tissues and organs becoming specialized.

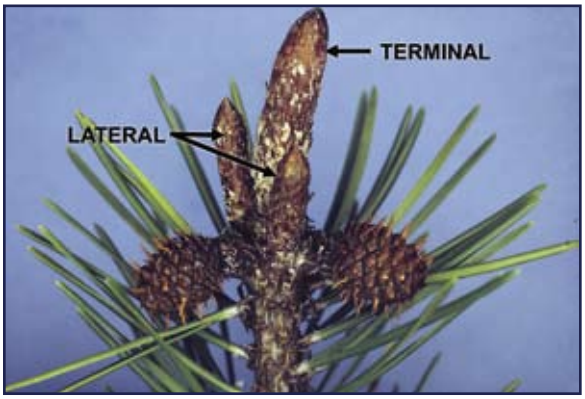


Figure 6. *Dormant terminal and lateral long-shoot buds and dormant first-year cones.*

At the tip of each bud, concealed beneath the bud scales, is a dome-shaped mass of embryonic cells called the shoot apical meristem (apex) (Fig. 7). This meristem forms all of the structures found in the LSB. It initiates a series of small, scale-like leaves (cataphylls) throughout the growing season (Fig. 8 A–E). An axillary bud is initiated just above most of these cataphylls (Fig. 8 C–E). The axillary buds initiate several bud scales then develop into one of four types of buds (Fig. 8 G, I, L, O): (G–H) pollen-cone buds; (I–K) short-shoot buds (SSB) (also called dwarf-shoot buds or needle fascicles); (L–N) seed-cone buds; or, (O–Q) LSB that develop into lateral branches. Some cataphylls, mostly at the base and tip of the LSB, initiate no axillary meristems and remain as sterile cataphylls and some function as bud scales for the LSB (Fig. 8 E–F).

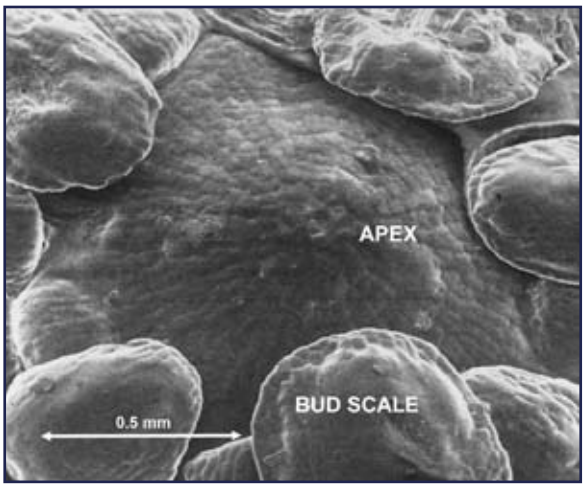


Figure 7. *Scanning electron micrograph of a dormant long-shoot bud apex (apical meristem) after most bud scales were removed. Compare this highly magnified mound of cell with the small apex at the tips of LSB in Figure 8.*

In young trees and on many branches of older reproductively mature trees, the dormant LSB (Fig. 8 F) contain mostly SSB that will become short shoots and lateral LSB that will become lateral branches. In reproductively mature trees, many LSB in upper regions of the crown contain SSB along most of their length and either lateral LSB or seed-cone buds near the distal end of the LSB, just below the terminal apex. Many LSB in lower regions of the crown produce some SSB and occasionally LSB near the tip and a cluster of pollen-cone buds near the base. In mid-regions of the crown, LSB may contain both pollen-cone and seed-cone buds.

Because cataphylls are initiated throughout the growing season, axillary buds also are initiated and develop throughout the growing season. However, axillary buds near the base of the LSB differentiate first and those near the tip differentiate last (Fig. 8 E). Pollen-cone buds usually differentiate in July and August (Fig. 8 D, E) and seed-cone and lateral branch buds differentiate in September or October, just before winter dormancy (Fig. 8 E, F). This phenology of LSB development is similar to that in other temperate hard pines that have been studied.

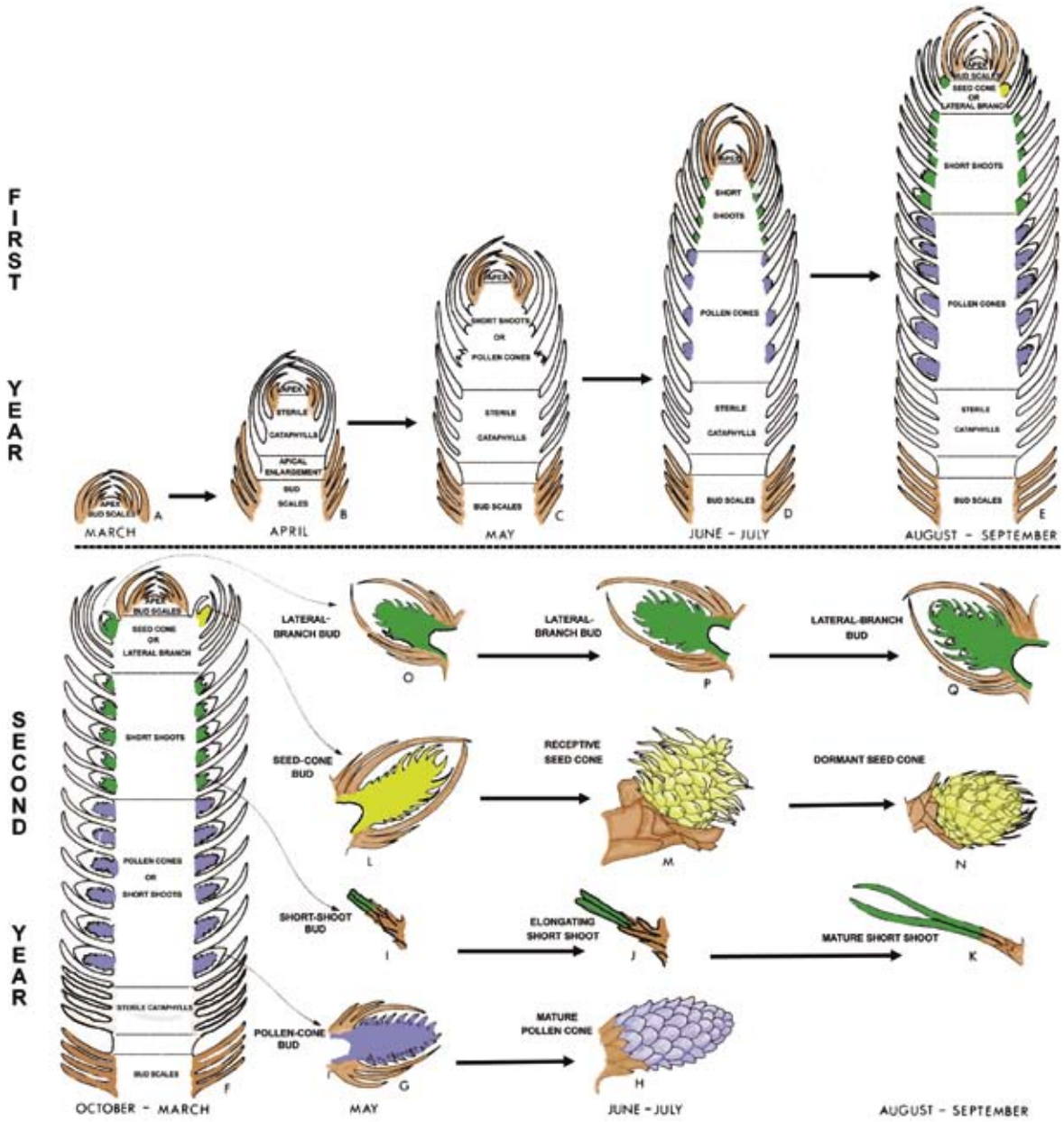


Figure 8. *Long-shoot bud structure and development as seen in longitudinal sections. The top row (A–E, above the dashed line) shows the first year of development and the lower rows (F–Q, below the dashed line) show the second year of development.* A. *The apex (apical meristem) of the dormant LSB is enclosed in bud scales before growth begins in the spring (see Fig. 7).* B. *When the LSB begins growth, the apex enlarges then initiates sterile cataphylls.* C. *Cataphylls continue to be initiated and buds begin to be initiated in the axils of most cataphylls.* D, E. *Axillary buds at the base of the LSB initiate bud scales then begin to differentiate into SSB or pollen-cone buds, then the rate of cataphyll initiation slows.* F. *A dormant LSB with differentiated basal pollen-cone buds, SSB and distal seed-cone, and vegetative-lateral-branch buds. The apex of the LSB is covered by bud scales. Pollen cones and seed cones are not commonly found in the same LSB but both are shown here for illustration.* G–Q. *Development of axillary buds after winter dormancy as the LSB is elongating. The months show the approximate time of development.* G, H. *Pollen-cone development.* I–K. *Short-shoot and needle development.* L–N. *Seed-cone development.* O–Q. *Lateral-branch-bud development follows the same sequence as the terminal LSB (A–E), but stages occur one year later.*

Lateral-bud Development

Lateral-bud primordia that are formed in the axils of most of the cataphylls in all LSB, initiate a spiral series of about 10 bud scales (Figs. 8 D, E; 9). Then the apical meristems initiate two opposite leaf primordia forming the SSB before winter dormancy (Fig.10). Other lateral-bud primordia in the basal half of some LSB in the lower crown initiate about eight bud scales, then a series of small microsporophyll primordia. These develop into pollen-cone buds (Figs. 8 D–F; 11). Lateral bud primordia formed in September in the axils of some cataphylls, near the top of the LSB, initiate about 20 bud scales then some initiate a spiral series of bracts up the flanks of the apex in October before becoming dormant (Figs. 8 E, F; 12). These develop into seed-cone buds. Other buds stop development before they fully differentiate and become dormant (Figs. 8 F, 13). These buds differentiate into vegetative long-shoot buds during the next growing season (Fig. 8 O–Q), but they do not burst until the following spring.

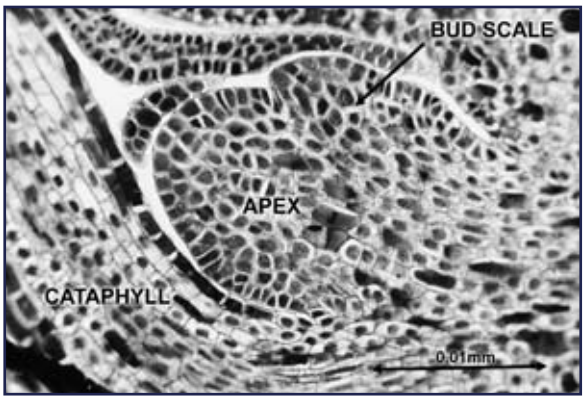
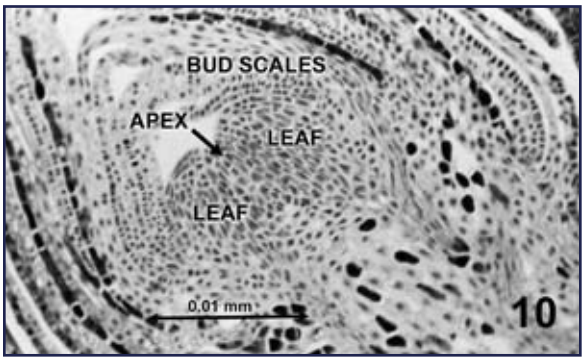
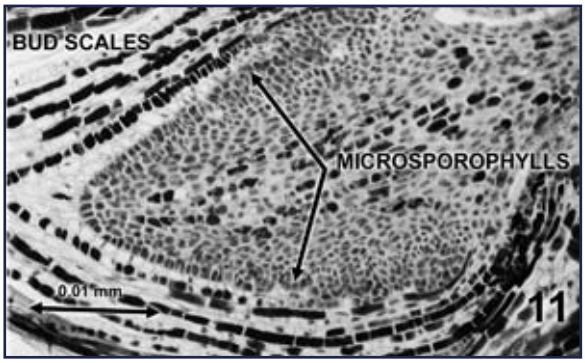
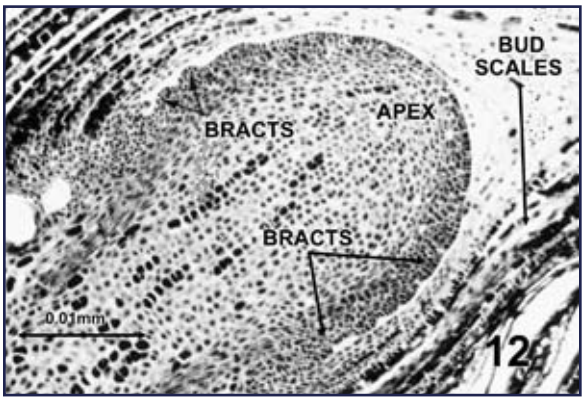
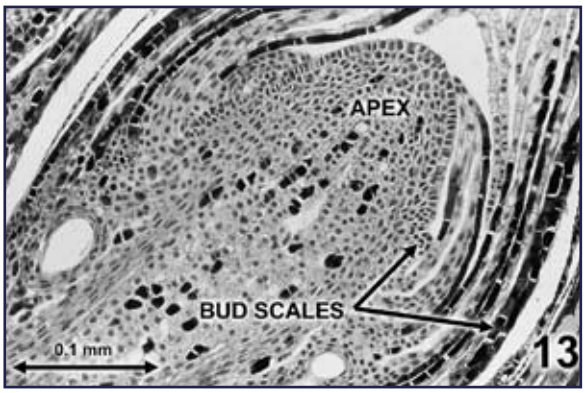


Figure 9. *Section through an axillary bud during bud-scale initiation before bud differentiation.*









Figures 10–13. *Sections showing types of lateral buds that may be present in the dormant LSB.* Fig. 10. *Dormant SSB with two leaf primordia and a small apical meristem (apex).* Fig.11. *Dormant pollen-cone bud with all the microsporophylls formed.* Fig. 12. *Dormant seed-cone bud with about half of the bracts initiated up the flanks of the large apex.* Fig. 13. *Dormant lateral-branch bud with bud scales but no cataphylls or short shoots have been initiated.*

Monocyclic and Polycyclic Shoot Development

The LSB development described above, and shown in Figures 6 and 8, form a "monocyclic" LSB. A moncyclic shoot has only one sequence of cataphylls and axillary buds formed in the one growing season. This results in a monocyclic shoot (Fig. 1). Many *P. contorta* trees growing in southern and mild coastal areas develop, in their upper crown, two or more sequences in the same LSB in one growing season and this results in a polycyclic bud that externally looks the same as a monocyclic LSB. However, it elongates to form a polycyclic shoot (Fig. 14) (Lanner and Van Den Berg 1975). Each sequence is separated by sterile cataphylls and below these is usually a whorl of seed-cone buds or lateral-branch buds. The second and third sequences are shorter than the first because they form later in the growing season over a shorter time period.

In interior lodgepole pine, polycyclic growth is not common but does occur in some provenances and varies with position of the branch within the crown, age of tree and weather conditions in the year of LSB development (O'Reilly and Owens 1987). Unlike many conifers, the polycyclic trait in lodgepole pine is often associated with better wood quality.

Temperate pines having polycyclic development generally produce more seed cones, one or more per cycle. The seed-cone buds enter dormancy at various stages of development, but the cones resume development in the second year. However, in some tropical pines the polycyclic nature can be extreme and result in many cycles and tremendous shoot elongation but few seed cones. It appears that a period of slow growth or dormancy towards the end of a cycle is needed for seed-cone bud differentiation (Greenwood 1978).

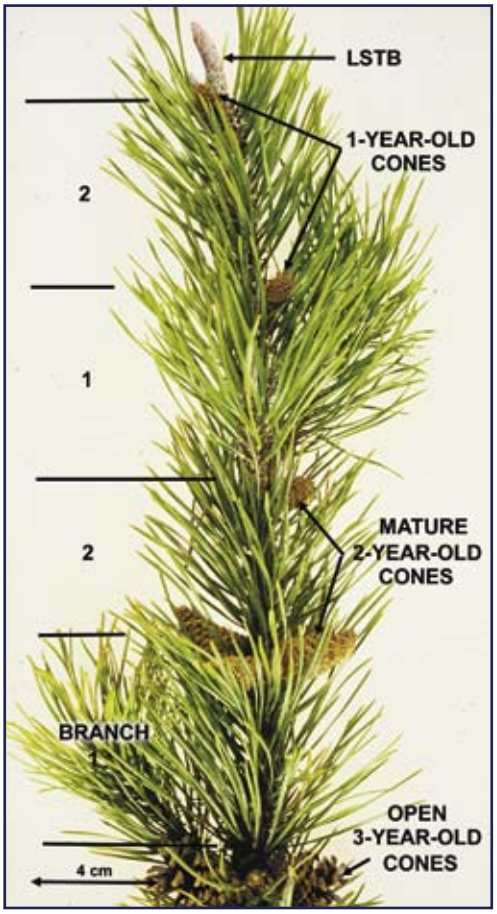
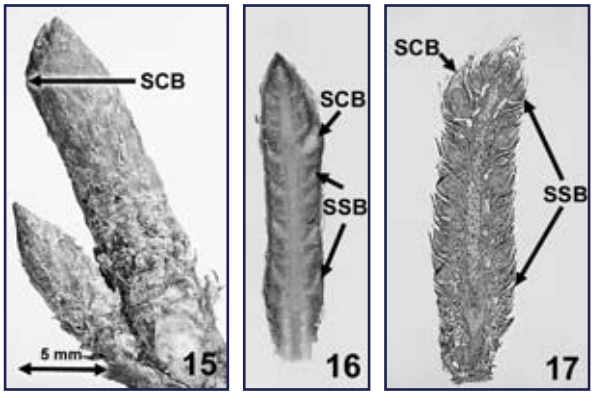


Figure 14. *A polycyclic long shoot at the end of the growing season showing a mature third-year open cone and lateral branch at the base. Above these are two polycyclic shoots, the lowermost bearing two sets of mature seed cones, one set terminating each of the two cycles for that year (1 and 2) and the uppermost bearing two sets of one-year-old seed cones, one set terminating each of the two cycles for that year (1 and 2). In one year, each cycle is usually shorter than the one produced before and usually terminates in lateral seed cones. At the tip of the shoot is a long-shoot bud in the terminal position (LSTB).*

Identification of Buds

It is often desirable in the fall to estimate the abundance of pollen cones and seed cones that will be available for pollination the following spring. This is more difficult in pines than in many other conifers because the cone buds are contained within the LSB. However, estimates can be made by examining the external shape of the LSB (Figs. 15, 18, 21) or by slicing the LSB longitudinally with a sharp razor blade then carefully observing the cut surface with a hand lens or a dissecting microscope (Figs. 16, 19, 22) (Owens and Molder 1975).

During late fall and winter, LSB that contain seed-cone buds appear swollen on one or more sides near the tip (Fig. 15). Each swelling is caused by a large seed-cone bud (Figs. 16, 17). The smaller, less-developed, lateral-branch buds cause no swellings.



Figures 15–17. *Dormant LSB containing seed-cone buds (SCB).* Fig. 15. *Whole large terminal bud showing a swelling caused by a SCB covered by cataphylls. The smaller lateral branch LSB bears no seed-cone buds and has no swellings.* Fig. 16. *A terminal LSB sliced down the centre with a razor blade to show the white seed-cone bud and the smaller SSB.* Fig. 17. *Sectioned and stained LSB showing a large SCB and many SSB.*

In the fall and winter, LSB that contain pollen-cone buds appear swollen at the base and often along most of their length (Fig. 18). When sliced longitudinally with a razor blade or sectioned and stained, several large pollen-cone buds are visible. (Figs. 19, 20).

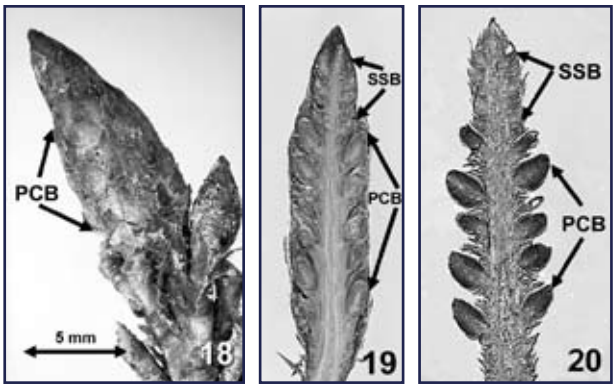


Figure 18–20. *Dormant LSB containing pollen-cone buds.* Fig. 18. *Whole large terminal bud showing the swollen base caused by many large pollen-cone buds (PCB) each covered by a cataphyll. The smaller lateral branch bud has no pollen-cone buds.* Fig. 19. *A terminal LSB sliced down the centre with a razor blade to show the large white pollen-cone buds and small distal SSB.* Fig. 20. *Sectioned and stained LSB showing pollen-cone buds and SSB.*

Figures 21–23. *Dormant terminal LSB and a small lateral branch bud. Both contain only short-shoot and lateral branch buds.* Fig. 21. *Whole terminal LSB and small lateral branch LSB, each showing no swellings.* Fig. 22. *LSB sliced down the centre with a razor blade to show only small SSB.* Fig. 23. *Sectioned and stained LSB showing only SSB.*

Long-shoot buds that contain no seed-cone or pollen-cone buds show no swellings and may be quite small on short branches in the lower crown (Fig. 21). When sliced longitudinally with a razor blade or sectioned and stained, they show only small SSB or branch buds (Figs. 22, 23). Using this technique on samples of LSB taken from different crown regions and from several trees, it is possible to determine if there will be cones for pollination the following spring.

Cone Induction

Cone induction and enhancement are terms commonly used to describe various hormonal and cultural treatments that cause trees to produce cones. Some workers may restrict the term cone induction to young trees that have never produced cones before and use the term enhancement for increasing cone production in trees that are already reproductively mature and have produced cones before. However, either term may be used with no distinction being made. In both cases, the same cultural or hormonal treatments may be used.

Flowering is a term usually restricted to flowering plants, including the hardwoods, but studies of the basic processes in flower initiation and cone initiation show that the processes are essentially the same at the early stages and flowers and cones become different during later stages of development. Thus, the terms "flowering" and "floral initiation" are commonly used in the conifer literature and designate a process and not a specific structure. However, using the term flower for cone is not accurate because, although functionally similar, they are structurally very different.

The abundance of cone buds on dormant LSB and, consequently, mature cone production two years later, is less cyclic in lodgepole pine and most other pines than in many other conifers. The physiological processes that control the differentiation of axillary bud primordia into any of the four types of buds—seed-cone, pollen-cone, SSB or LSB—are still poorly understood. This was an active area of research 20 years ago but once techniques for cone induction were developed that often worked, less emphasis was place on the search for the underlying causes.

Several environmental factors are known to promote cone-bud differentiation. These include high temperatures, high amounts of sunshine, and low rainfall, all of which may produce stress within the trees, thereby reducing vegetative growth during summer and fall when cone buds are differentiating. These stress conditions promote cone-bud rather than vegetative bud differentiation. As well, in pines abundant rainfall that promotes vegetative growth in the spring, when cataphylls and axillary bud primordia are being initiated, can increase the number of cone buds by increasing the number of cataphylls where axillary cone-buds may form. These factors, plus increased soil fertility, can increase the number of cone buds that differentiate. Consequently, some of the most successful and easiest ways of increasing cone-bud differentiation (flowering) in natural stands of pine is removal of competition by thinning and fertilizer application.

Many specific methods of cone induction have been tried in pines (Lee 1979, Owens and Blake 1985). Application of nitrogenous fertilizers is one of the oldest methods and has promoted seed-cone production in many pines. Nitrate fertilizer on lodgepole pine, for example, promotes female flowering but not male flowering. Stem or branch girdling or strangulation have promoted flowering in many pines, by interfering with the downward movement of food reserves and increasing the carbohydrate content above the constriction.

Branch pruning in pines has promoted male and female flowering by increasing branch formation and, therefore, the number of axillary buds. Branch pruning can also increase branch vigour, which is related to the types of cones induced in pines. Increased branch vigour promotes seed-cone production and decreased branch vigour promotes pollen-cone production. Treatments that limit root growth and water uptake, such as root pruning, root restriction and transplanting, have increased flowering in some pines. These simulate drought conditions. However, some of these treatments may result in some degree of permanent injury to the trees. Other injuries that may increase flowering can occur by frost, logging operations, insects, defoliation and disease (Lee 1979, Owens and Blake 1985).

In recent years, plant growth regulators (hormones) have been successfully used to induce and enhance flowering in conifers, including several

pines (Pharis et al. 1975). Early studies demonstrated that application of gibberellin A_3 (GA_3) as a foliar spray induced flowering in species within the Cupressaceae and Taxodiaceae families, but did not induce flowering in the Pinaceae. In the last 30 years, the less polar gibberellins (GA_4, GA_5, GA_7 and GA_9) have been tried in several species of the Pinaceae, including lodgepole pine. Other growth regulators, such as auxins and cytokinins have been ineffective or have given conflicting results. Growth regulators have been applied to conifers as foliar sprays or soil drench, or injected or fed into the xylem by various methods. Most gibberellin applications have given the best results when used in conjunction with other flower-promoting cultural treatments, such as fertilizer, girdling and root pruning, or forms of water stress (Pharis et al. 1975).

Lodgepole pine grows rapidly during its juvenile phase that lasts from four to 10 years for most provenances. During this time, flowering begins. Seed cones form first, followed by pollen cones a year or so later. Flowering in juvenile lodgepole pine has been induced by foliar spray applications of GA_4 and GA_7 combinations (Wheeler et al. 1980). These are called non-polar GAs because, in their molecular structures, they have only one hydroxyl group attached to the molecule. Presently these GAs are purchased as a mix of GA_4 and GA_7 because the two can not be readily separated. However, it has been shown that GA_7 is the active ingredient. Gibberellin A_3, a polar GA (having more than one hydroxyl group), is usually not effective in the Pinaceae. Gibberellins have been applied as foliar sprays or soil drenches, applied topically to the surface or incisions in young branches, and injected or fed by "intervenous" tubes into the stems of larger trees. Each technique may require different methods of preparation and different concentrations of GA and solvents for the GA (Owens and Blake 1985).

For small trees, a foliar spray may be applied that contains about 500 mg of $GA_{4/7}$ and 25 mg of napthalene acetic acid (NAA) dissolved in 1 litre of solvent consisting of 80 ml of 95% ethanol in 920 ml of distilled water with 0.1% Aromox-C, cationic detergent. Various detergents that have been reported in the literature promote penetration of growth substances into the foliage (Wheeler et al. 1980). Sprays are commonly given once per week. Spray applications have limitations due to weather (wind and rain) and absorption into the foliage. For large seed orchard trees with a diameter of 10 cm or more, the most successful method in recent years has been by stem injection (Ross and Bower 1989). This requires drilling holes downward at a 45 degree angle to the centre of the stem, about 1 m above ground level. Using a hypodermic needle or automatic syringe, a solution of $GA_{4/7}$ (34 mg/ml) in 80% ethanol is injected into the holes then holes are plugged with putty or grafting wax. The number of holes, and their depth and diameter varies with the tree diameter, as does the amount of $GA_{4/7}$ injected and the number of treatments per tree. For details of the methodology of stem injections see Ross and Bower (1989) or Owens et al. (2001b), and for foliar sprays see Wheeler et al. (1980).

Gibberellins are most successful when applied with adjunct treatments such as nitrate fertilizer, girdling, drought, root pruning, etc. (Owens and Blake 1985). The treatments usually are synergistic, meaning that together they are more effective than the sum of them when applied separately. The time of treatment application is critical and must be just before and during the period of cone-bud differentiation. For lodgepole pine in BC, this is early in August and lasts for about 11 weeks (Fig. 24). This time may vary from northern to southern provenances. The August treatments may enhance pollen-cone bud differentiation, whereas later fall treatments may enhance seed-cone bud differentiation. Results are extremely variable even within clones. There are differences between trees, locations, years, weather and the specific techniques used. Before any large-scale cone induction trials are begun, original reports should be consulted.

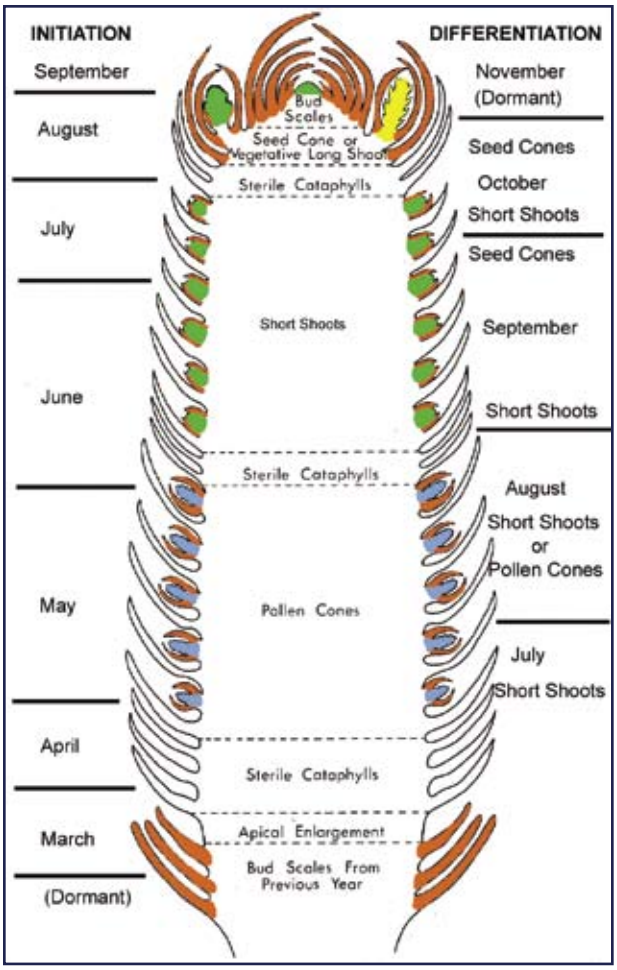


Figure 24. *Diagram of a long-shoot terminal bud showing the time of initiation of axillary buds and the approximate time of differentiation of these buds. Green indicates vegetative terminal and lateral long-shoot or short-shoot buds or apices, yellow indicates a seed-cone bud and blue indicates pollen-cone buds. Brown indicates the bud scales. (Redrawn from Owens and Molder 1975.)*

Treatments that promote flowering in pine do not greatly increase the number of axillary primordia initiated that could differentiate into cone buds. Rather, treatments appear to control the pathway along which the axillary primordia develop, within the limits established by the position of the primordia along the LSB (Figs. 8, 24), and the position of the LSB within the tree.

Pollen-cone and Pollen Development

Pollen-cone buds of shore pine resume development in coastal BC in mid-March and pollen is mature in mid-May, a period of about two months. In the interior of BC, pollen cones on lodgepole pine resume development in early April and pollen is mature in late May or early June, about two weeks later than in coastal regions. Despite differences in phenology, development (Fig. 25) is the same in trees from both regions (Owens et al. 1981).

Dormant pollen cones have about 140 microsporophylls, each of which has two microsporangia (pollen sacks) (Fig. 25 A). Microsporangia contain sporogenous cells that resume cell division following dormancy and increase to about 500 cells. These cells are the pollen-mother cells (microsporocytes) (Fig. 25 B) and they undergo meiosis, a special type of nuclear division in which chromosomes replicate once but the cell divides twice resulting in four haploid (N) microspores. Haploid microspores contain 12 chromosomes, half the usual diploid (2N) number of 24 chromosomes for lodgepole pine. The four microspores are held together for a short time within the pollen-mother cell wall. This group of four microspores is called a tetrad (Fig. 25 C). The microspores enlarge for a few days and burst the pollen-mother cell wall. This releases over 2000 microspores into the watery thecal fluid within each microsporangium. The period of meiosis to the formation of separate microspores (Fig. 25 B–D) lasts about two weeks and is the time of most rapid pollen-cone enlargement, although pollen cones still remain green (Fig. 39; see p. 19). During the next month microspores develop into mature pollen grains (Fig. 25 D–H).

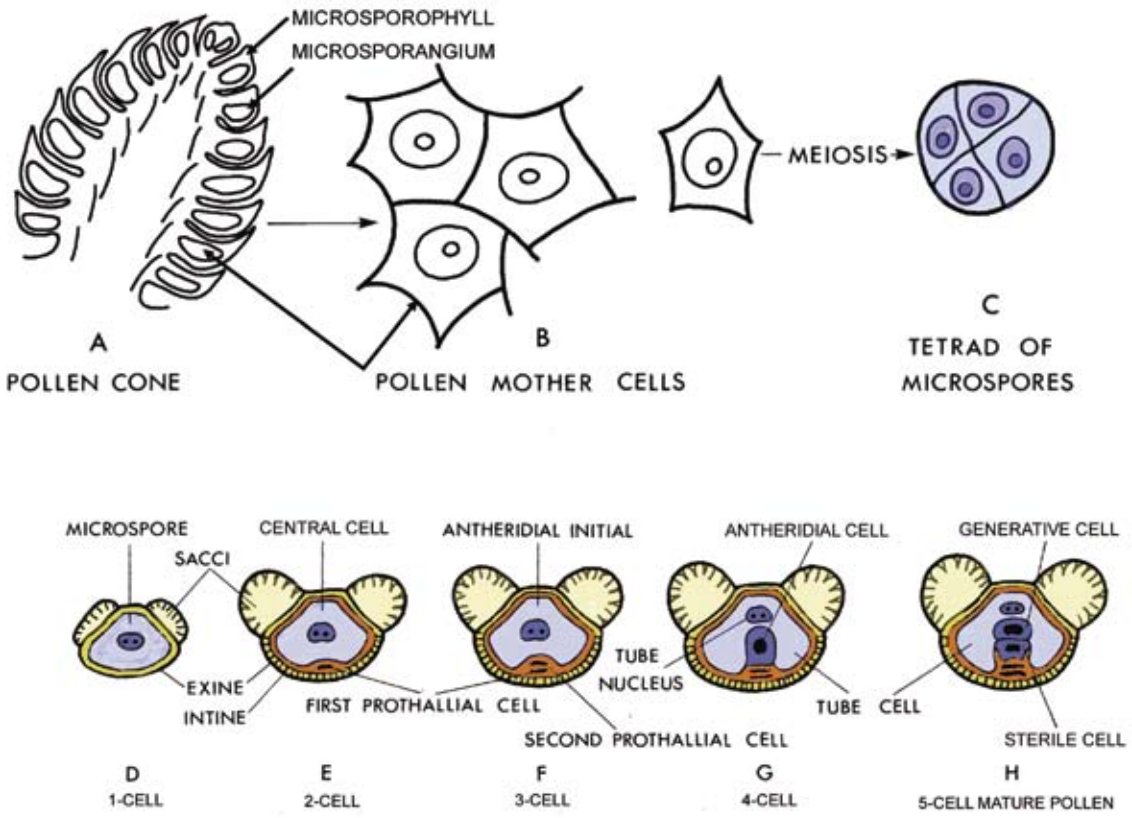


Figure 25. *Pollen development in pine. Haploid (N) gametophytic cells are shown in blue, the outer (exine) layer of the pollen wall is shown in yellow and the inner (intine) layer of the pollen wall is orange.*

During the first week of pollen development, the microspores become rounded, store food reserves (mostly as starch), and the pollen wall (exine) thickens and begins to form two small wings or sacci (Fig. 25 D). The microspore nucleus then divides by mitosis, a process in which both daughter nuclei each receive an identical set of chromosomes. But in this case, the two cells that form are unequal in size. A small first primary prothallial cell is formed to one side, opposite the wings, and a large central cell remains in the centre. A second pollen wall, the intine, forms inside the exine and encloses the first primary prothallial cell (Fig. 25 E). The central cell then divides unequally and forms a large antheridial initial and a small second prothallial cell on top of the first. This also becomes enclosed by the intine. Both prothallial cells remain small and lens-shaped and contain little cytoplasm and a nucleus that quickly degenerates (Fig. 25 F).

The antheridial initial then divides, forming a large tube cell and a smaller antheridial cell contained within the tube cell (Figs. 25 G, 26). The antheridial cell then divides forming a generative cell and a sterile cell (often called the stalk cell) that becomes enclosed in the intine on top of the two prothallial cells. The sterile cell has no further function (Figs. 25 H, 27). The generative cell divides to form two sperm after pollination.

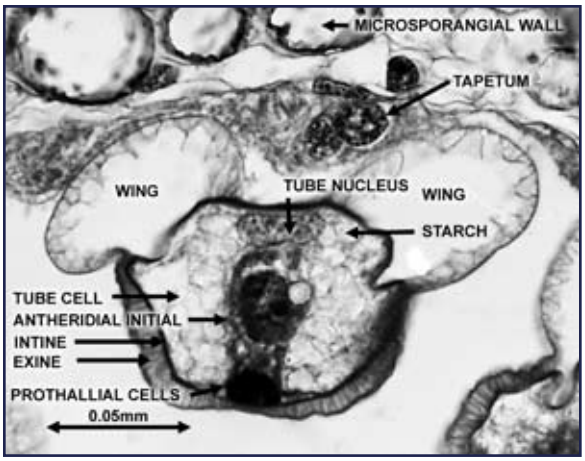


Figure 26. *Stained section of a microsporangium showing a four-cell pollen grain next to binuclear cells of the tapetum during last stages of pollen wall formation.*

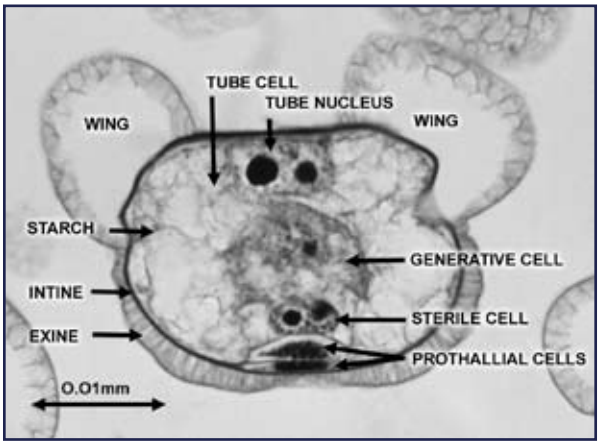


Figure 27. *Stained section of a microsporangium showing a mature five-cell pollen grain.*

Mature pollen has five cells (Figs. 25 H, 27), contains abundant starch and floats in the thecal fluid. The microsporangia enlarge as the pollen matures and the cells lining the inside of each microsporangium (the tapetum) degenerate (Fig. 26), releasing complex compounds including, sporopollenin, lipids and proteins, that coat the surface of the pollen. These coating compounds and the thick exine make the pollen very hard and resistant to pathogens and physical damage. During the last week of development the thecal fluid disappears and the pollen dries usually to less than 10% water content. Mature dry pollen is yellow, about 0.04 mm in diameter with a disk-shaped coarsely sculptured body and two finely sculptured wings (Fig. 28).

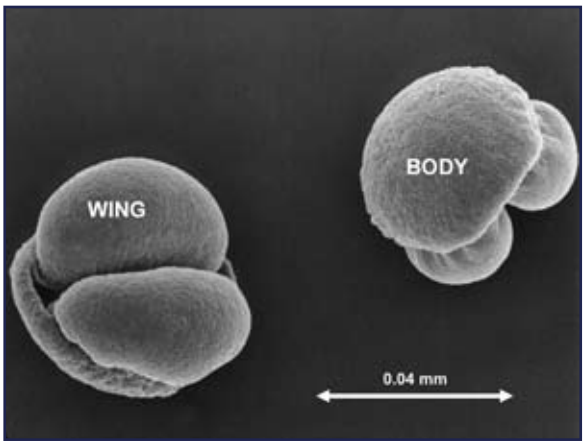


Figure 28. *Scanning electron micrograph of mature interior lodgepole pine pollen showing the body, two wings (sacci) and the different sculpturing on the body and wings.*

During pollen development, each microsporangium forms a line of dehiscence, consisting of specialized thin-walled cells that separate when they dry causing the microsporangia to burst open, releasing the dry pollen.

In interior lodgepole pine, meiosis usually occurs during the last week of April, the tetrad stage lasts just a few days, starch starts to accumulate and the exine and small sacci start to form during the week after microspores separate. Cell divisions begin about three weeks after meiosis and stops about one week before pollen is shed. Pollen is mature four to six weeks after meiosis depending primarily upon temperatures. During a warm spring, pollen may develop in only four weeks but this may take six weeks during a cool rainy spring. Although development starts earlier in coastal regions, it is usually slower and commonly takes six to eight weeks from meiosis to pollen release. Although pollen development is strongly affected by temperature, pollen release (dehiscence) is affected by both temperature and humidity. During warm dry springs, pollen will be released earlier than during warm wet or cool wet springs. At pollen maturity, a light rain or overhead sprinkling can delay pollen shed by one day.

Monitoring the stages of meiosis and pollen development is not difficult. Samples of pollen cones can be collected every few days and, from each cone, several microsporangia can be removed

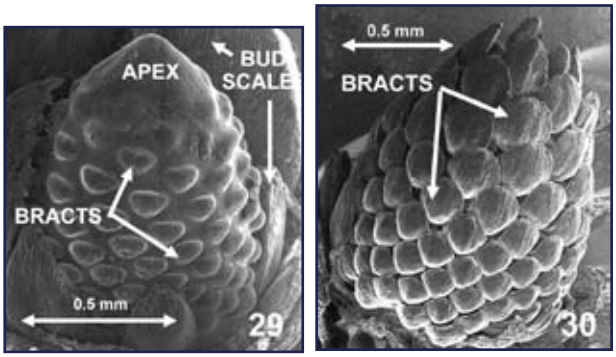
and squashed on a microscope slide. A drop of aceto-carmine stain is added, then a coverslip, and the cells can be observed using a compound microscope (see Appendix 2).

In lodgepole pine, the average number of pollen cones per shoot (cluster) is 15, the average number of microsporophylls is 140 per cone and pollen per microsporangium is 2100. It is estimated that each pollen cone produces about 590 000 pollen grains and each shoot almost nine million pollen grains. There may be hundreds of pollen-cone bearing shoots on a medium-size seed orchard tree resulting in several billion pollen grains per tree (Ho and Owens 1973).

Seed-cone and Ovule Development

Potential seed-cone buds are initiated in August or September, begin to differentiate in late September or early October and development may continue until about the end of October (Figs. 8, 24) depending upon the site and weather. At the start of differentiation, some of the large apices near the tip of the LSB enlarge forming a dome-shaped apex, then begin to initiate a spiral series of bract primordia up the flanks of the apex (Fig. 29). The number of bract primordia initiated before dormancy varies with the time of cone-bud differentiation, the position of the cone bud on the tree and the site and weather. Some seed-cone buds become dormant after initiating only a few bracts, whereas others may initiate nearly all bracts before winter dormancy. Other apices in the same region of the LSB slow in development in the fall. They initiate a few bud scales then become dormant. They differentiate into lateral LSB the next spring (Owens and Molder 1975) (Figs. 8, 24).

In seed-cone buds, bract primordia become broad and flat and curve upward, forming thin leaf-like structures (Figs. 30, 31). In the axil of the bracts in the distal half of the cone, a broad ovuliferous scale (scale) is initiated. It is thicker than the bract and a swelling appears on the two lateral portions. Each swelling develops into an ovule (Fig. 31). Most bracts in the basal half of the cone do not initiate scales. Many of the bracts in between the distal fertile region and the basal region initiate no scales or only rudimentary scales that bear no or only rudimentary ovules. The latter two regions make up the basal sterile two-thirds of the cone in lodgepole pine. The proportion of sterile and fertile regions of the cones varies among clones of lodgepole pine and varies considerably among species of pine. Scales in the fertile region of the cone each form a long tip or spine (Fig. 32), while more basal scales form shorter spines or no spines. The basal sterile region of the cone is narrow and remains covered by bud scales at pollination, whereas the distal fertile region is broad and exposed (Fig. 47, see page 21).



Figures 29 and 30. *Scanning electron micrographs of seed-cone bud development.* Fig. 29. *Seed-cone bud, with most bud scales removed, in the fall during bract initiation.* Fig. 30. *Seed-cone, with all bud scales removed, in the spring, following winter dormancy, after all bracts have been initiated and ovuliferous scale initiation has begun.*

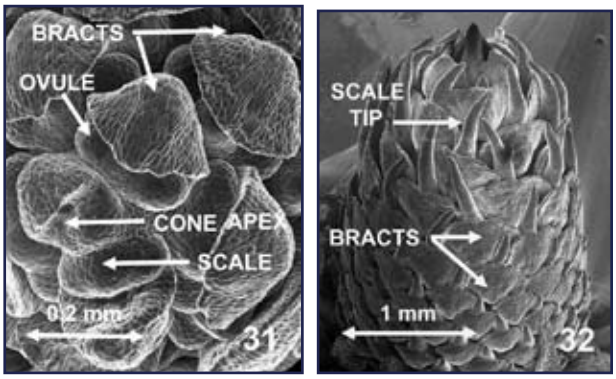


Figure 31 and 32. *Scanning electron micrographs of seed-cone buds with all bud scales removed.* Fig. 31. *The tip of seed-cone bud in the spring showing ovuliferous scale (scale) and ovule initiation. The cone apex has been used up in the initiation of bracts.* Fig. 32. *The tip of a seed-cone bud in the spring showing bracts and pointed tips of the scales.*

The outer layers (integument) of the ovule tip form two opposite finger-like projections, the micropylar arms, leaving a small mound of nucellus between. The arms elongate and a round opening, the micropyle, remains between the arms. The arms hang downward (Fig. 33) in the space next to the cone axis forming a spiral of long finger-like projections. The micropyle opens into a short, narrow, cylindrical micropylar canal that leads from the micropyle to the tip of the nucellus. As seed-cone buds burst and the seed cones elongate, cells covering the micropylar arms secrete many microdrops that are visible with the scanning electron microscope (Fig. 33) and cytochemically using specific stains. These are lipid drops to which pollen adheres (Owens et al. 1981, 2005).

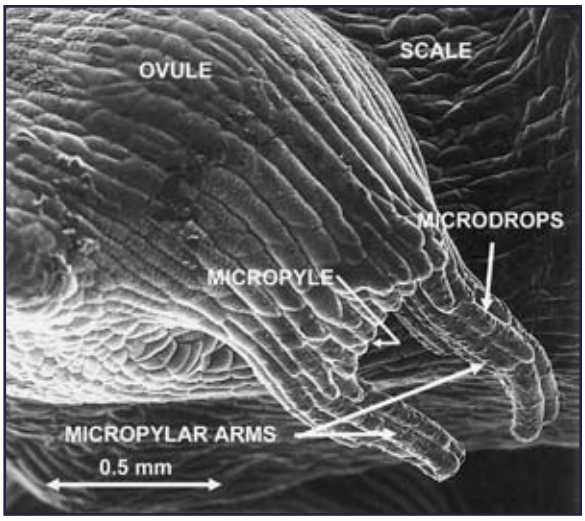


Figure 33. *Ovule tip just before pollination showing the two micropylar arms with microdrops. The micropyle is between the two arms.*

Pollination

Pines are monoecious, meaning that pollen cones and seed cones are found on the same tree. The rate at which cones develop in the spring is primarily determined by temperature—the warmer the temperature the more rapid the development. However, pollen release (dehiscence) is also dependent on drying of the pollen cones. In wet weather, pollen release may be delayed for several days and in dry weather it is hastened. This can lead to the temporal separation of the male and female functions. This may take three forms:

1. Female and male organs mature at the same time (homogamy) (Fig. 35);
2. Male organs mature before female (protandry) (Fig. 36); and,
3. Female organs mature before male (protogyny) (Fig. 37).

The reason that all three can occur in different years at one site is that, although the rate of both pollen-cone and seed-cone development is primarily determined by temperature—but pollen release is also determined by humidity—a light rain can delay dehiscence by one day or more but not slow seed-cone development.

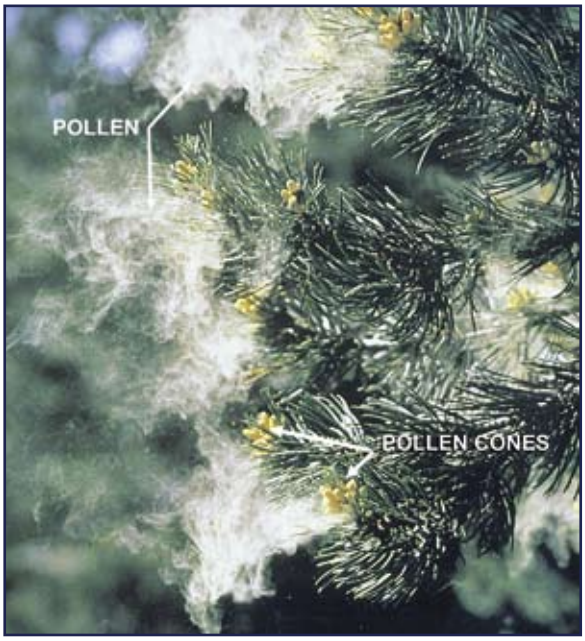


Figure 34. *The abundant pollen release "sulphur clouds" that may occur in pines.*

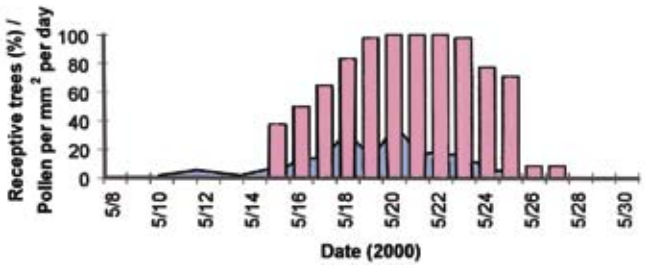


Figure 35. *Homogamy, shown as the amount of lodgepole pine pollen carried by wind and measured by the mean number of pollen per square millimetre per day using a pollen monitor (blue), compared with the number of trees having receptive seed cones (pink) at Pacific Regeneration Seed Orchard near Salmon Arm, BC in 2000 (Owens et al. 2005).*

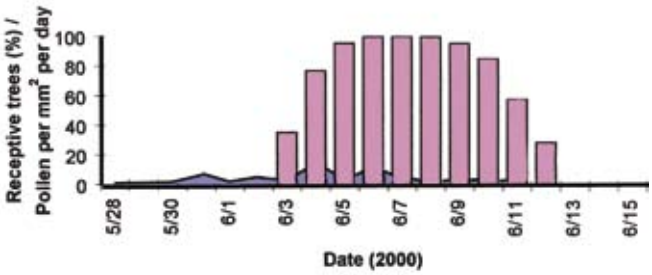


Figure 36. *Protandry in the seed orchard near Prince George, BC in 2000, measured as in Fig. 35. Small amounts of pollen were shed (Owens et al. 2005).*

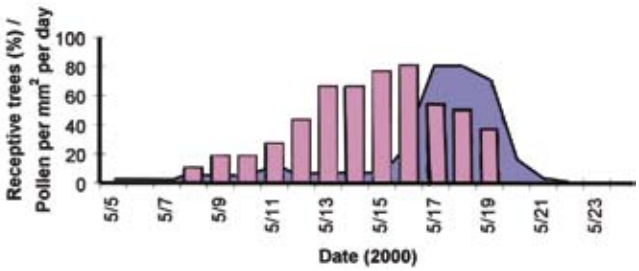


Figure 37. *Protogyny in Kalamalka Seed Orchard near Vernon, BC in 2000, measured as in Fig. 35. Abundant pollen was shed (Owens et al. 2005).*

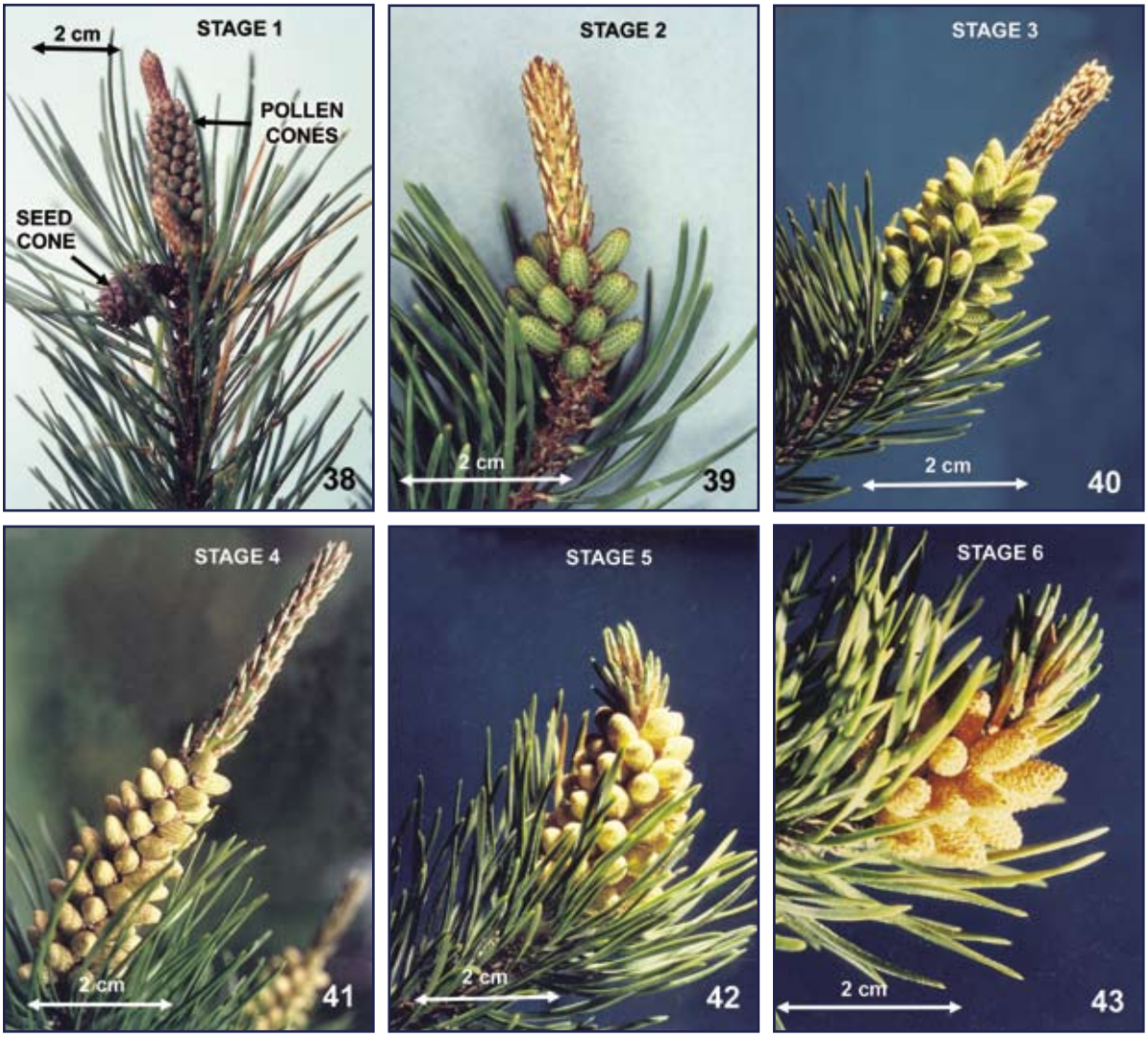
Detailed studies have been carried out on phenology of pollination using the same clones at a usually hot dry site near Vernon, BC and a cool wet site near Prince George, BC (Owens et al. 2005). At the Vernon site, weather can vary from hot and dry, resulting in early pollen release over a short time, to very cool and wet, resulting in late pollen release when only 20% of the seed cones have become receptive and over 80% of the pollen has been shed. In other years there is homogamy (Fig. 35), and in others slight protogyny (Fig. 37). At the Prince George site there is usually homogamy but occasionally slight protogyny (Fig. 37). Both protogyny and protandry favour cross-pollination in monoecious trees but can result in few ovules being pollinated by few pollen grains. Homogamy can result in many ovules in a cone being pollinated by many pollen grains, but in monoecious trees this can lead to high levels of self-pollination.

In pines, if many of the fertile ovules in a cone are pollinated (approximately over 80% in lodgepole pine), the cone will survive, but if fewer ovules are successfully pollinated, the seed cone will abort within a few weeks after pollination (cone drop). The critical number of ovules that must be pollinated for cone survival appears to vary among species (Owens et al. 2005). Cone abortion occurs over two to three weeks. Early stages of cone abortion are not obvious. The inner tissues (ovules) die first, but this is not visible unless the cone is sliced open. One or two weeks after ovule abortion the outer portions of the bracts and scales turn brown and the cone usually falls from the branch.

Successful pollination is usually measured by pollen flight or by pollen attached to the micropylar arms of dissected cones. However, pollen must be taken into the ovule rather than just adhere to the arms or cone surface. What is more important, but difficult to count, is the number of ovules that have taken in pollen. Although 10 to 20 grains may adhere to the micropylar arms, lodgepole pine has very small ovules and micropylar canals that will only take in one to four pollen grains (average 1.3 to 1.5). The rest of the pollen is left on the surface of the arms, ovule, bract or scale. Abundant pollen arriving too late will not be taken into the ovules (Owens et al. 2005).

Self-pollination is common in conifers because most species are monoecious and in many species of pine, pollen cones and seed cones are found in close proximity in the same region of the crown and often on the same branch. In pines, low to moderate levels of self-pollination do not cause cones to abort as long as the required number of ovules are pollinated for that species. In most ovules that are self-pollinated, pollen germinates, pollen tubes form and fertilization occurs, but the embryos and the megagametophytes abort soon after fertilization. This is about one year after pollination, leaving fully enlarged but empty seeds. Some early self-incompatibility reactions may occur in the first year, but little work has been done in this area. If only self-pollen is used in control pollinations some of the cones will abort (Owens et al. 2005). Further discussion of this topic is in the section on "Cone and seed production in seed orchards" (see p. 41).

It is convenient to assign stages to pollen-cone (Figs. 38–43) and seed-cone (Figs. 44–51) development before and during pollination. The following phenology (the relationship between development



Figures 38–43. *Stages of pollen-cone development before, during and after pollen release.* Fig. 38. *Stage 1 pollen-cone buds at the start of development in the spring before meiosis. Pollen cones are emerging from the bud scales.* Fig. 39. *Stage 2 pollen cones at meiosis and rapid cone growth and early LSB and SSB elongation.* Fig. 40. *Stage 3 pollen cones after meiosis and during early pollen development. Shoot elongation has started to separate the cones and cones are turning yellow.* Fig. 41. *Stage 4 pollen-cone buds during late pollen development. Cones are yellow and starting to dry. This is the best stage for collection of pollen cones for pollen extraction.* Fig. 42. *Stage 5 pollen cones during pollen dehiscence. This is too late to collect pollen cones as most pollen has been shed (Owens et al. 2005).* Fig. 43. *Stage 6 cones after most pollen has been released (from Owens et al. 2005).*

and time) is for lodepole pine growing in seed orchards in the interior of BC. Comparable stages occur about two weeks earlier near Vernon than near Prince George, and at either site the phenology may vary by about two weeks from year to year depending on spring weather (Owens et al. 2005).

Pollen-cone buds have few bud scales and the tip of the cone may be slightly exposed even during winter dormancy (Fig. 38). When growth resumes in the spring, pollen cones quickly emerge from their bud scales and may range from red, to brown, to green. Colour differences appear to be clonal and also result from slight differences in stage of development. Pollen cones grow rapidly at the time of meiosis and on most clones become light green (Stage 1, Fig. 38). Following meiosis and during early pollen development, pollen cones begin to turn yellow (Stage 2, Fig. 39). The long shoot on which the pollen cones are borne elongates, separating the pollen cones (Figs. 40, 41). During the last stages of pollen development the pollen cones begin to dry and turn yellow-brown (Stage 3, Fig. 40). This is the best time to collect pollen cones for pollen extraction. At Stage 4 (Fig. 41), pollen cones start to open and release pollen. This is usually too late to collect cones because some of the pollen has been shed, although pollen shed at this time is of high quality. At Stage 5 (Fig. 42), most of the pollen has been released. A pollen cone releases most of its pollen in one day and most pollen cones in a cluster release their pollen in two or three days. Dry pollen cones that have released their pollen (Stage 6, Fig. 43) soon turn brown but may remain on the branch for most of the summer (Owens et al. 2005).

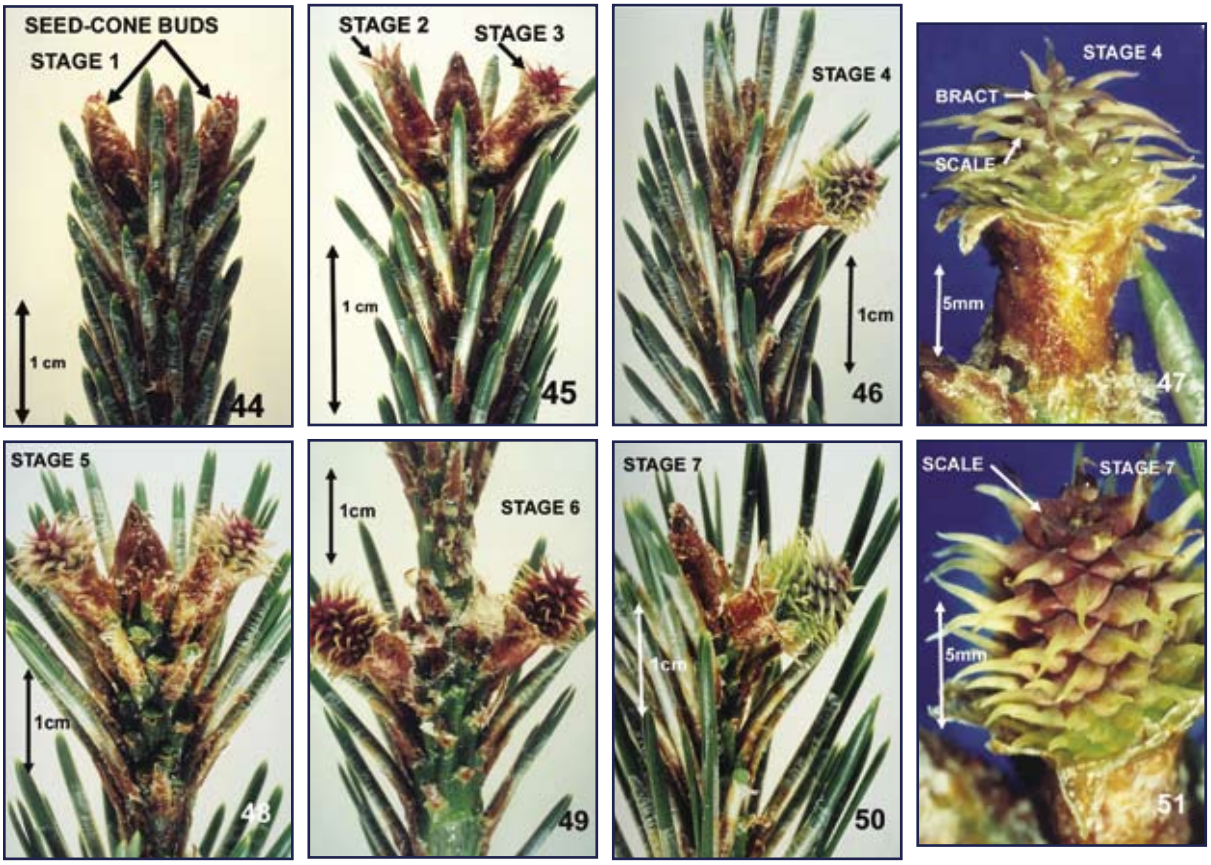
As seed cones elongate within their bud scales in the spring they may initiate their last bracts and scales. Then, the usually red cone tip pushes out from under the bud scales (Stage 1, Fig. 44). Seed cones continue to elongate and extend beyond the bud scales and at Stage 2 (Fig. 45, left), only the very distal scale tips are exposed and they are not yet receptive. By Stage 3 (Fig. 45, right) they are about one-third exposed and are receptive.

At this time the cone axis elongates quickly and the spaces (internodes) between the bract-scale complexes lengthen, separating them, allowing pollen to easily reach the bract-scale surfaces, ovules and cone axis. This is Stage 4 (Figs. 46, 47). Only the basal sterile scales remain covered by bud scales and the white cone axis and bracts are visible. Scales are commonly green to white near the pointed tip and at the base and red near the central portion that bears the ovules (Fig. 55). Stage 4 is the most receptive stage and individual cones remain at this stage for several days. Elongation of the cone axis then slows and the scales thicken, narrowing the spaces between scale-bract complexes (Stage 5, Fig. 48). Stage 5 cones are still receptive but generally less so than Stage 4 cones. Scales continue to thicken and seal the cone (Stage 6, Fig. 49) and the cones are no longer receptive. Seed cones then broaden and become oval and tightly sealed (Stage 7, Figs. 50, 51). A cone is receptive (Stages 3 to 5) for about five to seven days, with the most receptive stages lasting only two to four days depending on weather. Hot weather causes more rapid sealing of the cones (Owens et al. 2005).

Pollination success (PS) is a measure of the amount of pollen entering the cone and reaching the ovules. It can be measured directly by slicing a cone longitudinally down the centre with a razor blade, observing the ovules using a hand lens or dissecting microscope and counting the number of pollen grains attached to the micropylar arms (Figs. 54–57) (see Appendix 3 for the methods). The PS usually correlates well with the amount of pollen in the air, as measured using pollen monitors mounted in the orchard or stand. However, wind direction and even a very light rain can affect the amount of pollen reaching a tree or cones on different sides or levels of the crown. So it is wise to measure pollen abundance using pollen monitors, and samples of cones to measure PS.

Temperature sums in degree days (DD) may be used to predict the time of pollen release and seed-cone receptivity. In lodgepole pine near Vernon, BC, pollen release and seed-cone receptivity began

when the DD reached about 500 at a threshold of 5°C, but because pollen release is also governed by humidity, this can vary considerably. Use of DD is not as convenient as it first seems and it is usually much easier to monitor daily a sample of trees in the orchard that include early, late and more average clones. It takes only about one hour to monitor 20 trees each day and this allows orchard personnel to identify the early trees or clones so that they can be supplementally pollinated before they are past receptivity.



Figures 44–51. *Stages of seed-cone development before, during and after pollination.* Fig. 44. *Stage 1 seed-cone buds during early development and elongation showing the red tip of the cone emerging from the bud scales.* Fig. 45. *Seed cones at Stage 2 (left) and Stage 3 (right). Stage 2 seed cones are not receptive and Stage 3 cones are just becoming receptive.* Fig. 46. *Stage 4 seed cone with elongated cone axis causing wider spaces between bract-scale complexes. This is the most receptive stage.* Fig. 47. *Stage 4 seed cone showing the white bract and cone axis at the base of the scales. Most of the sterile base of the cone is covered by brown bud scales.* Fig. 48. *Stage 5 seed cones when scales are starting to thicken but the cone is still receptive.* Fig. 49. *Stage 6 seed cones showing thickened scales sealing the cone closed.* Fig. 50. *Stage 7 seed cone showing completely sealed cone. Cone may be red to green.* Fig. 51. *Stage 7 completely sealed seed cone with bracts covered by the thickened scales (from Owens et al. 2005).*

The Pollination Mechanism

The pollination mechanism in lodgepole pine is similar to that found in other members of the Pinaceae that have:

1. erect cones at pollination;
2. inverted ovules with micropylar arms hanging downward;
3. a pollination drop that is exuded out from the micropyle; and,
4. pollen with wings.

Airborne pollen blows into the cones for about one week, during Stages 3 to 5, but mostly during Stage 4. During Stages 3 and 4, the micropylar arms secrete lipid microdrops that remain on the surface of the arms. Pollen adheres to these microdrops (Fig. 52). Pollen settles on all cone surfaces but is easily dislodged, except from the sticky arms where pollen accumulates over several days (Figs. 54–56).

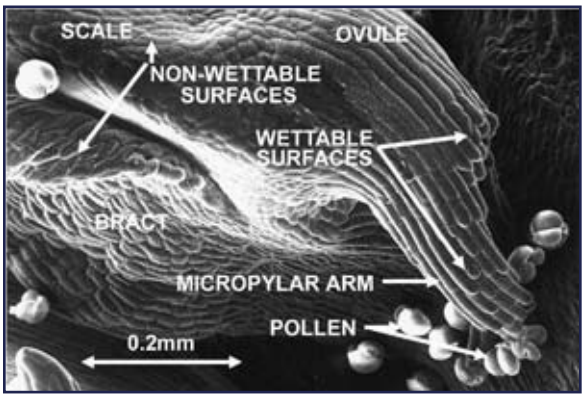


Figure 52. *Scanning electron micrograph showing a receptive ovule tip with pollen attached to the microdrops on the micropylar arms. Surfaces that bear no cuticular wax are wettable (near micropyle), whereas surfaces having cuticular wax are not wettable by rain or dew.*

At Stages 4, 5 and 6, the nucellus tip secretes a large pollination drop that fills the micropylar canal and is exuded out of the micropyle, usually filling the space between the arms and touching nearby surfaces of the scale, bract or cone axis (Fig. 53). It may scavenge pollen from these surfaces as has been shown in western white pine (Owens et al. 2001a) and interior spruce (Runions and Owens 1996). Pollen attached to the arms enters the pollination drop and, because the wings are filled with air, float up into the inverted drop, through the micropyle and to the surface of the nucellus. Also, once pollen has entered the pollination drop, the drop evaporates more quickly, decreases in volume and recedes up the micropylar canal, carrying any pollen captured within the drop. If there is no pollen on the arms or entering the pollination drop, the drop will evaporate during mid-day, decrease in size and recede up the micropylar canal only to reappear again that night or early the next morning. This process may continue for several days or until pollen is taken into the ovule. If pollen is taken into the ovule, no more drops are exuded from the micropyle. In lodgepole pine, only about 10–20% of the ovules have a pollination drop at one time but over several days all will form a drop and take pollen in if it is present (Owens et al. 2005). That is why it may be more effective to have pollen arrive in small quantities over several days, as happens with wind pollination, rather than a large amount of pollen applied at one time, as often happens with supplemental mass pollination (SMP) or breeding. This may be particularly true in lodgepole pine where the micropylar canal only holds one to four pollen grains.

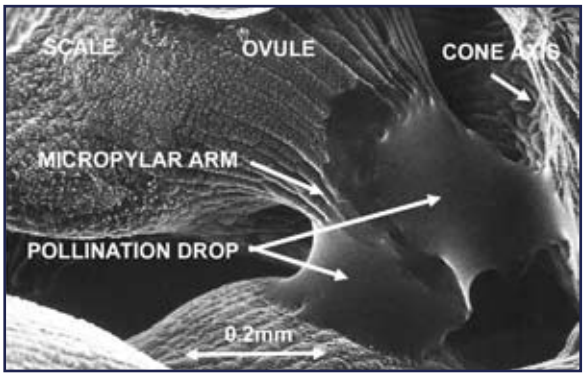
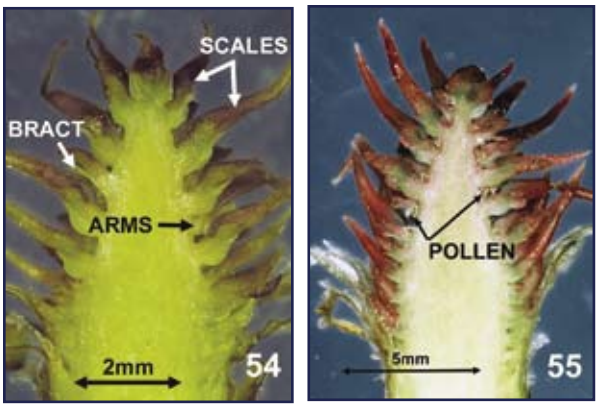
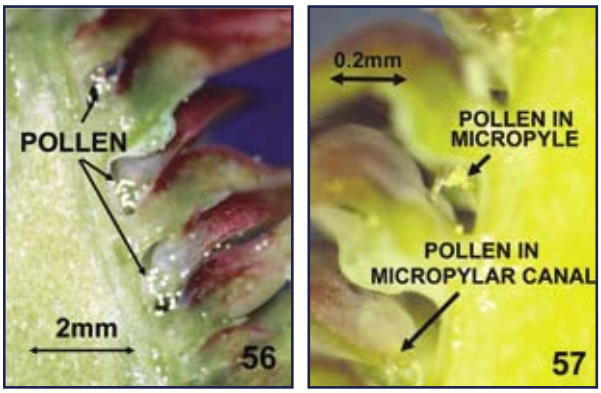


Figure 53. *Scanning electron micrograph of an ovule tip with a large pollination drop between the micropylar arms.*



Figures 54, 55. *Fresh Stage 4 seed cones sliced longitudinally.* Fig. 54. *Cone with no pollen on the arms.* Fig. 55. *Cone with pollen on the arms.*



Figures 56, 57. *High magnification of fresh seed cones sliced longitudinally.* Fig. 56. *Stage 4 cone showing abundant pollen attached to the arms.* Fig. 57. *Stage 5 cone showing pollen taken into the micropyle and below into the micropylar canal.*

Pollination drops are secreted from cells at the tip of the nucellus within the ovule. These cells collapse forming a depression in the tip (the pollen chamber) into which the pollen usually settles (Owens et al. 2005). The pollination drop contains about 8% sugars plus many amino acids and proteins. Pollination drops are secreted unless the tree is under severe water stress (4–5 Mpa in interior spruce, Owens et al. 1987). Drops evaporate very quickly and are very small and ephemeral in cones of lodgepole pine growing in the interior, but pollination drops are large and easily visible in cones from trees near Prince George (Owens et al. 2005).

Water from rain or sprinkling can fuse with and increase the size of pollination drops, form artificial drops or carry pollen from other cone surfaces to the ovule tips. Lodgepole pine cones have different textures on different surfaces within the cone. Cells covering the surfaces of the cone axis, bracts, and most of the scale and ovule have a cuticle that consists of fine wax rods of varying length that are only visible using the scanning electron microscope (Fig. 52). These "hairy" surfaces affect water that lands on them. The water does not wet the surface but forms tiny beads that may move over the surfaces, often downward in the erect cone toward the ovule tips. About the only cone surfaces lacking the wax rods are the micropylar arms. Water wets these surfaces and accumulates there. Water droplets reaching the arms have often picked up pollen from the cone surfaces. These droplets may fill the space between the arms forming an artificial pollination drop, or fuse with and add to existing small pollination drops (Fig. 53). Artificial or supplemented pollination drops appear to function in taking pollen into the ovule in the same manner as a drop secreted from the nucellus (Owens et al. 2005).

Experiments in 2000 using lodgepole pine in seed orchards in the dry interior near Vernon, BC and the more humid, wet northern interior near Prince George, BC demonstrated that water as rain, from sprinkling or misting (from 6:00 to 11:00 a.m.) increased the abundance and size of pollination drops on the dry site (Fig. 58). At the humid wet site where there was commonly light morning rains, seed cones at Stages 4 and 5 had pollination drops on 60–80% of the ovules in cones dissected in the early morning compared to 0–10% of ovules at the dry site. After early morning misting at the dry site, the frequency of pollination drops increased to about 45% of the ovules. Ground watering did not increase the frequency of pollination drops (Fig. 58) (Owens et al. 2005). It appears that lodgepole pine trees must be under very high water stress before this reduces the size or abundance of pollination drops, as has been shown in interior spruce where high water stress decreased the size and increased the viscosity of the pollination drops (Owens et al. 1987).

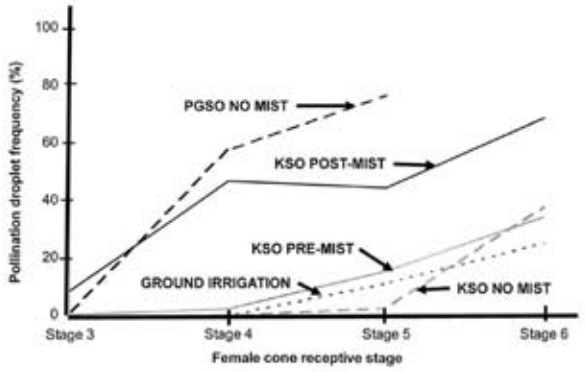


Figure 58. *The frequency of pollination drops observed in cones (percentage of ovules with a pollination drop) at Stages 3 to 6 at the non-misted, wet Prince George (PGSO) site and at the misted, non-misted or ground-irrigated dry Vernon (KSO) site before and after morning misting.*

In lodgepole pine near Vernon, BC, misting early in the morning did not prevent pollen release later in the day, but afternoon sprinkling delayed pollen release by about 24 hours (Owens et al. 2005). Pollen released in the afternoon would be taken into the ovule by pollination drops the following morning. The function and benefit of rainwater in pollination has also been observed in loblolly pine (Greenwood 1986, Brown and Bridgewater 1987) and in radiata pine (Lill and Sweet 1977).

Pollen Management

In young trees, few pollen cones are produced, and in young and older seed orchards, pollen release and female receptivity may coincide (homogamy) (Fig. 35), there may be clones that flower very late, after peak pollen release (protandry) (Fig. 36), or there may be clones that flower very early, before peak pollen release (protogyny) (Fig. 37). In the latter two cases, and where little pollen is produced, SMP is usually done to ensure that cones are adequately pollinated. In lodgepole pine, if too few fertile ovules (<80%) are pollinated the cone aborts within a few weeks. If ovules are self-pollinated the cone may not abort but most of the self-pollinated ovules within the cone abort within one year after pollination. If many selfed ovules abort early, soon after pollination, this may cause the cone to abort. If cones are pollinated with dead pollen, the ovules abort soon after pollination and most of the cones will abort (Owens et al. 2005). Therefore, SMP with high quality pollen from other clones well help decrease cone abortion and cone drop and increase the number of filled seeds per cone.

Supplemental mass pollination should be done two or three times, from Stage 3 through Stage 5, because cones are at different stages on a tree and on different trees. In lodgepole pine at the Vernon and Prince George seed orchards, a single pollination at Stage 3 gave the highest filled seed per cone compared to single pollinations at Stages 4 and 5. Also, cones pollinated at Stage 3 had a higher seed potential (fertile ovules) and filled seeds per cone than cones pollinated at Stages 4 and 5. It appears that pollen entering the cones at Stage 3 stimulates partially developed ovules, mostly near the tip of the cone, to complete development and become fertile. This significantly increased seed potential and thus increased filled seeds per cone from 18 or 20 to 26. It is a common seed orchard practice for many species to give SMP toward the end of the receptive period as one last attempt to increase seed production but this may be too late to have a positive effect in lodgepole pine. For lodgepole pine, SMP at the earliest stages of receptivity (Stage 3) should be more effective in increasing seed potential and filled seeds per cone than SMP given at later stages of receptivity (Owens et al. 2005).

Large amounts of pollen are often required to do SMP in seed orchards, necessitating collection of pollen often one or more years before the SMP. Pollen cones must be collected at the proper stage of development (see Figs. 38–43), dried and the pollen extracted. The extracted pollen may need additional drying to about 10% water content. Dry pollen may be stored in airtight containers at –20°C. If pollen is stored for several years it gradually loses its ability to germinate and form pollen tubes. Therefore, various testing methods for pollen quality have been developed. Some tests require expensive equipment and others simple equipment. The methods of pollen handling and testing described for interior spruce (Webber 1991) and Douglas-fir (Webber and Bonnet-Masembert 1989, 1993; Webber and Painter 1996)

work well for lodgepole pine. A pollen germination test developed for small pollen samples of lodgepole pine is given in Appendix 4.

Ovule and Megagametophyte Development

Ovules are initiated and ovule development begins during the year of cone initiation. In the next year —the year of pollination—some additional ovules are initiated, meiosis occurs, and early stages of formation of the female sexual reproductive structure (the megagametophyte or female gametophyte) occurs (Figs. 59, 60 A–E). Completion of megagametophyte development, fertilization, embryo and seed development occur in the third year (Fig. 60 F–H). Cones mature about two years after they were initiated and seeds mature about 15 months after pollination (Fig. 5) (Owens et al. 1981, 1982).

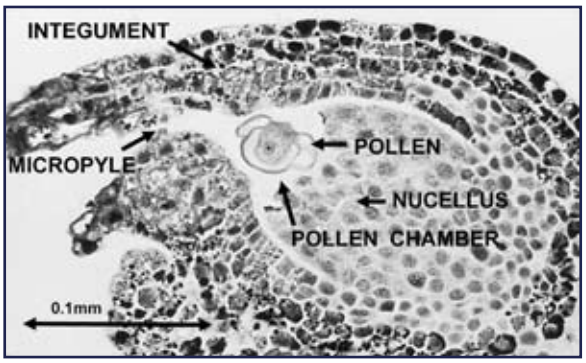


Figure 59. *Section of an ovule soon after pollination showing the closed micropyle and a pollen grain in the pollen chamber (see Fig. 60 B).*

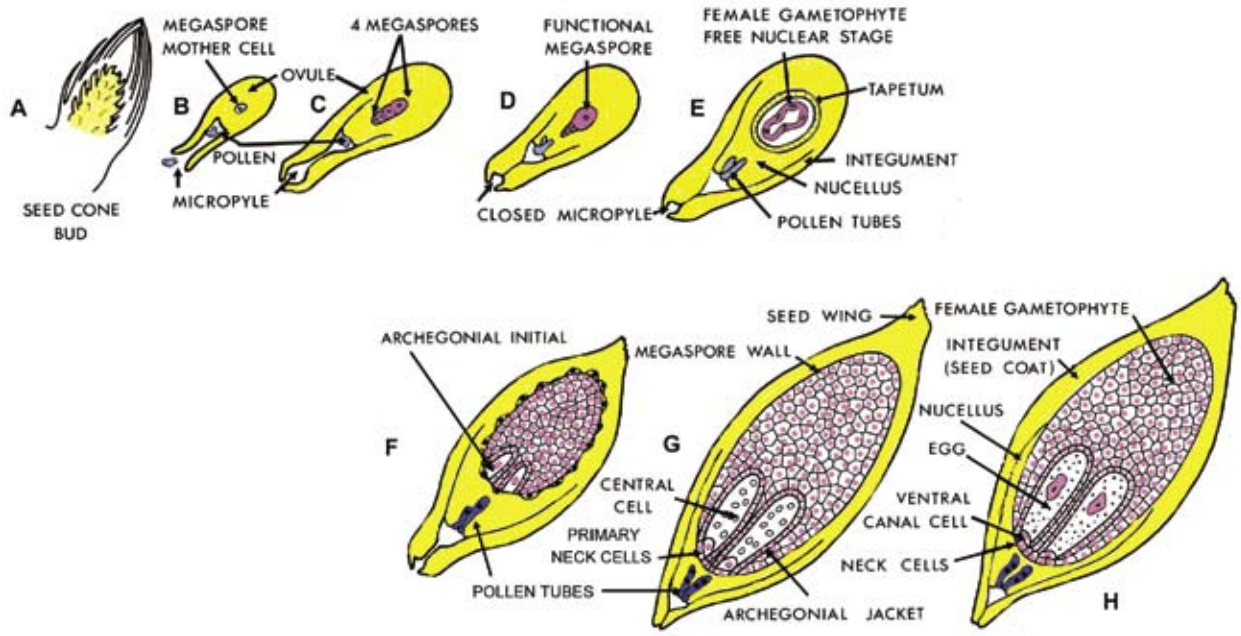


Figure 60. *Ovule and megagametophyte development in the second year when pollination occurs (A–E) and third year (F–H) when fertilization and embryo development occur.* A. *Dormant seed-cone bud.* B–E. *Post-dormancy meiosis and free-nuclear development from April through June of the year of pollination.* F–H. *Third-year ovule development, starting in April along the coast and May in the interior, and development of the cellular megagametophyte showing egg structure up to the time of fertilization in late May and late June on the coast and in the interior, respectively. Ovule and integument tissues are shown in yellow, haploid megagametophyte tissues are shown in pink and pollen and pollen tubes are blue.*

In the second year (Fig. 60 A–E), the ovules enlarge from late March through mid-August along the coast and early April through mid-July in the interior. During this time pollination occurs, pollen grains settle into the pollen chamber (Fig. 59) where they germinate and form pollen tubes (Fig. 60 D) that grow into the nucellus (Fig. 61). In the centre of the ovule, several sporogenous cells form and in the centre of these appears one enlarged megaspore mother cell (MMC) (Fig. 60 B). The MMC is comparable to the microspore mother cells in the microsporangium of the pollen cones except that there is only one MMC in the ovule. About mid-June, one or two weeks after pollination, the diploid MMC divides by meiosis forming four haploid megaspores in a row (Fig. 60 C). The outer three megaspores degenerate and the inner functional megaspore enlarges (Fig. 60 D) then undergoes a brief period of free nuclear division (mitoses without cell wall formation). This forms a sac-like structure, containing many nuclei suspended in a watery cytoplasm (Figs. 60 E, 62) within the megaspore wall. This wall is similar to the pollen wall in having an exine and intine but is thinner than the pollen wall. The sporogenous cells around the outside of the megaspore cell wall degenerate and, like the tapetum in microsporangia, function in formation of the wall. However, in the literature (Singh 1978) they are not called a tapetum, but spongy cells because of their large size and less compact arrangement compared to the outer nucellar cells. The seed cones and the free-nuclear megagametophytes within become dormant at this stage (Figs. 60 E, 62) in July or early August. Unpollinated ovules abort (Fig. 63). Dormant seed cones are brown and about 1.5 cm long (Figs. 1, 5 and 6).

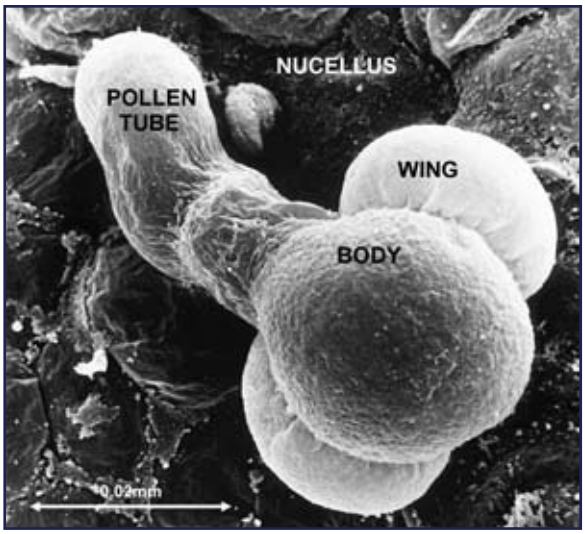


Figure 61. *Scanning electron micrograph of a pollen grain that has germinated in the pollen chamber and the pollen tube has grown into the nucellus.*

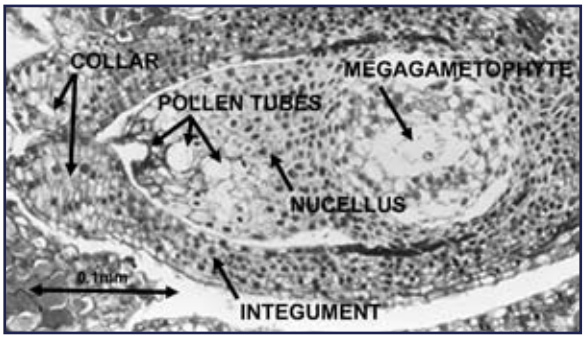


Figure 62. *Section through an ovule in June showing the early free nuclear megagametophyte in the nucellus in the centre of the ovule. A collar of cells has formed sealing the micropylar canal, and pollen tubes have grown into the nucellus tip.*

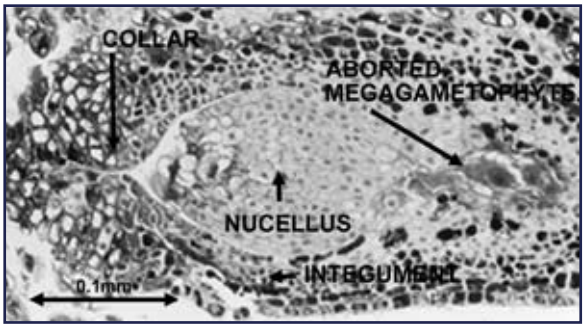


Figure 63. *Section of an unpollinated ovule in July of the year of pollination showing the aborted megagametophyte and surrounding sporogenous cells.*

In March of the following year along the coast and April in the interior, seed cones resume development and the megagametophyte is mature by mid-June (Fig. 60 F–H). The megagametophyte resumes free nuclear division, as shown in Figure 64, and development continues until sometime in May. It then enters a period of cell-wall formation. Cell walls form from the outside to the inside of the megagametophyte. These cells in turn divide, forming a few thousand small haploid prothallial cells. Several cells at the micropylar end enlarge and form archegonial initials (Figs. 60 F, 65). Each archegonial initial divides unequally forming a small primary neck cell and a large central cell. The primary neck cells form a layer of neck cells covering the entrance to the egg through which the pollen tube must pass (Fig. 66). Each archegonium is enclosed by an archegonial jacket (Figs. 60 G, 66). The central cell in each archegonium enlarges (Figs. 60 G, 67) and divides unequally to form a small ventral canal cell and a large egg cell. Other prothallial cells may become binucleate but do not accumulate starch, lipid or proteins before fertilization. This is the mature megagametophyte (Figs. 60 H, 68). Each ovule contains one megagametophyte, each megagmetophyte contains two to four archegonia, and each archegonium contains one egg.

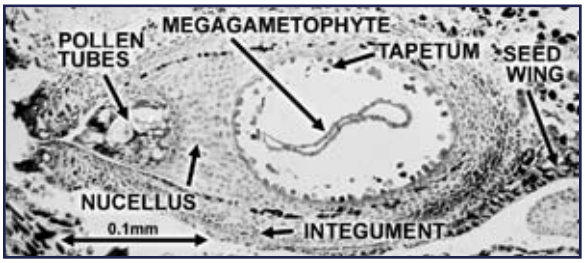


Figure 64. *Section of a pollinated ovule in August of the year of pollination, showing the flattened sac-like free-nuclear megagametophyte surrounded by cells of the tapetum and, outside that, the nucellus (see Fig. 60 E). Pollen tubes are present.*

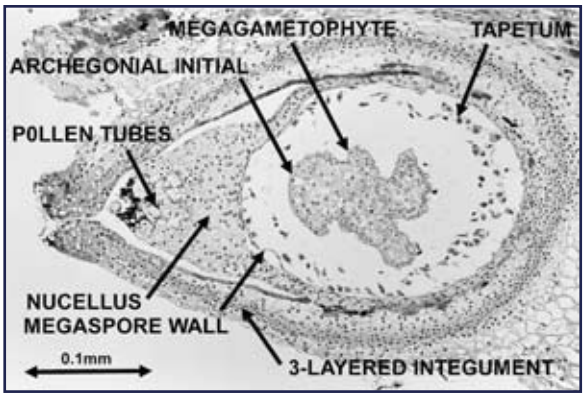


Figure 65. *Section of an ovule in May, one year after pollination, showing cellular megagametophyte with archegonial initials enclosed by the degenerating tapetum, the megaspore wall and three-layered integument.*

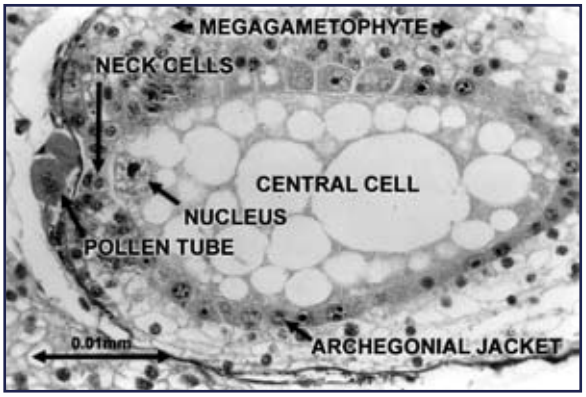


Figure 66. *Section of a megagametophyte in June, one year after pollination, showing a developing archegonium containing a large vacuolated central cell and its nucleus, and archegonial jacket and neck cells. A pollen tube is in the archegonial chamber.*

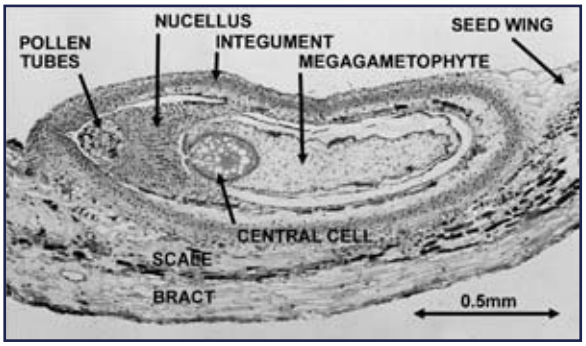


Figure 67. *Section of an ovule at the central cell stage in June, one year after pollination. Note that the integument has three layers and is attached to the seed wing.*

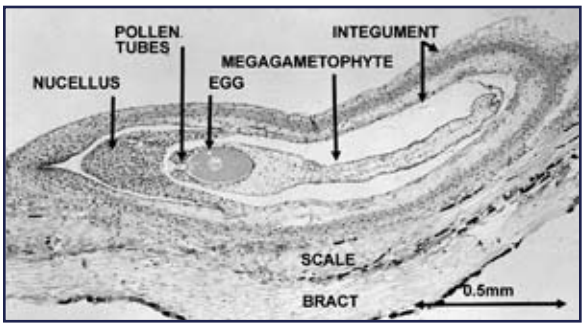


Figure 68. *Section of an ovule at fertilization in June. The megagametophyte and egg are mature and pollen tubes are in the archegonial chamber.*

In pines, during central cell and egg cell development, plastids migrate to the periphery (the outer zone) of the large cell, greatly enlarge and become transformed plastids (large inclusions in older literature; see Singh 1978). During egg development, the mitochondria migrate toward the egg nucleus and form a dense perinuclear zone (the inner zone) around the egg nucleus. Between the outer zone, containing transformed plastids, and the perinuclear zone, there is a mid-zone where there are few organelles. The mature egg is a very large complex cell. The significance of the complexity will be shown in the discussion of proembryo development and cytoplasmic inheritance.

From April until mid-June, one year after pollination, the seed cones enlarge from 1–2 cm (Fig. 6) to 3–4 cm long. Seed cones are green and usually pendant at fertilization (Fig. 69 A). Scale and ovule tissues are still soft and high in water content. The integument begins to differentiate into three distinct layers that form the seed coat and this is attached to the seed wing that is developing from the surface cells of the scale (Figs. 67, 68).

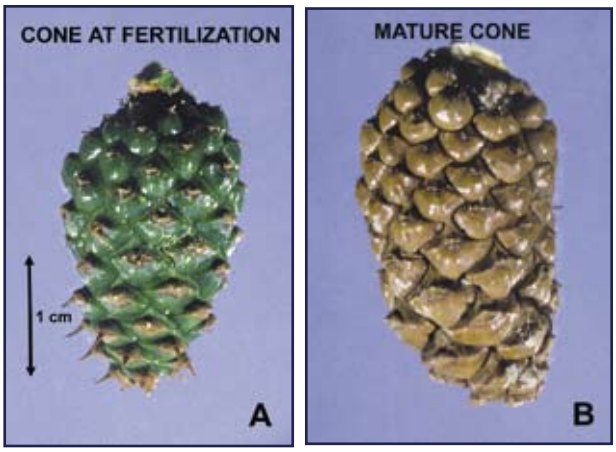


Figure 69. *Seed cones at fertilization (A), about one year after pollination, and at maturity (B) about 15 months after pollination.*

Fertilization

In late March along the coast and early April in the interior, seed cones resume development and the pollen tubes grow through the nucellus to the archegonial chamber of the megagametophyte (Figs. 60 E–H, 66, 68). In lodgepole pine, there may be up to four pollen tubes growing through the nucellus, each from a separate pollen grain. Early in pollen-tube development, the pollen tube branches in the nucellus tip and often forms an inverted funnel, the rim of which faces inward. This pollen tube is very irregular in shape with many short projections growing inward toward the megagametopgyte. After winter dormancy when pollen-tube growth resumes, usually one of these projections, the one containing the tube nucleus, grows more rapidly toward the megagametophyte forming a single pollen tube. Within the elongating pollen tube, the tube nucleus is near the tip and not far behind is the generative cell and the sterile cell. All three are suspended in the tube-cell cytoplasm. The generative-cell nucleus then divides forming two large equal-size nuclei that remain in the generative cell (Fig. 70) (Owens et al. 2005). This structure is often incorrectly called a binucleate sperm.

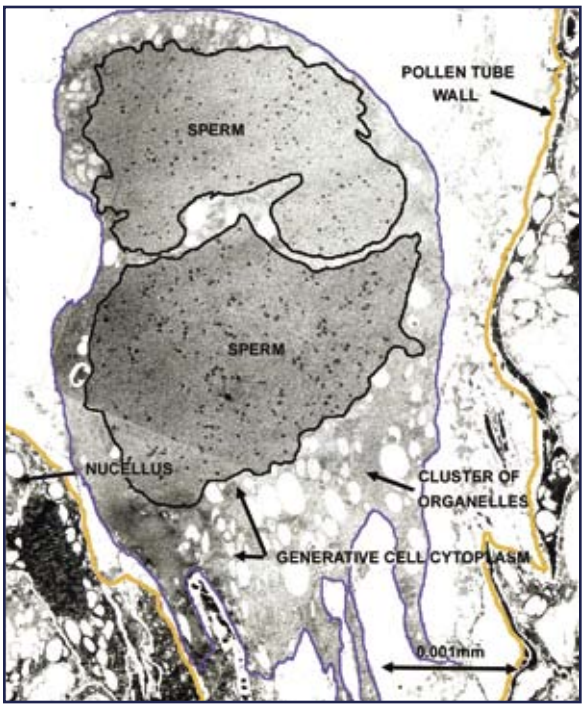
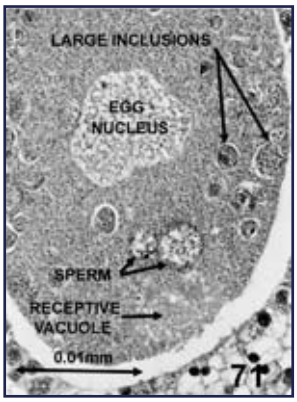


Figure 70. *Transmission electron micrograph of a portion of a pollen tube (outlined in orange) in pine showing the generative cell with its vacuolated cytoplasm and clusters of organelles (outlined in blue) containing two equal-size sperms (outlined in black). Sperms appear the same in all pines studied thus far. Compare with light micrograph in Figure 71.*

When the egg is mature about mid-June, a pollen tube penetrates between the neck cells and releases its contents into the egg. This causes one or more large receptive vacuoles to form within the egg (Figs. 71, 72). The contents released into the egg include the two sperms that burst out of the generative cell, the cytoplasm and organelles from the generative cell and pollen tube, the tube nucleus and sometimes the sterile cell. The two sperms move toward the egg nucleus (Fig. 71), usually in single file and accompanied by some of the pollen-tube organelles. One sperm soon lags behind, the leading sperm fuses with the egg nucleus (Figs. 72, 73) and the second sperm degenerates. The clusters of male (pollen-tube) organelles, including the plastids and mitochondria, intermingle with the egg mitochondria in the perinuclear zone around the egg nucleus (Figs. 73, 74 A).



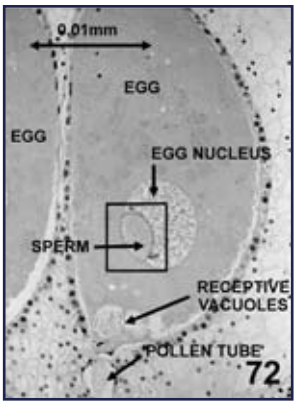


Figure 71, 72. *Sections of eggs at fertilization.* Fig. 71. *Egg showing two sperms moving through egg cytoplasm toward the egg nucleus. Apparent size difference results from the angle of the section.* Fig. 72. *Egg showing a sperm fusing with the egg nucleus and the remains of receptive vacuole and pollen tube. The square shows the approximate area that is highly magnified in Fig. 73.*

The sperm and egg nuclear membranes break down (arrows Fig. 73) and the 12 chromosomes from the sperm combine with the 12 chromosomes of the egg nucleus and the diploid (2N) condition is re-established. The diploid zygote nucleus is enclosed in dense cytoplasm (neocytoplasm) of the egg cell and contains all the maternal perinuclear mitochondria and many of the paternal mitochondria and plastids from the pollen tube. The zygote nucleus immediately divides by mitosis to form a two-nucleate proembryo enclosed in the neocytoplasm of the egg cell (Fig. 74 A).

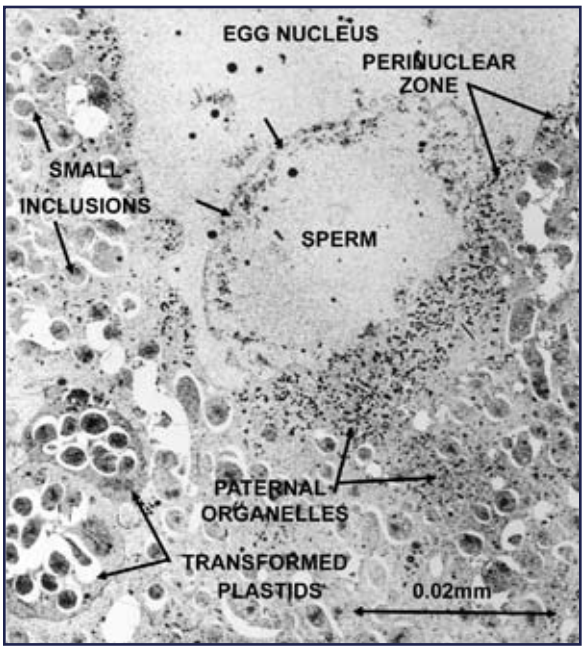


Figure 73. *Transmission electron micrograph of a sperm fusing with the egg nucleus as the nuclear membranes between them start to breakdown (arrows) and male and female organelles (small dots) intermingle. Transformed plastids and small inclusions become more conspicuous. See square in Fig. 72 for orientation.*

Cytoplasmic Inheritance

The chromosomes within the zygote and the proembryo nuclei contain most of the cellular DNA that is contributed equally—12 chromosomes from the sperm and 12 from the egg. However, the cytoplasm of egg and the cells within the pollen tube contain many small organelles. Some of these organelles, the plastids and the mitochondria, also contain small amounts of DNA that are not arranged in chromosomes. Their DNA is in fine single strands and consist of only a few genes. Plastids divide to form chloroplasts, responsible for photosynthesis in all the green tissues of the new tree and the mitochondria divide to form more mitochondria for energy release in all the living cells of the new tree. Plastid and mitochondrial DNA control the formation of many enzymes essential to the life of all the cells and the tree. But unlike the nuclear DNA, which is inherited equally from the male and female parents, cytoplasmic DNA is not contributed equally by the sperm and egg. Analyses of cytoplasmic DNA is useful for determining paternity in seeds, seedlings and mature trees.

The pattern of cytoplasmic inheritance varies among conifer families, but all of the Pinaceae that have been investigated are the same. Plastids, thus plastid DNA, are inherited from the male parent (paternally), whereas most mitochondria are inherited from the female parent (maternally). The structural mechanism by which this form of cytoplasmic inheritance occurs is seen in the formation and structure of the egg and the sperm and the fertilization process (Bruns and Owens 2000).

During egg formation all plastids are transformed (large inclusions of the older literature) and pushed to the periphery of the egg cytoplasm. At fertilization they are excluded from the new cytoplasm (neocytoplasm) that forms the cytoplasm of the embryo. Thus, maternal plastids are not usually found in any of the embryo cells. In contrast, most of the egg mitochondria migrate to the perinuclear zone, around the egg nucleus. At fertilization and the first mitoses of the new proembryo, they are incorporated into the cytoplasm of all the proembryo cells.

During pollen-tube growth through the nucellus, sperm formation occurs by mitosis of the generative cell nucleus but the two sperm nuclei remain within the generative cell. The generative cell cytoplasm surrounding the two sperm contains many plastids and mitochondria. At fertilization, these paternal organelles, containing paternal DNA, move to the egg nucleus with the leading sperm. When the neocytoplasm forms for the new proembryo, all of the plastids and about 10% of the mitochondria come from the male parent. The proportion of mitochondria in the new proembryo that are of paternal origin is variable because not all paternal mitochondria may reach the egg nucleus (Owens and Bruns 2000, Bruns and Owens 2000).

Molecular studies of the Pinaceae, using restriction fragment length polymorphisms (RFLPs), confirm this mechanism. From the information we have now, in the Pinaceae and most of the other

families of conifers, plastids are inherited paternally and mitochondrial inheritance is biparental. However, in the Cupressaceae and Taxodiaceae, both plastid and mitochondria are inherited paternally, whereas, in flowering plants, including hardwood trees, both are inherited maternally (Bruns and Owens 2000). The significance of this type of cytoplasmic inheritance will not be fully appreciated until we know what genes are present in conifer plastids (cpDNA) and mitochondria (mtDNA) and what they regulate physiologically and developmentally during the life of the tree. So far, molecular studies of mitochondrial DNA inheritance in the pines has been done for lodgepole pine, jack pine and their hybrids (Wagner et al. 1987, 1991). Studies of plastid DNA have been done for western white pine (White 1990).

The Embryo

Conifer embryo development (embryogeny) is commonly divided into four stages: proembryo, early-embryo, mid-embryo and late embryo. The proembryo includes the stages of development from the zygote (Fig. 74 A) through a four-tier, 16-cell stage (Figs. 74 B–D). The proembryo stages take place within an archegonium and end when the suspensors elongate and push the apical tier of cells out of the archegonium and into the megagametophyte tissue (Fig. 74 E). The early embryo includes elongation of the suspensor system, cleavage and the formation of a multicellular, club-shaped embryo in which the first specific meristems form that produce the major plant organs (shoot and root) (Fig. 74 E–H).

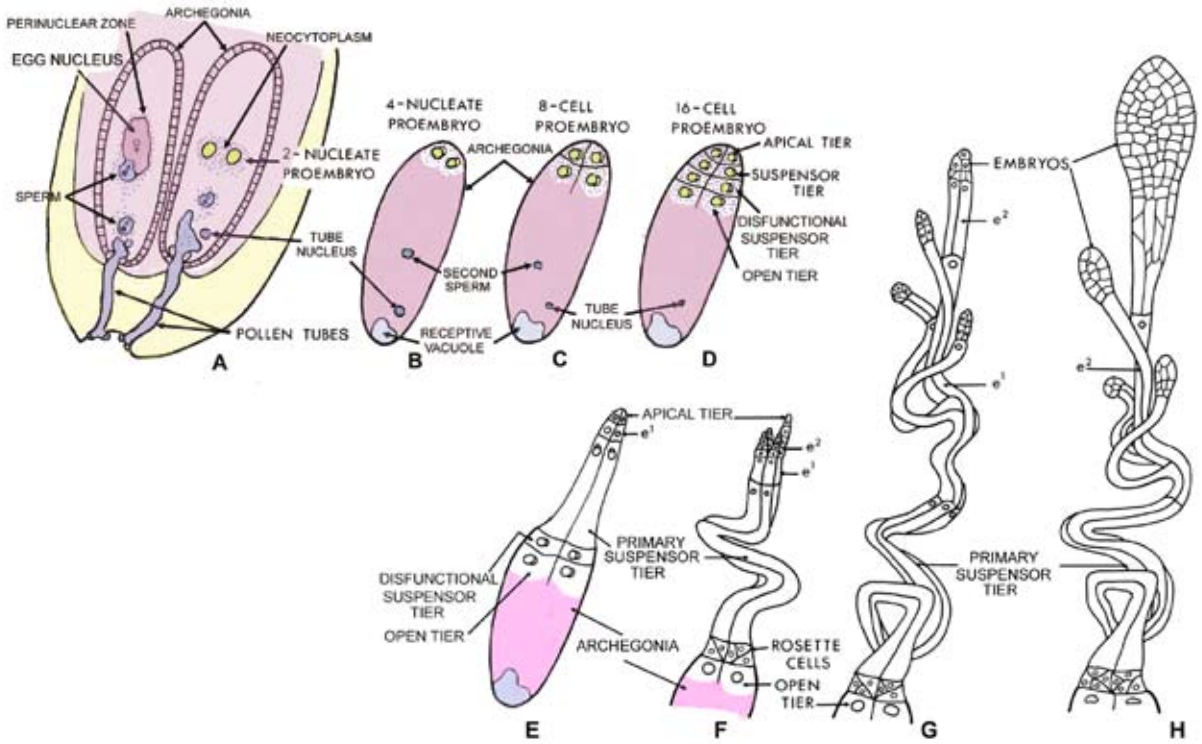
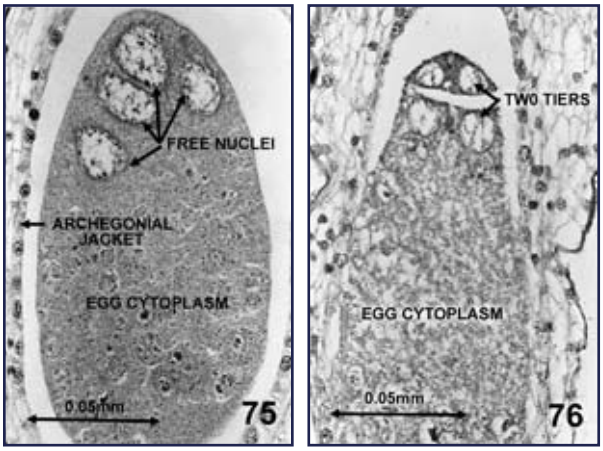


Figure 74. *Fertilization, proembryo and early embryo development.* A. *At fertilization the two sperms and their organelles (blue) enter the egg cytoplasm creating a receptive vacuole and one sperm and organelles from the pollen tube fuse with the egg nucleus. The maternal and paternal organelles form the neocytoplasm around the zygote nucleus. The zygote nucleus divides forming two free nuclei.* B. *The two free nuclei divide to form four free nuclei that migrate to the distal (chalazal) end of the egg cytoplasm.* C. *Mitosis of all four free nuclei is followed by cell-wall formation to produce a two-tier, eight-cell proembryo.* D. *Mitosis and cell division of the eight cells form a four-tier, 16-cell proembryo. The lower open tier remains open to the egg cytoplasm.* E. *The suspensor tier elongates, forcing the apical tier through the archegonial jacket and into the megagametophyte. This ends the proembryo stage. The apical tier then divides to form the apical cells and the embryonal tubes (e).* F. *Cleavage polyembryony occurs when the apical cells and the embryonal tubes elongate and separate forming four files of cells.* G, H. *Apical cells divide forming multicellular early embryos that are pushed further into the megagametophyte by the elongating embryonal tubes and suspensors that begin to coil. Some of the early embryos begin to degenerate and usually only one becomes dominant and survives to the mid-embryo stage.*

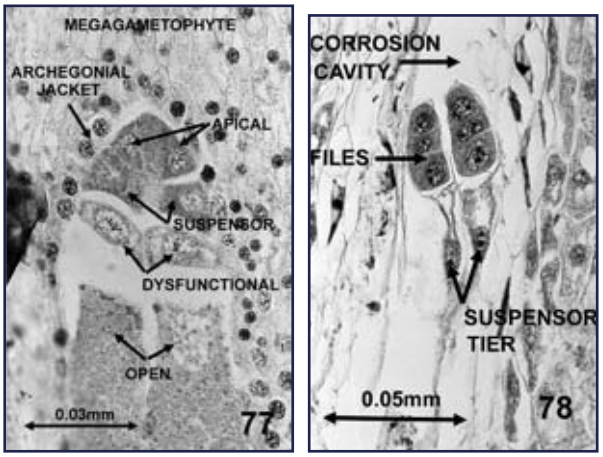
The mid embryo includes the rapid enlargement of the embryo and the formation of the root or radicle, shoot and cotyledons. The late embryo includes the completion of development of these structures and their maturation—the accumulation of storage products and the dehydration of the embryo and megagametophyte (Singh 1978, Owens et al. 1982, 1993).

The zygote nucleus divides immediately by mitosis forming two nuclei in the centre of the egg but no cell walls form. This is the two-free-nucleate stage (Fig. 74 A) in which the nuclei are enclosed in dense neocytoplasm made up of many maternal and paternal organelles. These two free nuclei divide simultaneously by mitosis forming four free nuclei in the centre of the egg. The four free nuclei and their enclosing neocytoplasm migrate to the chalazal end (opposite of the micropylar end) of the archegonium (Figs. 74 B, 75). Each nucleus divides by mitosis and two tiers of four nuclei each form. Cell walls quickly appear resulting in a two-tier, eight-cell proembryo (Figs. 74 C, 76). The four cells in each of the two tiers divide simultaneously producing a four-tier, 16-cell proembryo. The four tiers are the apical tier, the suspensor tier, the dysfunctional suspensor tier (sometimes called the rosette tier) and the basal open tier (Figs. 74 D, 77). The open tier remains open to the egg cytoplasm and may be the means by which materials are taken into the proembryo from the egg cytoplasm.



Figures 75, 76. *Sections of archegonia showing proembryo development.* Fig. 75. *Four free nuclei have migrated to the distal end of the archegonium (see Fig. 74 B).* Fig. 76. *Two-tier, eight-cell proembryo (see Fig. 74 C).*

The suspensor tier elongates and pushes the apical tier through the archegonial jacket and into the megagametophyte, ending the proembryo stages and starting the early embryo stages. Each apical cell divides to form embryonal tube cells below (Fig. 74 E–F). A corrosion cavity forms in the megagametophyte just ahead of the apical cells (Fig. 78). The cavity results from a breakdown of the megagametophyte cells and the subsequent utilization of its stored food for the developing embryo.



Figures 77, 78. *Sections of a proembryo and an early embryo.* Fig. 77. *Four-tier, 16-cell proembryo in the archegonium (see Fig. 74 D).* Fig. 78. *Early embryo in the corrosion cavity showing the first stages of cleavage between the four files of cells (see Fig. 74 F).*

Most conifers undergo one or two types of polyembryony—a process by which several embryos can develop within one ovule (Singh 1978). In pines, both types of polyembryony take place. "Simple polyembryony" occurs when more than one egg is fertilized within an ovule. Lodgepole pine has two to four archegonia and takes in no more than four pollen grains per ovule. There are usually only one to three fertilizations per ovule. The eggs in different archegonia must be fertilized by sperm from different pollen tubes. There are usually only two or three genetically different zygotes and proembryos formed per ovule. These genetically different embryos may compete during early development and usually only one develops past the early embryo stage.

Pines also undergo "cleavage polyembryony." This occurs after the primary suspensors elongate

forcing the apical cells into the megagametophyte. The apical tier then separates into four files of cells (Figs. 74 E–G, 78). Each file from one proembryo may develop into a separate but genetically identical embryo to the other three derived from the same proembryo. Despite their nuclear genetic identity, one of these early embryos soon becomes more vigorous and larger, and the other three degenerate (Figs. 74 G, 79). In a lodgepole pine ovule having three archegonia that are all fertilized, there would be three early embryos having different genotypes as a result of simple polyembryony. After cleavage there would be 12 early embryos but still showing only three different geneotypes. Of these 12 early embryos, usually only one will fully develop or all may abort.

Simple polyembryony allows for genetic selection during early embryo development in the seed. The selective advantage for cleavage polyembryony remains largely a mystery. It simply may be that the cleavage of one early embryo into four genetically identical early embryos increases the chance of one surviving until maturity even though their nuclear genomes are identical. Embryo development is a complex genetically and physiologically directed process in which the fundamental tissues of the tree form. Early embryo abortion is extremely common in conifers and a large number of early embryos per ovule might ensure the production of more viable and well-adapted seeds. In pines, usually only one embryo survives per seed but twins sometimes occur. If they do, whether they are identical or fraternal twins can only be determined using molecular techniques.

In the early embryo, following cleavage, the embryonal tube cells divide and elongate pushing the apical cells further into the megagametophyte. The apical cells divide to form a file of cells (Fig. 74 E, F) and these divide in all directions to form a small multicellular embryo (Fig. 74 G). The suspensor cells and embryonal tube cells coil as they elongate (Fig. 74 G, H) pushing the embryos deep into the megagametophyte (Fig. 79). Cell divisions within the multicellular embryo cause it to enlarge forming a club-shaped embryo (Fig. 74 H). At first, cell divisions within the club-shaped embryos are rapid and in all directions, then some of the cells become mitotically less active and the directions of cell divisions become more restricted. As a result, the embryo elongates, polarity becomes established and meristems begin to form. This is the start of the mid-embryo stage (Fig. 80).

Development from fertilization through the early embryo stages takes only about three weeks, from mid-May until early June along the coast and from mid-June to early July in the interior. During this time, megagametophyte cells around the embryos lose their cytoplasm, become translucent and collapse, forming the corrosion cavity. Stored food is absorbed from these cells by the developing embryos. Archegonia also collapse creating a small cavity in the megagametophyte at the micropylar end of the seed. Most megagametophyte cells begin to transform the soluble stored materials into less-soluble lipid and protein bodies (Figs. 79, 80).

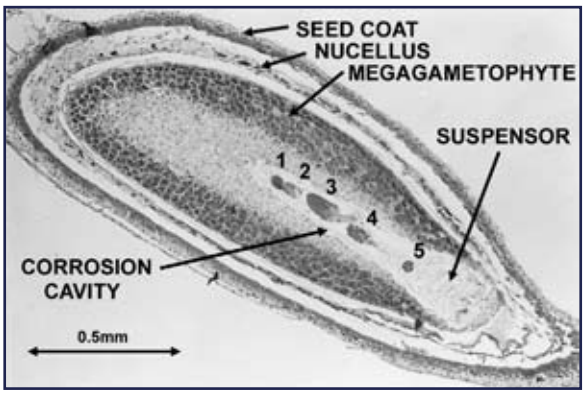


Figure 79. *Longitudinal section of a lodgepole pine ovule showing five early embryos, resulting from simple and cleavage polyembryony, in the corrosion cavity of the megagametophyte. The seed coat is well differentiated.*

The mid-embryo stage lasts only a few weeks. The first meristem to form is the rib meristem that separates the embryo into proximal (basal) and distal regions. The proximal region forms the root cap, contributes additional cells to the suspensor system and forms the root generative meristem that is responsible for all further root development. The distal region forms the hypocotyl-shoot axis that has several meristematic regions responsible for the formation of all the tissues in the

hypocotyl, shoot and cotyledons (Fig. 80). This is a period of rapid elongation and formation of a cylindrical embryo that, because of this shape, is sometimes called the torpedo stage.

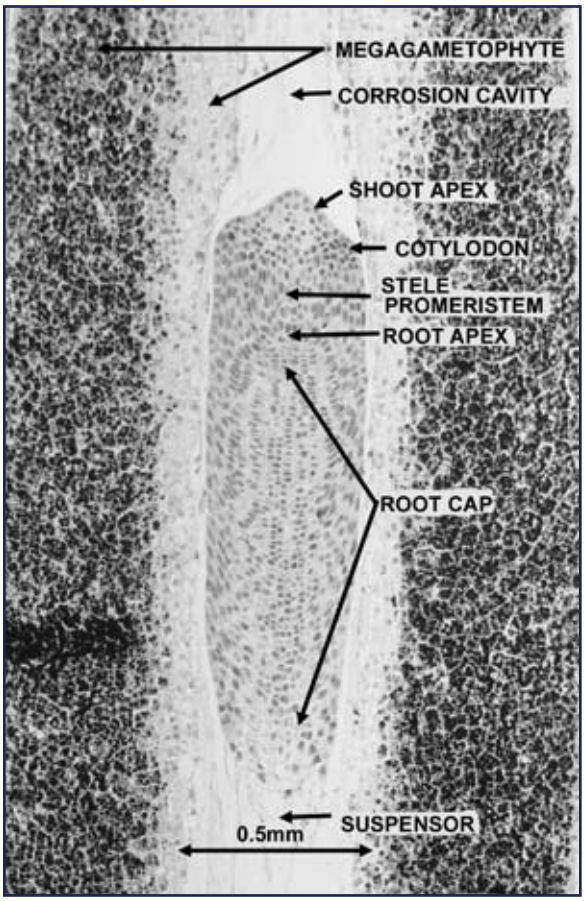


Figure 80. *Longitudinal section of a pine embryo at the end of mid-embryo and the start of late-embryo development. A long root cap has formed below the root apex, the stele promeristem is short and the small shoot apical meristem (apex) and cotyledon primordia are at the top. Megagametophyte cells have formed abundant small grey lipid bodies and large black protein bodies that are not yet present in the embryo.*

The late-embryo stage is characterized by the formation of more specific meristems for different structures and differentiation of some tissues. The root apical meristem forms as a small lens-shaped region of initial cells surrounded by mitotically active cells. These active cells produce cells toward the base forming the root cap. The tip of the root cap is continuous with the suspensor system. The root cap elongates during embryo development and occupies about one-third of the length of the mature embryo. Above the root apical meristem is the stele promeristem. It forms a central core of cells, the embryonic stele, that develops into a central column of pith surrounded by a cylinder of procambium. Outside the procambium is a cylinder of embryonic cortex and outside that a single layer of protoderm that forms the epidermis. At the tip of the hypotocyl-shoot axis is a dome-shaped group of cells that form several small primordia (Fig. 80), each of which develops into a cotyledon. The ring of cotyledons leave a small shoot-apical meristem in the centre (Figs. 81, 82). The shoot-apical meristem contributes no cells to the formation of the hypocotyls-shoot axis or cotyledons during embryo development. It only becomes active at germination when it forms the primary needles and stem tissues of the seedling.

The mature embryo is divided about equally in length into the root-apical meristem and root cap, the hypocotyl-shoot axis, and the cotyledons (Figs. 81, 82). During late embryo development, storage products become very abundant in the megagametophyte and to a lesser extent in most tissues of the embryo.

Storage products that began to form in the megagametophyte and embryo during the early embryo stages continue to accumulate through the late-embryo stages. The main storage products are lipid bodies that fill most of the cells. These usually surround large protein bodies. Both are only visible microscopically using specific histochemical stains. Both obscure most other cell contents (Owens et al. 1993). Starch is not common but occurs in collapsing megagametophyte cells around the corrosion cavity and in the root cap of the embryo.

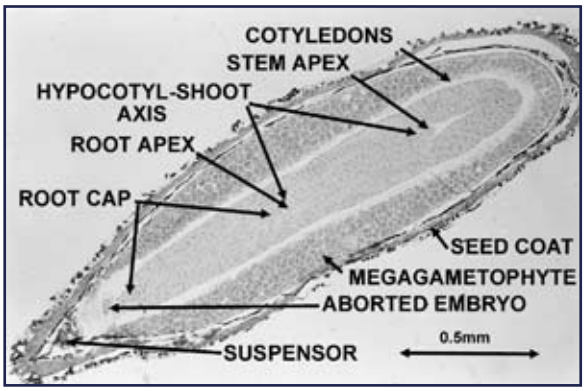


Figure 81. *Longitudinal section of a mature lodgepole pine seed.*

The Seed

A seed is a mature fertilized ovule and consists of a highly differentiated integument that forms the seed coat, a thin nucellus and inside that, a thin megaspore cell wall (membrane). All of these layers enclose the central megagametophyte that contains the embryo (Figs. 82, 88). The seed coat (testa) develops from the integument and differentiation into three layers begins before fertilization (Figs. 67, 68). The megaspore wall lines the inner surface of the seed coat and encloses the megagametophyte. It differentiates from the original megaspore cell wall, the surface area of which enlarges several hundred times during megagametophyte growth (Singh 1978). It is similar in formation, structure and chemistry, but thinner than the pollen wall. Megaspore wall formation involves the degeneration of the surrounding sporogenous cells that release their contents as they degenerate. Sporogenous cells appear to have a function similar to the tapetum in microsporangia but the details of this development have not been determined for conifers. These complex compounds, consisting of many lipids and proteins, move to the outer surface of the megaspore wall causing the wall to thicken as it forms a lightly sculptured exine and a thin intine.

Most of the nucellus becomes very thin as the seed develops (Figs. 79, 82, 88), and in the mature seed it is very thin (Fig. 82) and may only remain over the micropylar half of the megagametophyte. The nucellus at the micropylar end of the seed may become thick and, with the coiled and degenerated suspensor system, form a nucellar cap or "plug"

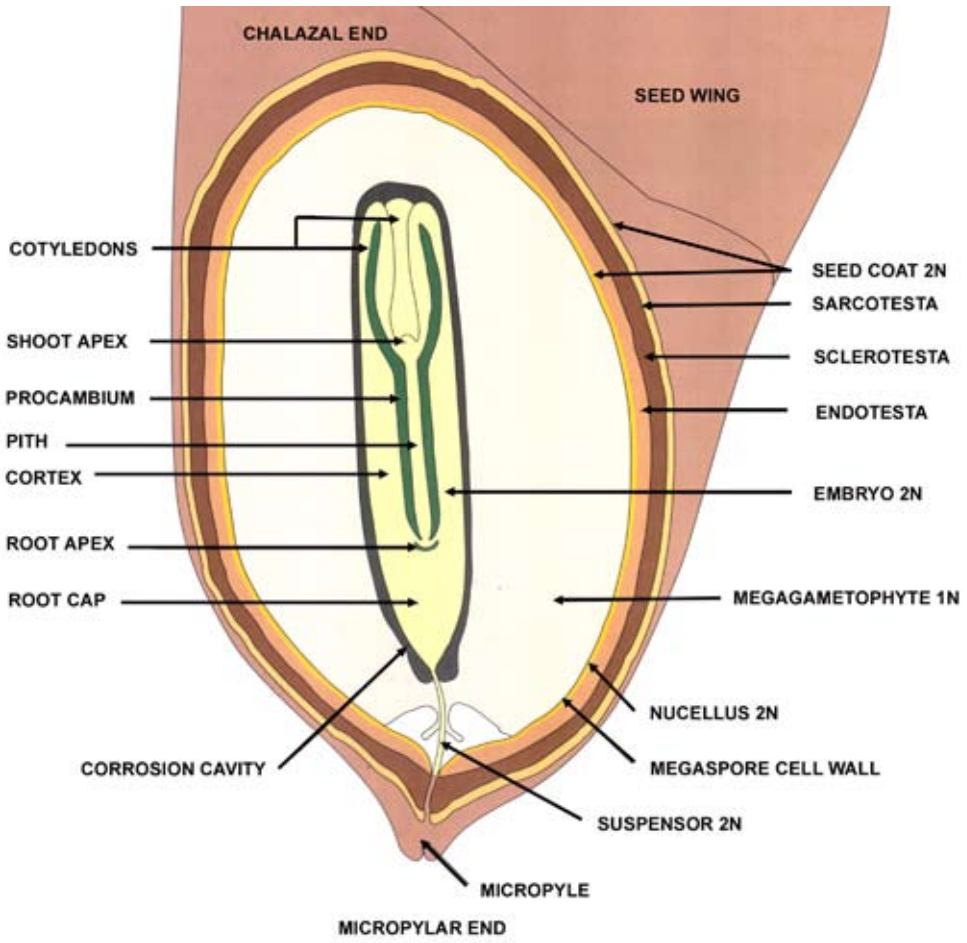
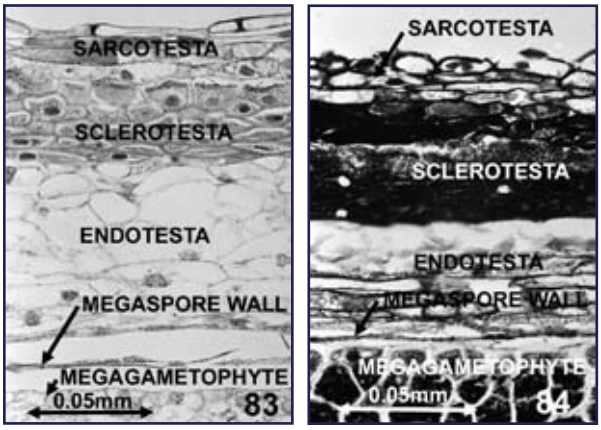


Figure 82. *Diagram of a mature seed showing the layers of the seed coat, the megagametophyte, and the embryo in the corrosion cavity. (Modified from Kolotelo, 1997.)*

(Fig. 88). This cap and the megaspore wall may serve as an impediment to germination in some species (Kolotelo 1997).

The integument has three layers that become visible during early megagametophyte development (Fig. 65). Cell differentiation within these three layers begins about the time of fertilization (Figs. 68, 83). Lodgepole pine seeds have the same three layers as in other conifer seeds:

1. The outer layer (sarcotesta) is thin, dark coloured, with a waxy cuticle;
2. The middle layer (sclerotesta) is thick and hard; and,
3. The inner layer (endotesta) is thin and lies just outside the nucellus (Figs 83, 84).



Figures 83, 84. *Sections of developing pine seed coats.*
Fig. 83. *Early differentiation of seed coat layers at fertilization.*
Fig. 84. *The same layers shown in the mature seed.*

The outer sarcotesta consists of several layers of parenchyma cells that contain tannins and give the seeds a dark brown to black colour. The outermost cell layer has a waxy cuticle and is somewhat impermeable to water until dried or scarified. The sarcotesta becomes loosely fused with the seed wing. The middle sclerotesta is the thickest layer and consists of hard woody sclerids that are dead when they are mature. This is the layer that gives hardness to the seed coat and protects the delicate megagametophyte and embryo from physical damage, insects and disease. Sclerids are hydrophilic and swell and soften during hydration of seeds. The endotesta is thin and papery, consisting of several layers of thin-walled parenchyma cells that contain few tannins. It may have little function in mature seeds (Fig. 84).

The seed wings in pines, as in other members of the Pinaceae, develop from the ovuliferous scale and not the integument of the ovule. In some pines it may be absent (pinyon pines), in others it may be loosely attached to the ovule, and in others firmly attached to the ovule. In lodgepole pine, the seed wing is loosely attached to the mature seed (Fig. 85). The seed wing begins to develop from the surface cells of the scale before fertilization. The wing tissue is forced up from the surface of the scale and attaches to the chalazal end of the seed. The wing consists of only two or three layers of cells through most of its length. There is a specialized separation layer between the wing and the scale that also extends beneath the seed separating it from the scale. The wing is thickest next to the seed.

As the seeds and wings mature and dry, the separation layer breaks down, in the same way as the abscission layer at the base of a leaf, and the wings and seeds are no longer fused to the scale. Because seed wings begin to develop very early, unfertilized seeds also have wings and many poorly developed or non-pollinated seeds have very small wings. Some scales may have small wings with no ovules. The function of the seed wing is to slow the rate of fall of the seed from the open cones allowing the seeds to be carried further by wind.

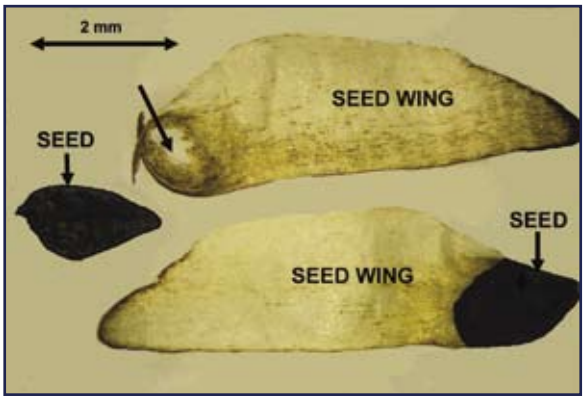


Figure 85. *Seeds attached (below) and detached (above) from seed wings. Arrow (above) shows the position of seed. (From Kolotelo 1997.)*

Serotiny

Seed cones mature in late July to early August along the coast and early August to early September in the interior, depending on the site and the summer weather during seed and cone development. Seed cones turn brown as they mature (Figs. 1, 86). Seed cones of shore pine and most lodgepole pine growing in coastal regions of BC begin to open in late summer as they mature and dry. Lodgepole pines growing in the interior tend to be serotinous, meaning that mature cones do not open unless exposed to high temperatures from prolonged high summer and fall temperatures or due to fire or insect damage. Most serotinous cones eventually open but this may take several years. The degree of serotiny appears to be mediated physiologically, but genetically and environmentally controlled (Gauthier et al. 1993)—some clones are more serotinous than others when grown at the same site and serotinous clones grown in milder coastal climates lose some degree of serotiny. Serotiny results partly from development of abundant woody tissues at the base of the cone scales. When dried, these tissues shrink and mechanically reflex the scales opening the cone. Also, additional resin secretions produced during cone maturation stick scales together. High temperatures affect both the drying and reflexing of scales and the melting of sticky resin.

Lodgepole pine (subspecies *latifolia*) is an aggressive pioneer species in interior regions and serotiny is the most significant causal factor that appears to be largely an evolutionary response to frequent destructive fires (Lotan et al. 1985). It is absent in areas where lodgepole is not a fire-related pioneer species. Fire-initiated stands contain more closed-cone trees (Muir and Lotan 1985). Therefore, it is important for the forest manager to understand the population structure of lodgepole pine regarding serotiny. Non-serotinous clones probably should not be planted in fire-prone areas.

Figure 86. *Mature serotinous cone of lodgepole pine.*

Seed Quality

Seed quality is a measure of several traits including whether seeds are filled, partially filled but aborted, incompletely developed, insect-damaged or disease-damaged. Ultimately, the most important criteria are if the seeds store well and germinate well. Determinations of seed quality must take into consideration that in pines seed development occurs over about 15 months and is interrupted by 8 or 9 months of late summer through fall and winter dormancy. A decrease in seed quality and loss of filled seeds can occur at any time during this long developmental period (see Fig. 5). Early ovule and cone abortion may occur during the first year, between the time of pollination and late-summer dormancy. Ovules during this developmental period are small and undergoing early free-nuclear division which is the beginning of the formation of the haploid megagametophyte. Abortion during this time may result from a lack of ovule pollination, frost, early self-or other incompatibility mechanisms and insect damage. Ovules that abort at this time remain as small rudimentary seeds. They are called seeds because they have a thick, three-layered seed coat but usually contain only a small aborted nucellus and megagametophyte.

During the first months of the second year of cone development in pines (April through mid-June), ovules complete free nuclear division then a few weeks before fertilization, cell walls form and the separate cells divide forming a mature cellular megagametophyte consisting of several thousand cells. These are cells that have large central fluid-filled vacuoles but they lack solid lipid and protein storage bodies. Ovule abortion may occur during this time as a result of developmental anomalies, early incompatibility mechanisms and insect- or disease-damage. This results in an array of aborted, full-sized seeds with megagametophytes and seed coats at various stages of development. These have often been called "empty seeds" because the fluid-filled megagametophyte collapses into a thin brown sac made up of the megaspore cell wall but it contains no living megagametophyte cells, no solid storage products and no embryos (Owens et al. 1993).

Abortion may also occur from the time of fertilization through seed maturation. For about the first two weeks after fertilization, embryos are very small and there are no solid storage products in the megagametophytes or embryos. This is the time when most self-fertilized megagametophytes abort leaving only a collapsed brown sac—the megaspore wall. These are the "empty seeds" referred to in the older literature (see review by Dogra 1967). After this initial stage of embryo development, lipid and protein bodies develop within the cells of the megagametophytes and embryos. Abortion during this developmental period may result from developmental anomalies, late incompatibility mechanisms and insect- or disease-damage. Abortion may result in embryo and/or megagametophyte degeneration but there are some remnants of these within the seeds. The remains from both pre- and post-fertilization abortions may appear in sliced seeds as coloured fluid material, collapsed and dried brown material, white or cream-coloured partially developed megagametophyte or embryo tissues, resinous crystalline material, or the seed coat may incase and insect, the remains of an insect or insect fras. Rarely is the seed coat truly empty, unless the seeds have been fixed and embedded for sectioning and microscopic observation. When this is done, aborted, dead or dieing megagametophytes and embryos do not preserve well with chemical fixatives and cannot be properly infiltrated with the embedding medium, thus they simply fall out during the sectioning or staining processes. It is from such processed seeds that the term "empty seed" was originally used and not for fresh dissected seeds (Dogra 1967).

The use of the term "empty seed" can be traced back to the research on pine by Dengler (1932) as referenced by Dogra (1967) and Singh (1978) but in the early literature it was used specifically to describe collapsed megagametophytes that resulted from self-fertilization, based on studies of seed development and structure, not on radiographs of mature seeds. Over the years, the term "empty seed" has often been incorrectly used and by the time of Dogra's (1967) thorough literature review it was already being used for certain other stages of aborted seeds but still in the context of self-fertilization. More recently, "empty seeds" has been used for almost any seed that is not filled or presumed viable or obviously insect-damaged as determined from radiographs of mature seeds. Radiographs, photos taken using x-rays, are not good for soft animal tissues or plant tissues such as seed contents. Once the x-ray beam has penetrated the 15 to 20 cell layers of the seed coat, most of which are dead and heavily sclerified, subtle differences in the soft inner tissues (megagametophyte and embryo) cannot be distinguished. Radiographs are not clear enough (Bates et al. 2001), especially if seeds are hydrated, to clearly distinguish differences in post-dormancy aborted seeds. Consequently, radiographs are often used only to distinguish filled from deteriorated seeds or to identify mechanical damage to the seed coat and some insects (i.e., *Megastigmus* sp.) (Kolotelo 1997). But, unfortunately there are many categories of deteriorated seeds resulting from different causes. An equally fast, cheaper and more accurate method for determining seed quality and causes of seed loss are those described by Kolotelo (1997). This simple cutting test has been used

for lodgepole pine (Owens et al. 2005) and other conifers in which mature cones are dried, all seeds removed and each seed placed on masking tape attached to a small board (Fig. 87). Each seed can be quickly sliced (Fig. 88) with a razor blade and the contents observed using a dissecting microscope. Seeds may then be placed in one of several seed categories (Owens et al. 2005) that can be related to the time and possible cause of the seed loss, rather than classifying them as simply filled or empty.

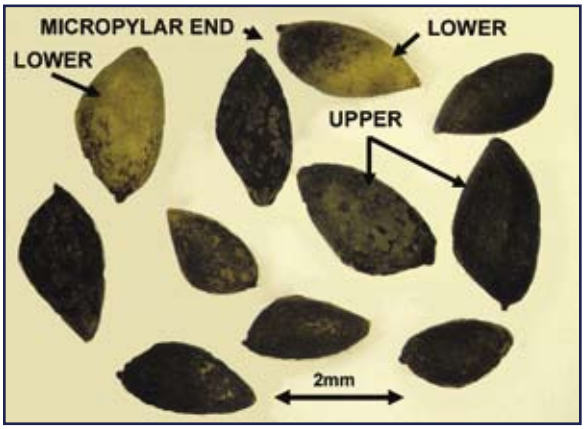


Figure 87. *Dewinged lodgpole pine seeds showing light-coloured lower and dark upper surfaces. (From Kolotelo 1997.)*

Kolotelo (1997) uses the terms healthy and deteriorated seeds and categories within each of these based on the cutting test. This is a good method for conifer seed analysis. In sliced healthy lodgepole pine seeds, the seed coat appears brown to black, the megagametophyte cream to white and the embryo yellow. The embryo is only slightly shorter than the seed (Fig. 88). The seed coat may appear the same in both healthy and deteriorated

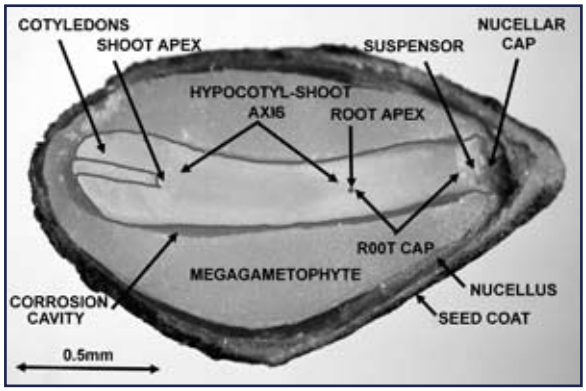
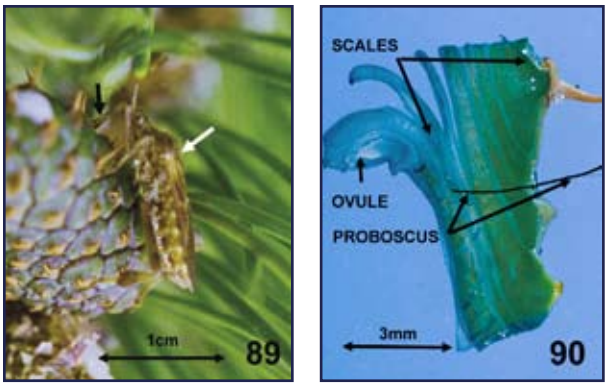


Figure 88. *Mature healthy lodgepole pine seed sliced with a razor blade to show easily visible structures.*

seeds because deterioration did not begin until after the seed coat was fully formed, but often the megagametophyte is various colours and the embryo is smaller in the latter. Deteriorated seeds may contain disease or saprophytic organisms (fungi or bacteria), or result from self-pollination that has caused reduced embryo growth but not embryo abortion (Kolotelo 1997; Kolotelo et al. 2001; Owens 2005). Other seeds may be very small (rudimentary) and result from early abortion in the year of pollination. Many seeds may be normal in size and external appearance but when sliced contain a dry, collapsed sac-like megagametophyte. In pines and other conifers (Dogra 1967 and references therein), these commonly result from incompatibility mechanisms causing the megagametophyte to abort before fertilization (Runions and Owens 1998) or self-incompatibility in which all early embryos abort soon after fertilization (Owens et al. 2005 and references therein). Still other seeds may have insect damage in which the larva is still present in the seed or has emerged leaving a hole in the seed coat or an external insect has fed on the contents of the developing seed.

Damage from the insect *Leptoglossus* feeding on developing cones and seeds may be very serious in lodgepole pine on certain sites in some years (Krugman and Kroeber 1969; Hedlin et al. 1980; Bates et al. 2000; Strong et al. 2001). *Leptoglossus occidentalis*, the western conifer seed bug, is 15–18 mm long and reddish-brown to grey (Fig. 89). It may feed on first year seed cones and cause them to abort (Hedlin et al. 1980) but is more commonly known to feed on seeds from before the time of fertilization until cones are nearly mature by inserting a long thin stylet into the cone scales (Fig. 90) and into the seeds (Krugman and Koerber 1969). The insect secretes enzymes out through the hallow stylet then sucks the digested contents from the megagametophyte usually causing it and, if present, the embryo within to abort and collapse (Bates et al. 2000). This feeding may also introduce micro-organisms into the developing seeds. In a lodgepole pine seed orchard in 1997 (J.N. Owens, unpublished data), in which cones were not enclosed in nylon mesh



Figures 89, 90. Leptoglossus *feeding.* Fig. 89. *An adult insect (white arrow) feeding with its proboscis (black arrow) on a lodgepole pine cone at fertilization.* Fig. 90. *A portion of a seed cone at fertilization sliced and stained to show the proboscis penetrating into the scale and close to the ovule.*

bags and *Leptoglossus* was abundant, there were many seeds that aborted during pre-fertilization megagametophyte development, before storage protein and lipid bodies, or early embryos had formed. These seeds contained spongy or fluid material that usually did not fill the seed cavity and varied in colour from white, cream, yellow, orange to rust but they contained no remains of embryos. These were interpreted as megagametophytes that had aborted before early embryo development and the variable colour may have resulted from the secretion of enzymes into the seeds (Bates et al. 2000) by *Leptoglossus* or from various micro-organisms introduced into the seeds, possibly by *Leptoglossus* feeding. Some of these early aborted seeds were indented (Fig. 91), apparently because they were damaged before the seed coat was fully developed (Kolotelo 1997). Later seed damage by *Leptoglossus* is described by Bates et al. (2000) using feeding experiments on mature Douglas-fir seeds and radiographic analyses of the damaged seeds. Feeding on mature seeds left no obvious external damage to the seed coat but from the radiographs the internal damage looked similar in form to seeds in which megagametophyte and embryos had aborted or development was retarded due to incompatibility mechanisms or unspecified developmental anomalies (Owens et al 2005). However, from radiographs the internal texture or colour of the contents of the seeds could not be determined.

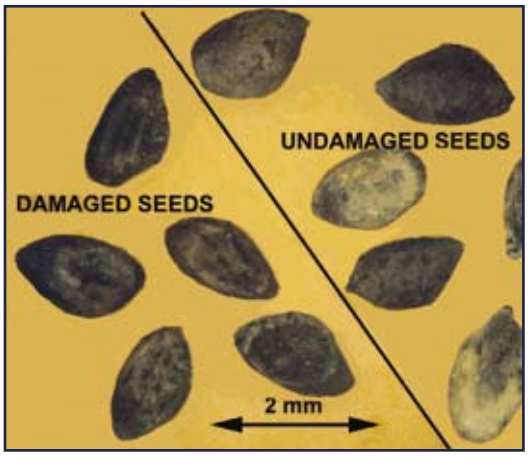


Figure 91. *Mature lodgepole pine seed damaged by* Leptoglossus *feeding as indicated by the indented seed coat. (From Kolotelo 1997).*

Cone and Seed Production in Natural Stands

Cone and seed production in lodgepole pine is not as cyclic as it is in many other conifers. Failure to initiate cones in one year is one cause of low or no cone production 27 months later (see Fig. 5). However, in most mature pines, some cone buds are initiated every year and a main cause for reduced cone production is the abortion of cones soon after pollination, due to low pollination success or frost at pollination. Cone drop soon after pollination is commonly observed, especially in coastal areas, but this has not been measured in interior natural stands of lodgepole pine. For interior lodgepole pine, serotiny may result in several years of unopened cones that accumulate on trees in a stand. Release of the seeds from these cones, usually due to fire, results in a massive shedding of seeds at one time.

The first seed cones may appear on trees as young as five years old and seeds from these cones are as viable as those from older trees (Fowells 1965). Consequently, the total seed production for the lifetime of a single lodgepole pine tree is very high but seeds may be released in large quantities only every few years. It is not uncommon for mature trees to bear several hundred cones produced in different years but each cone may not produce many seeds. Filled seeds per cone may be five or

less, but may be up to 30 per cone where pollination success is high, and self-pollination, insect and disease damage are low.

Although the lodgepole pine seeds are small with small wings, they are relatively heavy, thus they disseminate short distances. In still air seeds fall at a rate of about 0.8 metres per second, and most seeds are scattered within 50–90 m from the tree (Fowells 1965). Some seeds may be scattered further with high winds or be disseminated by birds and squirrels. Because not all trees are serotinous and there are varying degrees of serotiny, seeds may be disseminated throughout the year but not at a uniform rate. Even if a fire does not occur, many cones partially or completely open and shed seeds in the fall and, as with other conifers, small amounts of seeds will be shed during the winter. Cones that partially open may shed a few seeds then, with rain, they close but, due to the mechanical tissues in the scales, they open more widely a second or third time during dry, warm periods releasing more seeds.

Most viable seeds germinate in the spring following dispersal, but a small amount may germinate a year later. Best seedling establishment occurs in full sunlight on mineral soil or disturbed duff, free of competing vegetation (Fowells 1965).

Cone and Seed Production in Seed Orchards

Nine seed orchards were established in the British Columbia interior in the 1980s and 1990s and these have come into cone production in the last few years. Most of the newer orchards are located in the Okanagan Valley with some of the older orchards near Prince George. Anatomical studies of ovule and seed development and pollination experiments were done using lodgepole pine in the seed orchard near Prince George in the 1980s (Owens et al. 1981, 1982). These studies determined the normal sequence of ovule, embryo, seed and cone development over the 27-month reproductive cycle for interior lodgepole pine. In the mid-1990s it became apparent that, although lodgepole pine at the orchards near Prince George commonly produced 20 to 25 seeds per cone, lodgepole pine in the newer seed orchards in southern dry interior produced less than half that amount. However, the number of cones per tree near Prince George was commonly less than half that of trees near Vernon. Some of the same clones were in both orchards. Starting with pollination in 1996, studies were begun to determine why lodgepole pine in orchards in the southern region produced so few seeds per cone. There were many variables to consider including site, weather, natural pollen flight and cone receptivity, cone and seed abortions and insects.

Developmental studies and experiments on pollination and seed production were done at the Kalamalka Seed Orchard (KSO) near Vernon, BC in 1996–1997 and at KSO and PGTIS from 1999–2002. Cone production per tree and seed production per cone were determined and developmental studies were done to determine when and why cone and seed losses occurred (Owens et al. 2005). Several of the same clones were in the KSO and PGTIS seed orchards. Trees at KSO were top-pruned to about 1.5 m tall (Fig. 92) in 1995 in order to make cone collections easier, whereas those at PGTIS were not (Fig. 93).

Figure 92. *Top-pruned lodgepole pine orchard near Vernon, BC.*

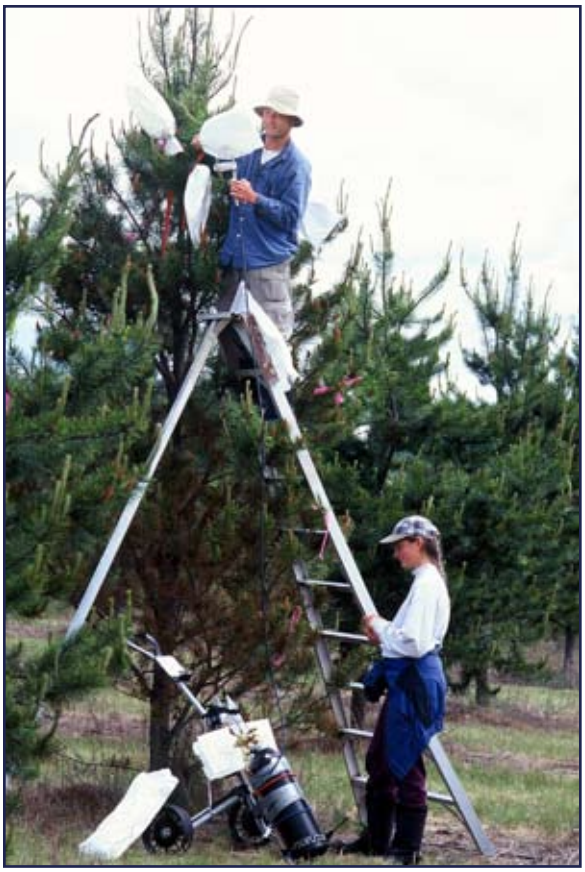


Figure 93. *Pollination experiments on non-pruned trees in the seed orchard near Prince George, BC.*

Seed cones at all sites were quite variable in size, weight and number of fertile scales. There was a significant clonal difference in all cone traits studied. Cones averaged about 100 scales of which 75 to 80 were sterile, meaning that they had no ovules or only rudimentary ovules that could not be pollinated. The 20 to 25 fertile scales per cone gave a seed potential of 40 to 50 seeds per cone. Larger cones produced larger seeds but not more seeds per cone. The seed efficiency (SEF) at PGTIS ranged from 44 to 56% with an average of about 20 to 25 seeds per cone in 2000 and 2001. The SEF in the trees at KSO in 1997 was only 20% and about 10 seeds per cone but these had increased in 2000 and 2001 to 67% SEF and about 25 to 30 filled seeds per cone (Owens et al. 2005).

Cone survival was a different story. At PGTIS the number of cones initiated per tree was low, commonly 50 to 100. Also, cone survival in open-pollinated trees was the lowest of all sites with only 35% of cones reaching maturity—a 65% loss from the time of pollination until cone maturity. The greatest loss was soon after pollination. From dissections and developmental studies, this was shown to result from too few ovules being pollinated (about 80% of fertile ovules must be pollinated for cone retention). Dissections of cones at pollination revealed that the amount of pollen on the micropylar arms (PS) was also low (usually five or less) because the pollen supply was low in the orchard, as measured by pollen monitors (Fig. 36). Additional but smaller cone losses occurred over the winter and from the time of fertilization until cone maturity. Losses were similar in single or multiple (three) pollinations per cone at seed-cone Stages 3, 4 and 5 (Fig. 94) (Owens et al. 2005).

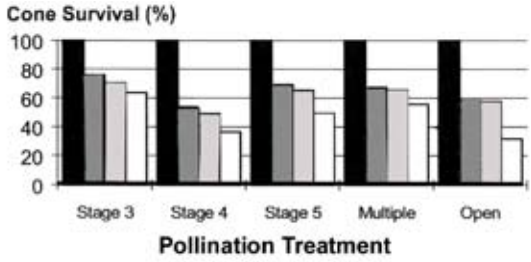


Figure 94. *Cone retention (survival) at Prince George Seed Orchard (PGTIS) for cones pollination once at Stages 3, 4, or 5, multiple at all three stages or open (wind) pollinated. Black bars are the numbers of cones present at pollination, dark grey bars are post-pollination counts done about two weeks after pollination, light grey bars are post-dormacy counts, and clear bars are mature cone counts (Owens et al. 2005).*

In contrast, at KSO (Fig. 95), the number of cones initiated per tree was very high, commonly 400–500. Also, about 95% of the cones survived to maturity in open-pollinated trees. Dissections of cones at pollination revealed that nearly all ovules in a cone were pollinated usually by 10 or more pollen grains. In control-pollinated trees 80 to 90% of cones survived to maturity. In all of the experiments dealing with time of pollination, the largest cone loss occurred soon after pollination and this was attributed to insufficient ovules being pollinated in control-pollinations. Cone losses due to insufficient pollinations in open-pollinated trees were negligible (Fig. 95) (Owens et al. 2005).

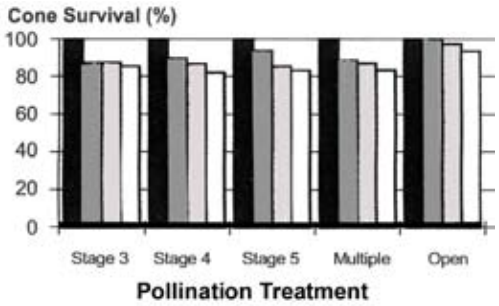


Figure 95. *Cone retention (survival) at Kalamalka Seed Orchard (KSO) for cones pollinated once at Stage 3, 4 or 5, multiple at all three stages, open (wind) pollinated, or in which a water mist was applied. Bar colours shown are counts made at the same stages as in Figure 94 (Owens et al. 2005).*

Various insect bagging experiments were done at KSO in 1997and 1998 to determine the effect of *Leptoglossus occidentalis* on seed production (Strong et al. 2001). In 1997, second year cones enclosed in insect exclusion bags produced about 20 filled seeds per cone. Cones produced about 10 filled seeds per cone if they were enclosed by bags in which a single adult female *Leptoglossus* and up to 14 nymphs were included and replaced when they died. In 1998 two ramets from each of 10 clones, that bore at least 50 second-year seed cones each, were selected and one ramet of each clone was sprayed twice with fenvalerate insecticide (Ciba-Geigy Canada Ltd) that was considered to be 90% lethal to related species of *Leptoglossus*. Control trees were sprayed with water. Cones on trees sprayed with fenvalerate averaged about 12 filled seeds per cone and water-sprayed trees only about two filled seeds per cone. In both experiments seeds were analyzed by radiographs and placed into two categories, filled or empty. These results demonstrated that *Leptoglossus* may have caused heavy loss of filled seeds in 1997 and 1998 at KSO.

In 1997 at KSO, open-pollinated second-year cones, that were not enclosed in insect bags, were sampled from one ramet from each of four clones during seed development and five mature cones were collected from three ramets of each of the four clones for a total of 60 mature cones. The cones were analyzed for the different categories of seeds using the cutting test. As was shown in earlier studies of lodgepole pine (Owens and Molder 1997), sectioned ovules were observed to have aborted at various stages from pre-fertilization through fertilization to seed maturity. Unfortunately, aborting seeds usually do not infiltrate and the seed contents are lost during sectioned or staining, making it is difficult to determine the cause of death. The cutting test, using seeds extracted from mature cones collected in the fall in 1997 revealed that cones had a seed potential of about 100 seeds per cone but 58 of these were rudimentary seeds (ovules that were not pollinated) leaving about 40 seeds per cone that could be filled. An average of five seeds aborted soon after pollination due to early factors such as early incompatibility mechanisms (Dogra 1967). An average of 10 seeds per cone were filled and the remaining seeds aborted during or after winter dormancy. About 10 seeds per cone had aborted at or soon after the time of fertilization, as indicated by the very uniform collapsed, dried and brown megagametophyte. Another 15 seeds were insect- or disease-damaged at various stages of second-year (post-dormancy) development, and an average of two seeds contained insects. Many of these 15 seeds per cone could have been damaged by *Leptoglossus* feeding. The variation in the number of insect- or disease-damaged seeds among cones and trees was high—there were commonly either only a few damaged seeds in a cone or most of the seeds in a cone were damaged. This may fit well with the feeding behaviour of *Leptoglossus*—if undisturbed it may feed on many seeds per cone but if disturbed it quickly flies away.

Developmental studies (Dogra 1967, and additional references given below) have shown that self-pollination results in ovule abortion at various stages of development but mostly soon after fertilization. The gene-regulation of seed development in selfed seeds causes a consistent type and time of abortion—as the new diploid embryos began to develop in the haploid maternal megagametophyte aborts. A study was set up in 1999 to determine the effects of self-pollination on seed development in two seed orchards (KSO and PGTIS). Cones in some clones at both orchards were self-pollinated, cross-pollinated or left for open pollination.

Developmental studies and dissection of ovules and seeds demonstrated, that most cross-pollinated ovules developed normally until seed maturity, whereas most self-pollinated ovules developed normally only until fertilization or soon thereafter. At that time, most self-pollinated ovules aborted leaving normal appearing seeds that contained a collapsed sac-like, dried, brown, megagametophyte. Most cross-pollinated ovules developed normally forming filled mature seeds, comparable in number to open-pollinated cones. The results indicate that, in open-pollination, there was a higher level of self-pollination leading to self-fertilization at KSO than at PGTIS and that self-pollination was a possible cause for low SEF at KSO (Fig. 96). Most self-pollinated ovules develop normally for the first year and, consequently, cones did not abort as they would have had ovules not been pollinated (Owens et al. 2005). But, during fertilization and early embryo development, after egg and sperm and their genes combine, the deleterious effect of the sub-lethal genes causes abortion of the embryo and the megagametophyte.

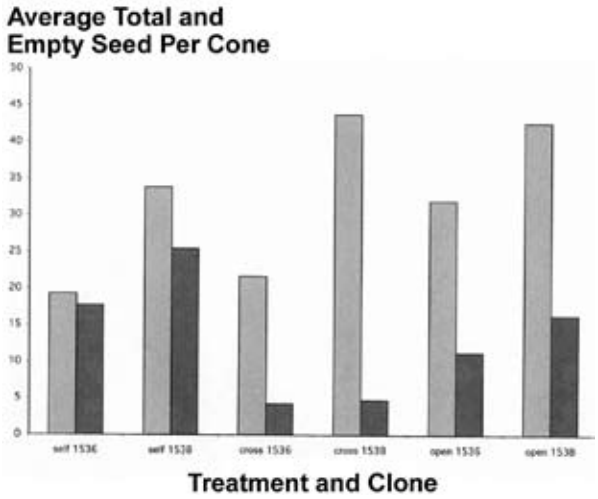


Figure 96. *Average total (grey bars) and "empty seeds" (black bars) per cone after self-, cross- and open-pollination at KSO. The "empty seed" category may include a few abortions due to unknown developmental anomalies in addition to those due to self-fertilization. All cones were enclosed in insect bags during first- and second-year cone development (Owens et al. 2005).*

These results, based on pollination experiments using self- and cross-pollinations are not limited to lodgepole pine and are not new for other conifers in which cross- and self-pollinated seeds have been studied anatomically and developmentally. It has been shown over the last 70 years that self-pollination in conifers in general (see review by Dogra 1967) and specifically in *Pinus* (Dengler 1932; Bingham and Squillace 1955) usually results in embryo and megagametophyte abortion, and the aborted seeds were originally called "empty seeds." It has since been reported that such "empty seeds" result anatomically and developmentally from abortion of the early embryos and megagametophytes within a few weeks after fertilization in *Pinus* (Ehrenberg et al. 1955; Ehrenberg et al 1957; Sarvas 1962; Hagman and Mikkola 1963; Fowler 1965; Plym-Forshell 1974; Lindgren 1975), *Picea* (Mergen et al. 1965; Fowler and Park 1983; Cram 1984), *Pseudotsuga* (Orr-Ewing 1957) and *Abies* (Sorensen et al 1976). The data from lodgepole pine experiments and developmental studies and earlier studies (Dogra 1967) indicate that less than 20% of self-pollinated ovules abort early (before fertilization) causing early ovule abortion that anatomically appears similar to abortion of unpollinated ovules. In the remaining 80% of self-pollinated ovules, fertilization occurs and early embryos develop, then most early embryos abort. This resulted in a uniform collapse of the megagametophyte within a well-developed seed coat in a fully enlarged seed in the lodgepole pine experiments (Owens et al. 2005). It is only a small percentage of embryos resulting from self-pollination that develop leading to the formation of filled seeds. It is these survivors that geneticists measure to determine the degree of selfing.

Why should some self-pollinated ovules develop into seeds in essentially a self-inviable species? This relates to simple polyembryony. In self-fertilized ovules there may be several early embryos but there are no competing cross-fertilized genetically different embryos. Thus, even poorly adapted embryos might survive. As in several other conifers that have been studied developmentally, it appears that in lodgepole pine about 15% of pollinated ovules abort for, as yet, undetermined reasons. Developmental studies in other conifers (Dogra 1967; Runions and Owens 1998) show that this may result from subtle early incompatibility

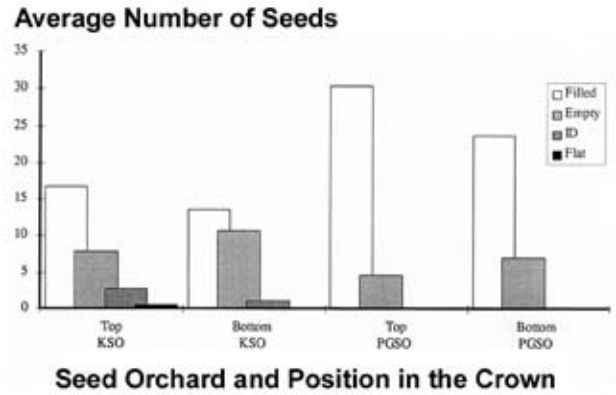


Figure 97. *The average numbers of seeds per cone are shown by position of the cones in the crown at KSO and PGTIS. The "empty seed" category consists of seeds considered to have aborted at about the time of fertilization due to self-fertilization and a few seeds that aborted due to unknown developmental causes. All second-year cones were enclosed in insect bags (Owens et al. 2005).*

mechanisms acting during pollen-tube growth and fertilization, as commonly occurs in flowering plants (Sedgley and Griffin 1989).

Selfing is usually measured by molecular or isozyme studies of mature seeds. The number of seeds having selfed embryos using these techniques is commonly only a few percent. However, these are only the survivors—a measure of the number of self-fertilized ovules and embryos that survive to seed maturity. Most selfed seeds abort during embryo development. In a stand or tree of normal form, the open-pollinated cones may have only a few ovules that are self-pollinated and as a result a very small percentage of mature filled seeds contain embryos resulting from self-pollination. However, in an isolated tree or one in which pollen cones and seed cones are intermingled (top-pruned), the number of self-pollinated ovules may be greatly increased, thus the percentage of self-pollinated ovules and "empty seeds" is higher. Some pioneer species (e.g., red pine; Fowler 1965) have adaptations allowing for much higher rates of development of filled seed from self-pollinated ovules, but this does not appear to be the case for lodgepole pine.

In the above comparison of cone production at KSO and PGTIS from 1997 to 2002, seed production per cone at the Prince George site remained high, at 20–25 seeds per cone but cone survival was not measured until 2000 to 2002. During the same time, seed production per cone at KSO increased from about 10 seeds per cone to 25–30 seeds per cone and cone retention was very high. During this time, conditions changed little at PGTIS—trees got taller and seed-cone and pollen-cone production increased. The main factors that changed at KSO were that cone and seed insects such as *Leptoglossus* may have decreased and that the trees grew from about 2 m and their shrub-like, top-pruned form (Fig. 92) to 4 to 5 m tall with a tree-like crown (Fig. 98) and both pollen- cone and seed-cone production increased (Owens et al. 2005). Trees that have not been top-pruned not only have a greater number of filled seeds per cone, but also a greater difference between the numbers of filled and empty seeds per cone (Fig. 97).

It is well accepted that cone and seed insects must be controlled in seed orchards but consideration should also be given to insuring that there is adequate cross-pollination. These observations and data resulted in recommendations being made not to severely top-prune young lodgepole pine trees in order to make cone collection easier (Owens et al. 2005). Top-pruning may reduce filled seed production for several years, not only because of possible increased self-pollination. An earlier study of top-pruning at 50% in a 10-year-old orchard and 25 and 40% in a 14-year-old orchard showed a decrease in seed-cone and, to a lesser extent, pollen-cone production over controls during the three to five years following top-pruning (Stoehr et al. 1995).

Figure 98. *KSO trees in 2001 showing crown form and blowing of pollen using a tractor-pulled blower.*

In seed orchards, where pollen supply is limited or winds are consistently light during pollination, existing pollen should be blown around every day during the receptive period using a helicopter or a tractor-drawn blower (Fig. 98). A trial was done using a tractor-pulled blower at KSO and PGTIS in 2000. Cones were sampled to determine pollination success in 2001 and for seed analyses in 2002. Blowing of existing pollen did not increase pollination success at either of these established orchards nor did it increase the number of filled seeds per cone in the upper crown or lower crowns (Owens et al. 2005). However, this may be a useful technique in young orchards where there are only small amounts of natural pollen.

On hot dry sites, such as KSO, a sprinkling system should be used to control protandry that occurs in hot dry springs. Sprinkling for a few hours in the morning also increases the humidity and the frequency and size of pollination drops. These may increase pollen uptake by ovules but does not prevent pollen shedding.

Seed Extraction, Dormancy, Storage and Germination

Mature lodgepole pine cones may be harvested in the interior starting about mid-August. Collections should begin before cones begin to open and this depends on the degree of serotiny of trees in the stand. In most natural stands of lodgepole pine that are known to have serotinous cones, collections are done during the winter because cones then snap more easily from the branches and winter collections are more easily scheduled compared to the busy late summer and fall schedule of most cone collectors. Collected cones may be stored in burlap bags placed on racks in an open covered area where there is good air circulation. The method of storage of collected cones is not as critical as in most other conifers since mature cones are hard, dry and less likely to become mouldy.

Cones are usually kiln-dried at 60°C for 16 hours then tumbled to remove the seeds. Because of the serotinous cones, some facilities moisten then scorch the cones, or use infrared heaters to heat and open the cones. After seeds are released, they may be coarsely screened (scalped) to remove broken portions of cones, scales and coarse debris. Seed dewinging is done by wetting the seeds then tumbling them in a rotary drum—often a small commercial cement mixer. This removes the loosely attached wings. This must be done carefully so that no damage is done to the seed coat that would reduce storage time and germination. The seeds and detached wings are dried then given fine screening to remove wings and debris. The final stage of cleaning is done using a gravity table to remove any non-viable seeds or other impurities. Clean dry seeds with less than 10% water content may be stored at -18°C for many years without appreciable deterioration in plastic bags or containers that can be tightly sealed to prevent air and moisture from entering (Kolotelo 1997, Kolotelo et al. 2001).

There are about 250 seeds per gram in interior and 375 seeds per gram in coastal lodgepole pine. Seed length averages 3.4 mm with a range of 2–5 mm). Embryo length is about 2.5 mm and cotyledon number ranges from 3 to 5 (Kolotelo 1997).

Procedures for germination and testing of lodgepole pine seeds have been prescribed by the BC Ministry of Forests (Kolotelo 1997). These procedures are constantly being tested and may be revised periodically. For current recommendations workers should contact the Tree Seed Centre, BC Ministry of Forests, Surrey, BC. The stratification currently recommended for lodgepole pine is to soak seeds in water for 24 hours then surface dry them and place in plastic bags at 2°C for 28 days. Germination tests are done at 20–30°C for 21 days.

Seedling Development

The demand for seedlings for reforestation of lodgepole pine in BC is high because the species is the most widely distributed, harvested and planted species in the province. Current requirements are about 98 million seedlings per year. With a sowing factor of 2.18 seeds per seedling produced, there is a need for about 213 million seeds per year. The

current document regarding this for 2004 is on the BC Government web site (http://www.for.gov.ca/hti/publications/updates/vol5no2.pdf). Because requirements and numbers change frequently, new similar documents will be provided on the same web site.

Nearly all seedlings planted in BC are grown in Styroblock® containers, but some small bareroot nurseries may still exist. Sowing for plastic or glasshouse seedling production is usually done in January and February. Later in the summer when seedlings are developing and temperatures increase, the containers may be moved outdoors or the sides of plastic houses rolled up or removed. For lodgepole pine some seeds are sown and germinated in Styroblock® containers in open compounds from March to May because the cost is less than that for those sown in plastic or glass houses (pers. comm., D. Kolotelo, 2004).

If seeds are of high quality and suitably stratified, a high percentage will germinate within two to three weeks. Germination is epigeal, meaning that the cotyledons appear above the surface of the ground, but as in most conifers they remain enclosed within the seed coat for a short time. The radicle, or primary root, emerges and elongates (Fig. 99). The seed coat is raised above the soil surface and elevated further as the hypocoty elongates. Cotyledons enlarge and emerge from the seed coat that often remains covering the tips of the cotyledons. During germination the stem apex initiates a spiral series of leaf primordia that immediately elongate (Fig. 100). The seed coat falls from the tips of the cotyledons when the seedling is only about 2–4 cm tall. Cotyledons remain green and photosynthetic for most of the first year then turn brown and wither late in the growing season (Fig. 101).

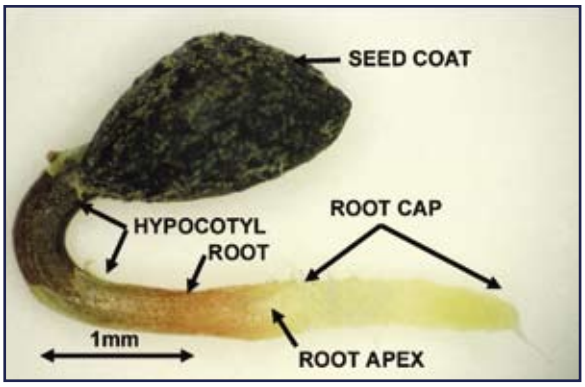


Figure 99. *Germinating lodgepole pine seed. Cotyledons and shoot apex are still covered by the seed coat (Kolotelo 1997).*

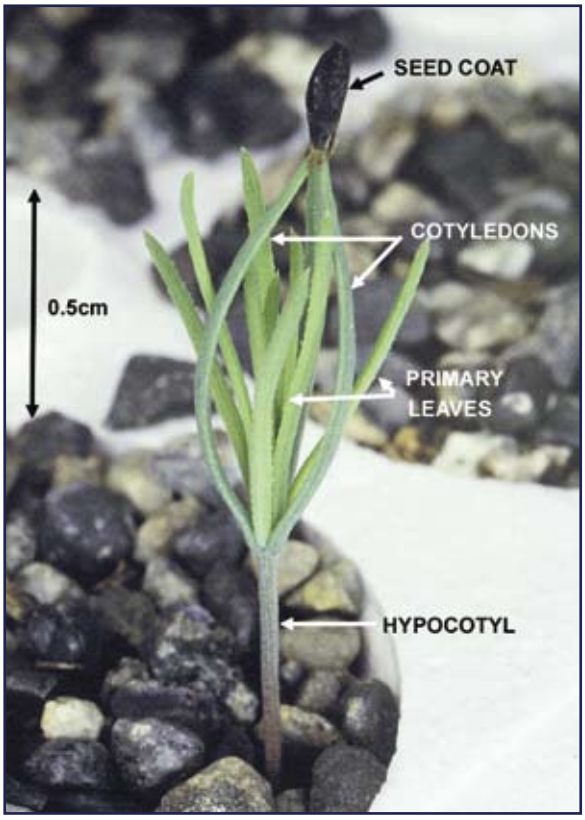


Figure 100. *Lodgepole pine seedling a few weeks after germination in a Styroblock® container.*

As the primary shoot elongates the apical meristem continues to produce primary leaves that elongate. Axillary shoot (branch) apices may be initiated in the axils of some primary leaves. These are sylleptic, meaning that they elongate immediately without passing through a dormant phase, and form rather weak lateral branches. The lateral branches also bear primary leaves. During the latter half of the growing season, as daylength shortens, some of the distal primary leaves on the stem initiate axillary buds that develop into short shoots. These buds immediately develop into short shoots bearing two needles (Fig. 101). At about the same time, the terminal apex on the stem initiates smaller primary leaves and then scale-like leaves (cataphylls) that are green to brown and enclose the stem apex. During the remainder of the growing season, the stem apex initiates cataphylls most of which bear an axillary SSB. By mid-fall a complete LSB has formed at the tip of the stem and on some of the more vigorous lateral shoots. The LSB are small but similar in structure to those described for mature trees (Figs. 8, 23).

The period of primary leaf initiation (free growth) may be extended increasing shoot length and number of primary leaves by maintaining seedlings under long days and without drought. When out-planted in the second year, the LSB elongates and flushes. The shoot apex initiates a series of sterile cataphylls followed by cataphylls that bear axillary short shoots. Long-shoot bud development proceeds as it does in more mature trees in which all structures are initiated within the LSB before it becomes dormant. This is proleptic development in which structures that are initiated undergo a dormant period before elongating.

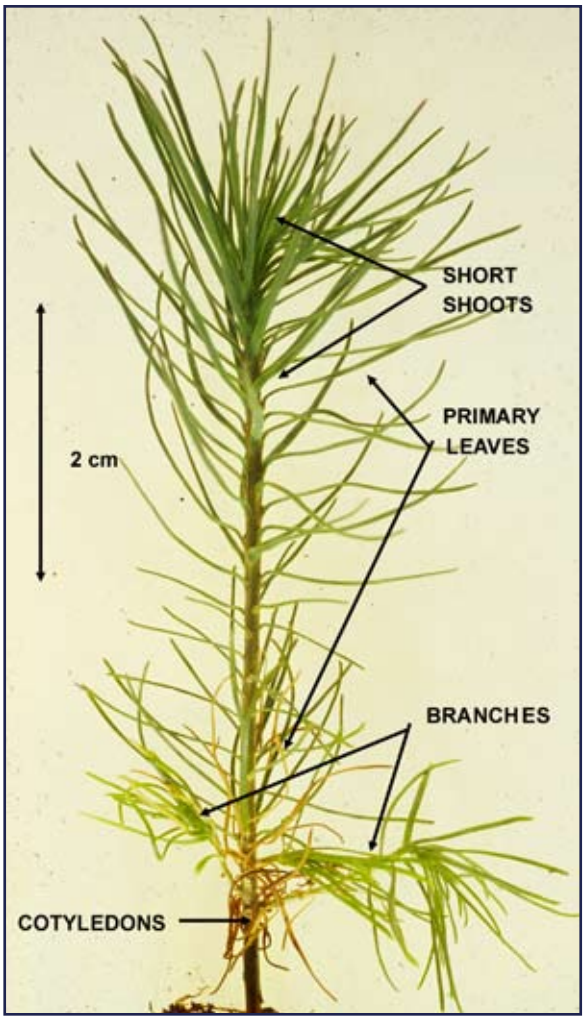


Figure 101. *Dormant seedling grown in a Styroblock® container for one growing season. Cotyledons have withered and the primary shoot bears primary leaves, lateral branches and developed short shoots.*

Summary

Lodgepole pine is the most widely distributed, extensively harvested and reforested conifer in BC. It is a pioneer species that regenerates quickly after fires forming dense even-aged stands. This is primarily a result of the serotinous seed cones that may remain on trees for many years, opening only in response to extreme heat or drying. The reproductive cycle is typical of pines and extends over 27 months from cone initiation to cone maturity and 15 months from pollination to seed maturity. Pollination is by a pollination drop mechanism and airborne pollen. Ovules are small with few eggs and each takes in few pollen grains. Cones bear many scales but few (20 to 25) are fertile thus, seed potential is low (40 to 50) per cone but high per tree because of the abundant cones that may form almost every year and accumulate in the crown over many years. Ovules that are not pollinated quickly abort and, unless about 80% of fertile ovules in a cone are pollinated, the cone aborts soon after pollination. This cone-drop is due to lack of pollination but some results from frost at some sites. Later winter losses may result from low temperatures or nutritional factors. These several factors can cause the loss of only a few of the cones as at KSO or most of the cones during development as may occur at PGTIS. Most self-pollinated ovules develop normally until after fertilization then abort. Selfing may cause cone drop if most of the self-pollinated ovules abort soon after pollination. However, most self-pollinated ovules become fully enlarged at fertilization thus, externally form normal appearing but aborted seeds.

Major constraints to cone production in interior lodgepole pine are insufficient cone initiation, cone drop due to inadequate pollination, frost and low winter temperatures and possibly poor nutrition. Major constraints to seed production are insufficient amounts of pollen in young orchards and at some sites, high levels of self-pollination and insects, especially *Leptoglossus*.

Several recommendations regarding lodgepole pine seed orchards have been made.

1. Samples of trees representing all major clones should be monitored every year to determine the phenology of pollen release and female receptivity and identify early- and late-flowering clones.
2. When evaluating the productivity of an orchard, cone survival should be measured as well as seed production.
3. Seed production should be calculated on a per-cone basis.
4. Trees on very dry sites may show extreme protandry in certain years. This can be corrected by sprinkling to delay pollen release. Sprinkling early in the morning at very hot dry sites will increase the humidity and the frequency and size of pollination drops that may increase pollination success.
5. Pollen in young orchards may be blown around by tractor-pulled blowers or by helicopters every day during receptivity. This will increase pollination success and may reduce selfing where there are limited amounts of pollen.
6. Supplemental pollinations should be done at early Stage 3 cones as well as later Stage 4 and 5 cones since cones pollinated at Stage 3 produce the highest number of filled seeds.
7. Crown topping and management regimes are currently being field tested for lodgepole pine seed orchards and may contribute to a cost-effective management strategy that may prevent a several-year decrease in cone and filled-seed production.
8. Seed orchard insect pests should be carefully controlled by the best current management practices.

Bibliography and References

Allen, G.S. and J.N. Owens. 1972. The life history of Douglas-fir. Environ. Can. For. Serv. Ottawa, Ont. 139 p.

Amman, G.D. and L. Safranyik. 1985. Insects of lodgepole pine: Impacts and control. *In* Lodgepole pine: The species and its management. D.M. Baumgartner, R.G. Krebill, J.T. Arnott and G.F. Weetman (eds). Symp. Proc., Spokane, Wash. pp. 107–124.

Bates, S.L., J.H. Borden, A. Savoie, S.E. Blatt, G.G. Blatt, C.G. Lait, A.R. Kermote and R.G. Bennett. 2000. Impact of feeding by *Leptoglossus occidentalis* (Hemiptera: Coreidae) on the major storage reserves of mature Douglas-fir (Pinaceae) seeds. Can. Entomol. 132: 91–102.

Baumgartner, D.M. (editor). 1975. Management of lodgepole pine ecosystems. Vols. 1 and 2. Proc. Pullman, Wash.

Bingham, R.T. and A.E. Squillace. 1955. Self-incompatibility factors and effects on self-fertility in western white pine. For. Sci. 1: 121–129.

Brown, S.D. and F.E. Bridgewater. 1987. Observations on pollination in loblolly pine. Can. J. For. Res. 17:299–303.

Bruns, D. and J.N. Owens. 2000. Western white pine (*Pinus monticola* Dougl.) reproduction: II. Fertilization and cytoplasmic inheritance. Sex. Plant Repro. 13:75–84.

Cram, W.H. 1984. Some effects of self-, cross- and open-pollination in *Picea pungens*. Can. J. Bot. 62: 392–395.

Critchfield, W.B. and E.L. Little, Jr. 1966. Geographic distribution of the pines of the world. U.S. Dept. Agric. Misc. Pub. 991.

Dengler, A. 1932. Kunstliche Bestaubungsversuche an Kiefern. Z. Forst.- u. Jagdwes. 64: 513–555.

Dogra, P.D. 1967. Seed sterility and disturbances in the embryogeny in conifers with particular reference to seed testing and tree breeding in Pinaceae. Studia For. Suec. No. 15: 5–96.

Ehrenberg, C.A., C. Gustafsson, C. Plym-Forshell and M. Simak. 1955. Seed quality and the principles of forest genetics. Hereditas 41: 291–366.

Ehrenberg, C.A., H. Eklund, and M. Simak. 1957. Flowering and pollination in Scotts pine. Medd. Stat. Skogforskin. Inst. 46: 1–27.

Farjon, A. 1998. World Checklist and Bibliography of Conifers. The Royal Botanical Gardens, Kew, UK. 298 p.

Fowler, D.P. 1965. Inbreeding in red pine, *Pinus resinosa* Ait. IV. Comparison with other northern *Pinus* species. Silvae Genetica. 14: 76–81.

Fowler, D.P. and Y.S. Park. 1983. Population studies of white spruce. I. Effects of self-pollination. Can. J. For. Res. 13: 1133–1138.

Fowells, H.A. (Compiler). 1965. Silvics of forest trees of the United States. U.S. Dept. Agric. Agric. Handb. 762 p.

Gara, R.I., W.R. Little, J.K. Agee, D.R. Geiszier, J.D. Stuart and C.H. Driver. 1985. Influence of fires, fungi and mountain pine beetles on development of lodgepole pine forest in South-Central Oregon. *In* Lodgepole pine: The species and its management. D.M. Baumgartner, R.G. Krebill, J.T. Arnott and G.F. Weetman (eds). Symp. Proc., Spokane, Wash. pp. 153–162.

Gauthier, S., Y. Bergeron and J-P. Simon. 1993. Cone serotiny in jack pine: ontogenetic, positional, and environmental effects. Can. J. For. Res. 23: 394–401.

Greenwood, M. 1986. Gene exchange in loblolly pine: the relation between pollination mechanisms, female receptivity and pollen viability. Am. J. Bot. 73: 1443–1451.

Greenwood, M.S. 1978. Reproductive development of loblolly pine. II. The effect of age, gibberellins plus water stress and out-of-phase dormancy on long shoot growth behavior. Am. J. Bot. 68:1184–1190.

Hagman, M. and L. Mikkola. 1963. Observations on cross-, self-, and interspecific pollinations in *Pinus peuce* Griseb. Silvae Genetica. 12: 73–76.

Hedlin, A.G., H.O. Yates III, D.C. Tover, B.H. Ebel, T.W. Koerber, and E.P. Merkel. 1980. Cone and seed insects of North American conifers. Can. For. Ser., U.S. Dep. Agric. For. Serv., Secretaria de Agricultura y Recursos Hidraulicos, Mexico. Victoria, BC.

Ho, R.H. and J.N. Owens. 1973. Microstrobili of lodgepole pine. Can. J. For. Res. 3:453–456.

_____. 1974. Microsporogenesis and pollen formation in lodgepole pine. Can. J. Bot. 52: 1669–1674.

Illingworth, K. 1971. Geographic variation in *Pinus contorta*. Proc. 13th Meet. Comm. Forest Tree Breed. Can. Part 1:107–112.

Kolotelo, D. 1997. Anatomy and Morphology of Conifer Seed. BC Min. For., Nursery and Seed Ops. Br. For. Nurs. Tech. Series 1.

Kolotelo D., E. Van Steenis, M. Peterson, R. Bennett, D. Trotter and J. Dennis. 2001. Seed handling guidebook. BC Min. For. Tree Imp. Br., Victoria BC. 106 pp.

Krugman, S.L. and T.W. Kroeber. 1969. Effect of cone feeding by *Leptoglossus occidentalis* on ponderosa pine seed development. For Sci. 15:104–111.

Lanner, R.M. and D.A. Van Den Berg. 1975. The vegetative buds and shoots of lodgepole pine. *In* Management of lodgepole pine ecosystems. D.M. Baumgartner (ed). Proc. Pullman, Wash. Vol. I: 68–85.

Lee, K.J. 1979. Factors affecting cone initiation in pines: A Review. Korean Inst. For. Gen. Res. Rep. No. 15:45–85.

Lill, B.S. and G.B. Sweet 1977. Pollination in *Pinus radiata*. N.Z. Jour. For. 7:21–34.

Lindgren, D. 1975. The relationbship between self-pollination, empty seeds and seeds originating from selfing as a consequence of polyembryony. Studia For. Suec. Nr. 126: 126 1–24.

Little, E.L. and W.B. Critchfield. 1969. Subdivisions of the genus *Pinus* (Pines). U.S. Dept. Agric. Misc. Pub. No. 1144.

Lotan, J.E., J.K. Brown and L.F. Neuenschwander. 1985. Role of fire in lodgepole pine forests. *In* Lodgepole pine: The species and its management. D.M. Baumgartner, R.G. Krebill, J.T. Arnott and G.F. Weetman (eds). Proc. symp., Spokane, Wash. pp. 107–124.

Mergen F., J. Burley and G.M. Furnival. 1965. Embryo and seedling development in *Picea glauca* (Moench) Voss. after self- and wind pollination. Silvae Genetica. 14: 188–194.

Muir, P.S. and J.E. Lotan. 1985. Disturbance history and serotiny of *Pinus contorta* in western Montana. Ecology. 66: 1658–1668.

O'Reilly, C. and J.N. Owens. 1987. Long-shoot-bud development, shoot growth and foliage production in provenences of lodgepole pine. Can. J. For. Res. 17: 1421–1433.

Orr-Ewing, A.L. 1957. A cytological study of the effects of self-pollination on *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genetica. 6: 179–185.

Owens, J.N. 2004. The reproductive biology of western white pine. For. Genet. Counc. BC, BC Min. For., Victoria, BC Ext. Note 04. 40 pp.

_____. [2006]. The reproductive biology of western larch. Inland Empire Tree Improve. Coop. Publ. Univ. Idaho Press, Moscow, Id. (In press). 70 p.

Owens, J.N., J. Bennett and S. L'Hirondelle. 2005. Pollination and cone morphology affect cone and seed production in lodgepole pine seed orchards. Can. J. For. Res. 35: 383–400.

Owens, J.N. and M.D. Blake. 1985. Forest tree seed production. A review of literature and recommendations for future research. Can. For. Ser. Inf. Rep PI-X-53. 161 p.

Owens, J.N. and D. Bruns. 2000. Western white pine (*Pinus monticola* Dougl.) reproduction: I. Gametophyte development. Sex. Plant Repro. 13:61–74.

Owens, J.N., G. Catalano and J.S. Bennett. 2001a. The pollination mechanism of western white pine (*Pinus monticola*). Can. J. For. Res. 31:1–11.

Owens, J.N., L.M. Chandler, J.S. Bennett and T.J. Crowder. 2001b. Cone enhancement in *Abies amabilis* using $GA_{4/7}$, fertilizer, girdling and tenting. For. Ecol. and Manage. 154: 227–236.

Owens, J.N. and M. Molder. 1975. Development of long-shoot buds of *Pinus contorta* ssp. *contorta*. *In* Management of lodgepole pine ecosystems. D.M. Baumgartner (ed). Proc. Pullman, Wash. Vol. I:86–104.

_____. 1977a. Development of long-shoot terminal buds of western white pine *(Pinus monticola)*. Can. J. Bot. 55: 1308–1321.

_____. 1977b. Seed-cone differentiation and sexual reproduction in western white pine *(Pinus monticola)*. Can. J. Bot. 55: 2574–2590.

_____. 1984. The reproductive cycle of lodgepole pine. BC Min. For., Res. Br., Victoria, BC. 29 p.

Owens, J.N., S.J. Morris and S. Misra. 1993. The ultrastructure, histochemical and biochemical development of the post-fertilization megagametophyte and zygotic embryo of *Pseudotsuga menziesii* (Mirb.) Franco. Can. J. For. Res. 23:816–827.

Owens, J.N., S.J. Simpson and G. Caron. 1987. The pollination mechanism of Engelmann spruce (*Picea engelmanii* Parry). Can. J. Bot. 65:1439–1450.

Owens, J.N., S.J. Simpson and M. Molder. 1981. Sexual reproduction of *Pinus contorta*. I. Pollen development, the pollination mechanism and early ovule development. Can. J. Bot. 59:1828–1843.

_____. 1982. Sexual reproduction of *Pinus contorta*. II. Postdormancy ovule, embryo, and seed development. Can. J. Bot. 60:2071–2083.

Pfister, R.D. and R. Daubinmire 1975. Ecology of lodgepole pine. *In* Management of lodgepole pine ecosystems. D.M. Baumgartner (ed). Proc. Pullman, Wash. Vol. I: 27–46.

Pharis, R.P., R.L. Wample and A. Kamienska. 1975. Growth, development, and sexual differentiation in *Pinus*, with emphasis on the role of the plant growth hormone, gibberellin. *In* Management of lodgepole pine ecosystems. D.M. Baumgartner (ed). Proc. Pullman, Wash. Vol.1:106–134.

Plym-Forshell, C. 1974. Seed development after self-pollination and cross-pollination of Scots pine. *Pinus sylvestris* L. Studia For. Suec. Nr. 118: 1–35.

Ross, S.D. and R.C. Bower. 1989. Cost-effective promotion of flowering in a Douglas-fir seed orchard by girdling and pulsed stem injections of gibberellin A4/7 treatment. Silva Genet. 38:189–194.

Runions, C.J. and J.N. Owens. 1996. Pollen scavenging and rain involvement in the pollination mechanism of interior spruce. Can. J. Bot. 74:115–124.

_____. 1998. Evidence of pre-zygotic self-incompatibility in a conifer. *In* Reproductive biology. S.J. Owens and P.J. Rudall (eds.). Royal Botanic Gardens, Kew, UK. pp. 255–264.

Sarvas, R. 1962. Investigations on the flowering and seed crop of *Pinus sylvestris*. Comm. Inst. For. Fenn. 53(4).

Sedgley, M. and A.R. Griffin. 1989. Sexual reproduction of tree crops. Academic Press, New York, NY.

Singh, H. 1978. Embryology of gymnosperms. Borntraeger, Stuttgart. W. Germany.

Sorensen, F.C., J.F. Franklin and R. Wollard. 1976. Self-pollination effects on seed and seedling traits in noble fir. Forest Sci. 22: 155–159.

Stoehr, M., C. Hollefreund, J.E. Webber, C. Hewson and S.D. Ross. 1995. Effects of crown-pruning on seed and cone production in two lodgepole pine seed orchards in British Columbia. New Forests 10:133–143.

Strong, W. B., S.L. Bates and M.U. Stoehr. 2001. Feeding by *Leptoglossus occidentalis* (Hemiiptera: Coreidae) reduces seed set in lodgepole pine (Pinaceae). Can. Entomol. 133: 857-865.

van der Kamp, B.J. and F.G. Hawksworth. 1985. Damage and control of major diseases of lodgepole pine. *In* Lodgepole pine: The species and its management. D.M. Baumgartner, R.G. Krebill, J.T. Arnott and G.F. Weetman (eds). Coop. Ext. Washington State Univ., Pullman Wash. pp. 125–132.

Wagner, D.B., G.R. Furnier, M.A. Sagai-Maroff, S.M. Williams, B.P. Dancik and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole pine and jack pines and their hybrids. Proc. Natl. Acad. Sci. USA. 84:2097–2100.

Wagner, D.B., J. Dong, M.R. Carlson and A.D. Yanchuk. 1991. Paternal leakage of mitochondrial DNA in *Pinus*. Theor. Appl. Genet. 85:510–514.

Webber, J.E. 1991. Interior spruce pollen management manual. BC Min. For., Victoria, BC Land Manage. Rep. 70.

Webber, J.E. and M. Bonnet-Masembert. 1989. Influence of moisture content of forest tree pollen on its response to different viability tests. Ann. Sci For. 46:605–638.

_____. 1993. The response of dehydrated Douglas-fir (*Pseudotsuga menziesii*) pollen to three *in vitro* viability assays and their relationship to actual fertility. Ann. Sci. For. 50: 1–22.

Webber, J.E. and R.A. Painter. 1996. Douglas-fir pollen management manual. Second Edition. BC Min. For., Res. Br., Victoria, BC Work. Pap. 02/96.

Wheeler, N.C. and W.B. Critchfield. 1985. The distribution and botanical characteristics of lodgepole pine: Biogeographical and management implications. *In* Lodgepole pine: The species and its management. D.M. Baumgartner, R.G. Krebill, J.T. Arnott and G.F. Weetman (eds). Coop. Ext., Washington State Univ. Pullman, Wash. pp. 1–14.

Wheeler, N.C., R.L. Wample and R.P. Pharis. 1980. Promotion of flowering in the Pinaceae by gibberellins. IV. Seedlings and sexually mature grafts of lodgepole pine. Plant Physiol. 50:340–346.

White, E.E. 1990. Chloroplast DNA in *Pinus monticola*. 2. Survey of within-species variability and detection of heteroplasmic individuals. Theor. Appl. Genet. 79:251–255.

Glossary

Abaxial: facing away from the axis of an organ

Abortion: loss of a structure during development

Adaxial: facing toward the axis of an organ

Apex: see apical meristem

Apical meristem: the apex region of embryonic tissue at the tips of roots, stems and cones

Archegonium: multicellular haploid female sex organ that produces the egg

Axil: area or location between a leaf or bract and the stem axis from which it arises

Basal: toward the base of a structure

Bract: modified leaf in a seed cone

Cataphyll: usually brown scale-like leaves formed in long-shoot buds of pine. They may bear axillary structures, remain sterile or function as bud scales.

Chalazal: the end of the ovule that attaches to the seed wing—opposite the micropyle

Cleavage polyembryony: where a single embryo separates into four embryos during early development

Cone: male or female strobilus (see strobilus)

Cone drop: abscission of seed cones soon after pollination

Cone enhancement or promotion: increasing cone production in trees that have previously produced cones

Cone induction: causing cone production in trees that have not previously borne cones

Corrosion cavity: space that forms around the embryo as it develops in the megagametophyte

Cortex: the portion of the embryo hypocotyl or older plant root or stem that lies outside the vascular tissue

Cotyledon: embryonic leaf formed on the embryo

Cytochemical: microscopic chemical tests for compounds in cells or tissues

Determination: time at which a cell, tissue or organ cannot be prevented from developing along a pathway or diverted into a different developmental pathway

Development: process of growth and differentiation of a cell, tissue, organ or plant

Differentiation: process of a cell, tissue or organ becoming structurally specialized

Diploid: having the full complement of chromosomes (2N)

Distal: toward the tip of a structure

Dormancy: when a plant, organ or tissue which is predisposed to elongate or grow in some manner does not do so

Embryo: young sporophyte plant in the seed that results from fertilization

Embryogeny: development of an embryo

Empty seed: seed in which all the contents have degenerated

Endotesta: inner of three layers that make up the seed coat

Exine: outer resistant layer of a pollen grain

Female gamete: haploid (1N) egg

Female gametophyte: megagametophyte or the haploid (1N) multicellular phase in the life cycle that produces the egg and is contained within the ovule

Fertilization: the union of male (sperm) and female (egg) gametes to form the zygote

Filled seed: seed containing a well-developed megagametophyte and embryo

Germination: beginning or resumption of growth of a pollen grain or seed

Homogamy: pollen is shed when seed cones are receptive

Hypocotyl: portion of the embryonic plant axis below the cotyledons and above the embryonic root

Incompatibility: physiological differences that prevent fertilization, embryo or seed development

Initiation: earliest stages of development of a structure

Integument: the outer layers that enclose the ovule and form the seed coat or testa

Intine: inner wall of the pollen that forms the pollen tube

Lipid bodies: small droplets of lipid in the cell serving as stored food that may be detected using cytochemical tests

Longitudinal section: cutting a plant or structure parallel to the long axis of that structure

Male gamete: haploid (1N) sperm

Meiosis: type of nuclear division in diploid tissues that results in the number of chromosomes being reduced by half to the haploid (1N) number

Megaspore: the haploid (1N) cells that result from meiosis of the megaspore mother cell

Megaspore cell wall: The thick cell wall that forms around the megagametophyte

Megaspore mother cell: the diploid cell in the ovule that undergoes meiosis to form the four megaspores, one of which forms the female megagametophyte

Micropylar: the end of the ovule or seed nearest the cone axis

Micropyle: small opening in the integument at the tip of the ovule through which pollen enters

Microspore: haploid (1N) cell that develops into a pollen grain

Microsporocyte: diploid (2N) pollen-mother cell that undergoes meiosis to form four microspores

Microsporophyll: modified leaf that bears the microsporangia

Mitosis: type of nuclear division that involves duplication and separation of chromosomes such that each of the two daughter nuclei receive a chromosome complement identical to that of the parent cell

Nucellus: the diploid tissue within the ovule that encloses the megagametophyte and is located just inside the integument

Nucleus: the large usually spherical cell structure that contains the chromosomes

Ovule: the structure that contains the megagametophyte and develops into the seed following fertilization

Ovuliferous scales: scales in seed cones that bear the ovules

Phenology: science that relates periodic biological phenomena such as bud burst to climate, especially seasonal changes

Pith: the central core of cells in the hypocotyl of the embryo or stem of an older plant

Plant growth regulators: naturally occurring as well as synthetic compounds (hormones) that affect plant growth and development

Pollen: haploid (1N) male gametophyte

Pollen cone: strobilus or male cone that produces pollen

Pollen-mother cells: see microsporocyte

Pollination: transfer of pollen from the male cone to the female cone

Pollination drop: watery secretion produced by the ovule that picks up pollen from the micropylar arms

Pollination efficiency: measure of the amount of pollen reaching the surface of the ovule or entering the ovule

Pollination mechanism: specialized structures or methods for the transfer of pollen into the ovule

Polyembryony: formation of more than one embryo per ovule. See simple and cleavage polembryony.

Postzygotic: stages of development following fertilization

Prezygotic: stages of development before fertilization

Proembryo: early stages of embryogeny that end with suspensor elongation forcing the embryo out of the archegonium

Protandry: pollen is shed before seed cones are receptive

Protein bodies: small bodies of concentrated protein functioning in protein storage within the cell which can be detected using cytochemical tests

Prothallial cells: small non-functional cells in the pollen grain and the many vegetative cells within the megagametophyte

Protogyny: seed cones are receptive before pollen is shed

Radicle: embryonic root of the embryo

Reproductive potential: the number of cones produced times the number of fertile ovules per cone

Reproductive success: the number of cones reaching maturity times the number of filled seed per cone

Root initials: meristematic tip (apex) of the root

Sarcotesta: outermost of the three layers of the seed coat

Scanning electron microscope (SEM): the type of electron microscope used to study the surfaces of structures

Sclerotesta: hard middle layer of the seed coat consisting of stone cells

Seed: fertilized ovule containing an embryo or in some cases an aborted embryo and megagametophyte—a mature ovule

Seed coat: envelope or covering that develops from the integument of the ovule

Seed efficiency: seed produced (yield) divided by the seed potential (fertile ovules) in a seed cone multiplied by 100

Seed potential: maximum number of seeds a cone can produce—number of fertile ovules

Seed yield: amount of seed produced

Self-fertilization: where sperm from pollen from the same individual or clone fertilizes the egg

Self-incompatibility: mechanisms that prevent pollen from germinating, forming a pollen tube within the nucellus, sperm from fertilizing the egg, or preventing the embryo from fully developing

Self-inviability: mechanism whereby embryos that result from self-pollination abort during embryo development—late-acting self-incompatibility

Self-pollination: where pollen from the same individual or clone pollinates the seed cone

Selfing: self-pollination whereby seed cones are pollinated by pollen from the same plant or clone

SMP: Supplemental mass pollination is the addition of extra pollen to the orchard at some time during the pollination period in order to supplement the pollen produced within the orchard

Sperm: the male gamete

Stratification: a technique used to overcome certain types of seed dormancy and involves moistening followed by chilling

Strobilus: the simple pollen cone or compound seed cone

Testa: seed coat that develops from the integument

Transmission electron microscope (TEM): the type of electron microscope used to study thin sections of specimens at high magnification

Viable: alive and capable of growth

Zygote: the fertilized egg

Sources of Illustrations

Illustrations are taken from many sources. Those used with permission from publications by other authors are listed with the figure description. Most of those used are from the author's publications and these are listed below. Those used with permission from other researchers and agencies are also listed. Many other illustrations are from the author's collection of unpublished photographs taken by the author over the past 40 years. Thanks are given to the all of the authors, agencies and other researchers who have contributed photographs. Published sources are given in the Bibliography and References section.

Critchfield and Little (1966): Fig. 3

John N. Owens' photograph collection: Figs. 2, 4, 6, 7, 26–34, 42, 43, 54, 57, 58, 60, 66, 69, 71, 86, 88–90, 92, 93, 96–98

Owens and Molder (1975): Figs. 9–13, 24

Owens and Molder (1977b): Figs. 80, 83–84

Owens and Molder (1984): Figs. 1, 5, 8, 14, 15–23, 25, 52, 53, 59, 62, 63, 67, 72, 74, 75, 81, 86, 101

Owens et al. (1981): Figs. 54, 61, 63, 64, 67

Owens et al. (1982): Figs. 64, 65, 67, 68, 75–79, 81

Owens et al. (2005): Figs. 35–41, 44–51, 55, 56, 94, 95

Owens and Bruns (2000): Fig. 70

Bruns and Owens (2000): Fig. 73

David Kolotelo (1997) (BCMOF Tree Seed Centre): Figs. 82, 85, 87, 91, 99, 100

John Runions: Fig. 61

Appendix 1: Determining Reproductive Potential and Reproductive Success

Reproductive potential (RP) is a function of the initial number of cones per tree at pollination and the potential number of seeds per cone, and Reproductive Success (RS) is a function of the final number of cones (cone surviving to maturity) per tree and the filled seeds per mature cone.

In order to determine how well an orchard, clone, family or a tree within the orchard is performing, it is necessary to do detailed counts of cones and analyses of seeds. This may be done using a sample of flagged branches on selected trees. The number of branches and cones should be fairly large (~50 cones/tree at pollination) to allow for the cone loss that may occur during development from pollination to cone maturity. If too few cones are initially counted there may not be enough left for cone and seed analysis at cone maturity. It is not necessary to make cone counts of entire trees because the numbers of cones may be huge and impossible to accurately count. Sample branches should be representative of the crown or the particular portion of the crown that is of interest (lower, higher, etc.). Branches should be labeled with metal tags because flagging tape may be lost when the cones over-winter. In order to obtain the most information, cones should be counted at several stages of development:

1. at pollination in order to determine the initial number;
2. about four weeks after pollination to determine the amount of cone drop that has occurred, due to inadequate pollination in pines, frost, etc. during or soon after pollination;
3. after winter dormancy in pines in order to determine the winter loss of cones; and,
4. at cone maturity, before cones open on the trees.

These data are used to determine the initial cone potential and the proportion of this potential retained at successive developmental stages. This can be represented as the mature cone (C) to the "flower" or conelet (Cl) ratio (C/Cl) that is usually much less than one.

At cone maturity, a sample of 5 to 10 cones from each tree, crown region, treatment, etc. should be collected before the cones start to open on the tree. Each cone should be placed in a separate envelope or bag that can be sealed so if the cone opens the seeds are not lost or mixed with those of other cones. The cones should then be kept separate until after they are dried in an oven until the cones open. To determine SP, the total cone scales are counted. To determine the total fertile scales (those bearing fertile ovules) the number of sterile scales at the base and tip of the cone are subtracted from the total scales. Sterile scales are those that bear no fertile ovules and at cone maturity may bear tiny wings but no seed or aborted ovule attached to the wing. In lodgepole pine, about 80% of the scales are sterile. Nearly all sterile scales are in the basal half of the cone, but some occur in the distal fertile portion of the cone. In other pines, such as western white pine, only about 10% of the scales, mostly at the base and tip of the cone, are sterile. Fertile scales times two gives seed potential.

All seed, wings with very tiny undeveloped ovules and wings with no ovules should be shaken from the cone and any of the above that stick within the cone should be pulled out with forceps. These can be quickly separated into:

1. wings only (those from sterile scales);
2. rudimentary seeds (wings with a tiny ovule at the tip) that was too poorly developed to be pollinated); and,
3. small to full-sized and normal-appearing seeds that usually remain attached to the wings.

Rudimentary and small to full-sized seeds should be counted and this number divided by two to determine the number of fertile scales. The total scales minus the fertile scales equals the number of sterile scales. Rudimentary seeds should be

placed in one category that we believe from developmental studies (Owens et al. 2005) results from the ovules being not well developed enough at the time of pollination to be pollinated. The small to full-sized normal-appearing seeds must be sliced open and the contents observed to determine if they are filled or if not, the possible cause for their failure to fully develop. These seeds should be placed on masking or strapping tape, or a double-sided tape strip attached to a small board. These seeds may then be sliced longitudinally to reveal the contents of the seeds. Slicing is best done with a sharp razor blade. After slicing and viewing with a dissecting microscope, these seeds may be placed into subcategories (3a, 3b, etc).

3a. Filled seeds will have a cream-coloured megagametophyte and white, yellow or greenish embryo and the embryo should be about 90% of the length of the corrosion cavity.

3b. Empty seed will contain a dried, brown, collapsed and empty megagametophyte. These result from abortion of the megagametophyte about the time of fertilization and are commonly caused by self-pollination or otherwise incompatible pollination. But, in pines this indicates that these ovules were pollinated; unpollinated ovules abort a few weeks after pollination.

3c. A few seeds may be round, flat or indented with varying degrees of megagametophyte development and these may have aborted during embryo development or result from feeding by the insect *Leptoglossus*.

3d. Other seeds will have the contents destroyed by insects and in some the larva may still be present or an emergence hole may be visible in the seed coat.

Further subdivision of Category 3 seeds may be possible and desirable in certain sites or circumstances but it is difficult to assign causes without more detailed developmental studies.

The Seed Efficiency (SEF) is calculated as the percentage of fertile ovules that develop into filled seeds that appears normal and is likely viable.

$$\text{SEF (\%)} = \text{Filled Seed/Fertile Ovules} \times 100$$

The Reproductive Success (RS) is the product of the cone:conelet ratio times the filled seed: fertile ovules ratio.

$$\text{RS} = \text{C/Cl} \times \text{FS:FO}$$

From experimental and developmental studies of lodgepole pine in Interior BC seed orchards (Owens et al. 2005) the RP was about 40 to 50 seeds per cone, the SEF was commonly about 50%, C/Cl ranged from from 50–95% giving a RS of about 25–50%. Although this may sound low, it is actually moderate to high for a conifer and quite high for seed plants in general. In most hardwood forest trees, RS is usually below 5% and commonly below 1%. This results from the extremely high abortion of fruits (analogous to cones) giving very low fruit to flower ratios. Any cultural treatments that can increase cone retention and seed efficiency, such as supplemental pollination or reduction of selfing or insect damage, may significantly increase filled seed production in an orchard. But some careful cone analyses, as described above, should accompany such trials.

Appendix 2: Monitoring Pollen Development

Monitoring the stages of meiosis and pollen development in conifers is not difficult and requires only an inexpensive compound microscope and simple dissecting tools. Pollen cones are sampled from branches, moistened, placed in plastic bags and taken in a cooler to the lab. There, each cone may be placed on a separate clean microscope slide and, using a sharp scalpel (pointed No. 11 blade), a few mirosporangia are removed from the cone. This may be more easily done by first slicing the cone in half. One or two microsporangia are then squashed on the slide and the creamy thecal fluid, which contains the pollen, comes out. Microsporangial walls and microsporophylls are removed from the slide leaving the small amount of contents (a drop 1–2 mm across) on the slide. A drop of aceto-carmine is placed over the material and a cover-slip placed on the drop. The slide is then gently heated over an alcohol burner (warmed but not boiled) for a few minutes. Boiling precipitates the stain and ruins the slide. The slide is then placed on a piece of paper towel on a flat surface and a second piece of paper towel is placed over the cover slip. Using your thumb, you can press firmly down on the towel to squash the cells in the specimen. Let the specimen stand for a few minutes or gently heat again to increase the staining intensity.

Observe the specimen using the compound microscope. Pollen-mother cells are large with large nuclei and thin walls (see Figure 25). Tetrads following meiosis show four angular cells within a cell wall. Separate microspores are round but smaller than pollen-mother cells and have a thickened wall. Wings develop on the pollen a few days after meiosis and cell divisions occur within the pollen two to three weeks after meiosis. For stages of meiosis and details of the technique, see Ho and Owens (1974).

Aceto-carmine stain is prepared by heating 45% acetic acid (45 ml glacial acidic acid in 55 ml of water) in a fume hood or lab with good ventilation then slowly adding 1% aceto-carmine (0.5 gm to 500 ml). This should be heated but not boiled for several minutes then allowed to cool. After cooling, it is filtered using filter paper and can be stored in a closed bottle at room temperature for several years. Staining actually improves with aging and oxidation of the stain. The stain is put into dropper bottles and stain placed on the slide using a dropper.

Appendix 3: Determining Pollination Success

The amount of pollen available in an orchard is commonly monitored using pollen monitors (Webber 1991) and this is usually a good indication of how well pollinated are the cones. However, for breeding techniques and supplemental pollinations individual cones may need to be sampled to determine how much pollen is in the cone. Pollination success (PS) is a measure of the number of pollen grains on the ovules of a cone. The method is simple and quick. A sample of cones at pollination can be sliced longitudinally down the centre and the two halves observed using a dissecting microscope (see Figures 54–57). Ten intact ovules are observed and the number of pollen grains per ovule counted. Pollen counted is that on the micropylar arms. As a general rule in pines, if there is an average of five pollen per ovule or less, the cone has been rather poorly pollinated and SMP is necessary otherwise cone drop will be high and there will be low seed set. Five to 10 pollen per ovule is satisfactory, but SMP might increase seed set and reduce cone drop. More than 10 pollen grains per ovule is very good and SMP is not needed. The proportion of ovules with pollen is also important—in lodgepole pine more than 80% must be pollinated or the cone aborts. With natural wind pollination most ovules usually receive pollen, but with SMP, in which pollen may be applied from limited directions and short often infrequent times, many ovule may receive few or no pollen.

More important than pollen on (PO) the ovule is pollen in (PI) the ovule—the number of pollen grains actually taken into the micropyle. However, counts of PI require careful slicing of tiny ovule tips to see the pollen taken in (Fig. 57), and is not a practical method for monitoring pollination.

Appendix 4: Pollen Germination Test

For breeding and SMP it is necessary to collect, store and test pollen. Several techniques are described in detail by Webber (1991), Webber and Bonnet-Masembert (1989) and Webber and Painter (1996). For lodgepole pine pollen, germination is an easy test, requiring simple equipment—only an inexpensive dissecting microscope and simple glassware. This also requires very small pollen samples which may be critical for breeders. The basic medium is modified from Brewbaker's medium. The Brewbaker's stock solution consists of the following dissolved in 100 ml of distilled water:

Boric acid	0.1 g
Calcium nitrate	0.3 g
Magnesium sulphate	0.2 g
Potassium nitrate	0.1 g

The working solution consists of the following brought up to a final volume of 300 ml with distilled water:

Hydrogen peroxide	1 ml
Brewbaker's solution	30 ml
Sucrose (table sugar)	10–15 g

Dispense about 25 ml of this solution into a flask and add about 0.1 g of pollen (the exact amount is not critical). Cover the flask with foil and incubate at 28°C for 48 hours. If incubated at room temperature this may take more than 48 hours. Mould on the surface of pollen grows rapidly, so incubation for too long will result in a lot of water mould that will inhibit pollen germination. If incubation is for about 48 hours or less, the hydrogen peroxide may be omitted. Reducing the sugar to 10% or replacing it with 20% polyethylene glycol may reduce fungal growth (Webber and Painter 1996). For each sample, shake the flask then remove a dropper-full of the pollen-medium mix and place it on a microscope slide and then cover the drop with a coverslip. Using a dissecting (or compound) microscope, observe the pollen. Count the number of pollen grains out of 100 in which the pollen tube is equal to or greater in length than the diameter of the pollen. Do four replications of each germination sample. Fresh high-quality pollen should have over 90% germination.