FGC extension note 04

The Reproductive Biology of Western White 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, 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.

Acknowledgements

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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 the book is based, and to the many research assistants and associates, technicians, graduate students, and post-doctoral fellows who carried out or assisted in much of the research. Special thanks to Jordan Bennett who carried out much of the pollination studies and recent field studies in coastal and interior seed orchards.

Preface

British Columbia has 25 native conifer species, of which 15 are commercially important and used in reforestation. Genetic tree improvement 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. A necessary prerequisite for extensive reforestation with genetically selected seed from seed orchard grown superior trees is an understanding of the reproductive biology of each species.

Until the mid-1960s, Douglas-fir was the only native species for which the reproductive biology was well studied. Since then extensive research has been done on the reproductive biology of 13 of the other commercially important conifers. 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, 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 separate or closely related species.

This book is intended to serve as a brief, readable, informative, and well-illustrated reference for foresters, silviculturists, physiologists, breeders, and seed orchard personnel involved in seed procurement, production, and handling. It contains a glossary of terms commonly used in conifer reproduction plus specific terms that are unique to the particular genus or species. This publication explains the complete reproductive cycle, provides useful guidelines for cone induction, assists in forecasting pollen, cone and seed production, enables more effective pollination and pollen management for breeding and seed production, and explains many of the causes for cone and seed loss in natural stands and seed orchards.

The Reproductive Biology of Western White Pine 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 reports.

Table of Contents

Introduction

The reproductive biology of a species is 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.

Western white pine begins to form seed cones at 10 to 15 years old, followed a few years later by pollen cones. Seed cones are large, often 15 cm or longer, and have 100 to 150 cone (ovuliferous) scales. Most scales (80%) are fertile and bear two ovules. A few scales at the tip and base of the cone are sterile, bearing no or only rudimentary unfertile ovules. This gives each cone a reproductive potential of 160 to 240 seed per cone. Young trees may bear none or few cones whereas mature trees may bear hundreds. The Reproductive Potential (RP) of a tree is the number of cones (C) times the average number of fertile ovules per cone (O/C). For more detailed methods see Appendix 1.

The number of cones formed from year to year depends on many exogenous factors (e.g., climate, soil) and endogenous factors (e.g., hormone levels, nutrition). Many cones may be lost (cone drop) soon after pollination and this is usually a result of cones being inadequately pollinated. Cones may also abort in the first year due to frost at pollination and low winter temperatures and in the second year of development due to stress, tree nutrition, insects, or disease.

The number of ovules that mature into seeds also varies from year to year. In pines, fertile ovules may abort soon after pollination or develop normally for the first year then abort in the second year. A common cause for ovule abortion shortly after the time of pollination is because some ovules were not pollinated. A common cause for ovule and seed abortion in the second year is self-pollination, which if it results in self-fertilization, usually causes embryos and ovules to abort soon after fertilization (about one year after pollination). Still other ovules and seed may be damaged or destroyed by insects and disease.

At the end of the reproductive cycle, cone survival commonly ranges from 35–45% and filled seed from 40–50% (60–110 seed per cone). The product of these two percentages is the RS. The RS of western white pine is commonly less than 25% of the RP in seed orchards and in the forest.

The goal of this book is to provide information about the reproductive biology of western white pine growing in seed orchards and natural stands. This information should make it possible to determine such things as RP, RS, and many of the causes for the loses, 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 problem.

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 similar control usually is not possible.

Taxonomy and Distribution

Western white pine (*Pinus monticola* Dougl.) (Figure 1) is in the Pinaceae, the largest family of conifers. The Pinaceae has 11 genera and over 200 species. 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 while it shows considerable variation, it also possesses so many uniquely derived characters that all 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 31 or so species of white pines are further divided into two sections: Strobus with 19 species separated into two subsections, Strobi with 14 species, and Cembrae with five;

Figure 1. *Mature open-grown coastal western white pine.*

and section Parrya with 12 species. Western white pine belongs within the section Strobi (Little and Critchfield, 1969).

Three species of white pines are native to British Columbia. *Pinus monticola* is distributed from the coast to the central BC interior and south into California and Montana through the Cascade and Rocky Mountains (Figure 2). *P. albicaulis* Engelm. (whitebark pine) is scattered at high elevations in the Coast Mountains of southwestern BC, the Cascade Mountains south through Oregon and into the Sierra Nevada Mountains of California. In the Rocky Mountains this species extends from the central BC and Alberta border south through Idaho and Montana into northeastern Nevada and northwestern Wyoming. *P. flexilis* (James) (limber pine) is found only in the southeastern corner of BC and extends south at high elevations in the Rocky Mountains into Arizona. A fourth species found in Canada, *P. strobus* L. (eastern white pine) is similar to western white pine but distributed from Ontario eastward in Canada and the northeastern United States and as far south as Georgia (Critchfield and Little 1966, Hosie 1979). Several other white pines are native to the United States and Mexico, and to Eurasia from the European Alps to Japan (Bingham 1983).

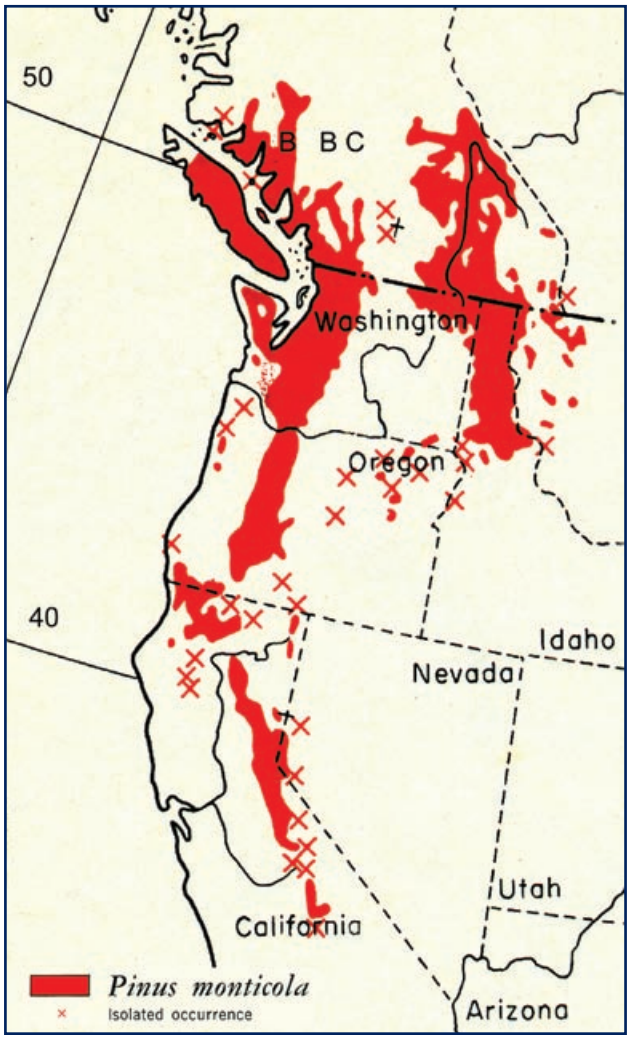


Figure 2. *Distribution of western white pine (from Critchfield and Little, 1966).*

Economic Importance

In BC and the Pacific Northwest, western white pine has considerable commercial potential. The wood is light, strong, and dries and takes paint well. It is ideal for furniture, window frames, and general construction. In the past western white pine lumber has sold for twice the price of most BC softwoods. It is a beautiful tree commonly used as an ornamental. It is fast growing and resistant to root rot making it ideal for reforestation of many sites where this disease occurs (Bingham 1983).

Until early in the last century, western white pine was extensively harvested throughout its range. About 1910, white pine seedlings infected with white pine blister rust (*Cronartium*) were imported from France to Vancouver's Point Grey. White pine blister rust spread rapidly through western white pine forests of BC and the Pacific Northwest and by 1930 most sawmills utilizing the species had closed. By 1937 (about 15 years after the entry of the rust into Idaho) the average level of infection on young western white pine reached 15% in the U.S. National Forests in that region and by the mid-1940s, infection had reached over 95% in some areas (Bingham 1983). To this day, despite its high commercial value, few western white pine seedlings are planted in BC because of the devastating effect of blister rust disease. All four Canadian white pines are classed as very susceptible, whereas many Eurasian and a few North American species are considered immune or resistant (Bingham 1983).

The rust spores are carried by wind to the leaves of young white pine trees where they germinate and penetrate the leaf surface. The fungal hyphae grow inside the leaf and down into the young stem then permeate the outer stem tissues. Hyphae (the microscopic fungal strands) grow within the branch and may grow down into the main stem. Hyphae often girdle the branch or main stem discoloring and reducing wood quality in the latter or if there is a more severe infection, girdling may kill the branch or main stem. An infected stem usually shows cankers and resin oozing from the surface (Figure 3) before the stem dies and turns brown.

Figure 3. *Stem of a young rust-infected western white pine tree showing the wound area (yellow) and resin produced (photo courtesy of Canadian Forest Service).*

The disease commonly kills the young trees within a few years (Bingham 1983).

In the 1950s the decision was made in the United States to start a rust-resistant white pine selection and breeding program. In the 1970s, a similar program was started in BC and a rust-resistant clone bank, selected from U.S. trees, was established near Salmon Arm, BC. During the last 10 years, additional rust-resistant clone banks and seed orchards have been established in Saanich, Sechelt, and near Vernon, BC. The seed orchards are beginning to produce cones and breeding for rust resistance has started. Successful breeding and efficient seed production in seed orchards requires knowledge of not only the basic reproductive cycle but as much as possible about the reproductive biology of the species, including cone induction, pollination mechanisms and factors affecting cone and seed production.

Reproductive Cycle

The reproductive cycle of western white pine is similar to most pines in that it extends over about 26 months from pollen-cone initiation to seed and cone maturity, and 15 months from pollination to seed and cone maturity (Figure 4). Pollen-cone buds are initiated late in the summer, become dormant and overwinter then pollen forms the next spring. Lateral buds that may form either vegetative long shoots or seed cones are initiated in the fall but can not be distinguished anatomically until the following April. Pollination occurs about June. Pollen grains enter the cone, form pollen tubes in the ovule tissues and the female tissues begin to develop but development stops about July of the year of pollination. Seed cones remain dormant until the following spring. Growth resumes in April and sperm and eggs form by June, when fertilization occurs. Embryo, seed, and cone development are completed by late summer when seed is shed.

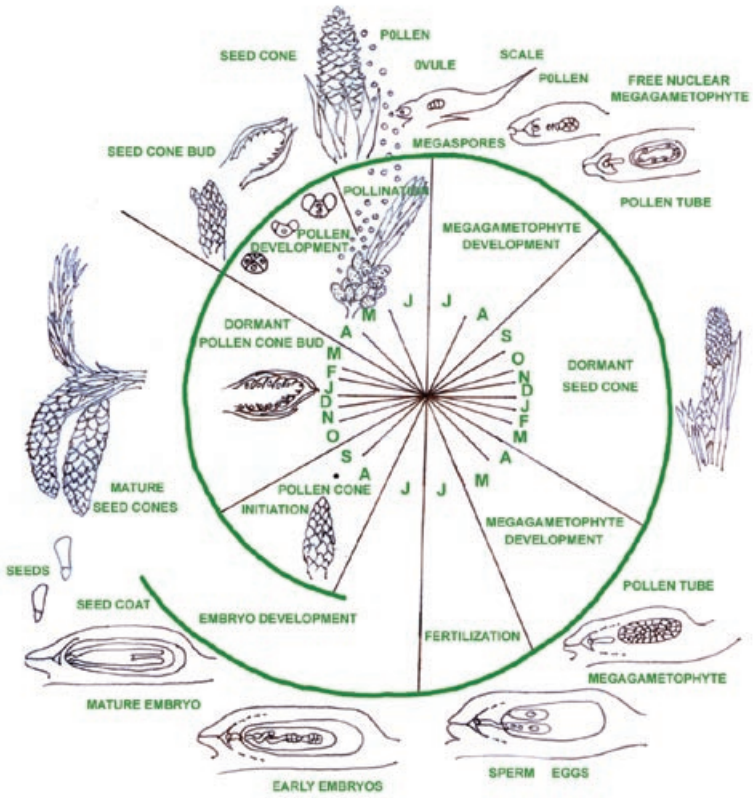


Figure 4. *The reproductive cycle of western white pine extends over about 26 months from pollen-cone initiation until cone maturity, and 15 months from pollination until seed maturity.*

Long-shoot Bud Development

Pines have very different types of vegetative buds compared to most other conifers. They are called long-shoot buds (LSB). During the spring and summer, western white pine forms LSB that contain all of the structures that will be found on the shoots as they elongate the following year (Owens and Molder 1977a).

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) (Figure 5). 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. An axillary bud is initiated just above most of these cataphylls (Figure 6). The axillary bud initiates a spiral of cataphylls then develops into one of four types of buds: 1. Short-shoot buds (SSB) (also called dwarf shoots or needle fascicles). These develop into short shoots that bear five leaves; 2. LSB that develop into lateral branches. 3. Seed-cone buds; or 4. Pollen-cone buds. Some cataphylls, mostly at the base and tip of the LSB, initiate no axillary meristem and remain as sterile cataphylls functioning as bud scales (Figures 7–9).

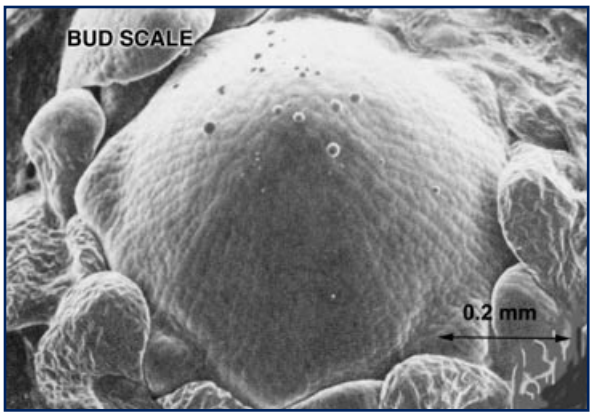


Figure 5. *Scanning electron micrograph of the shoot apex in a long-shoot bud (Owens and Molder 1977a).*

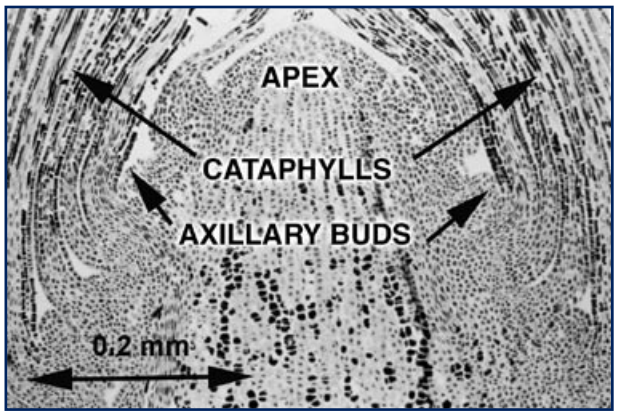
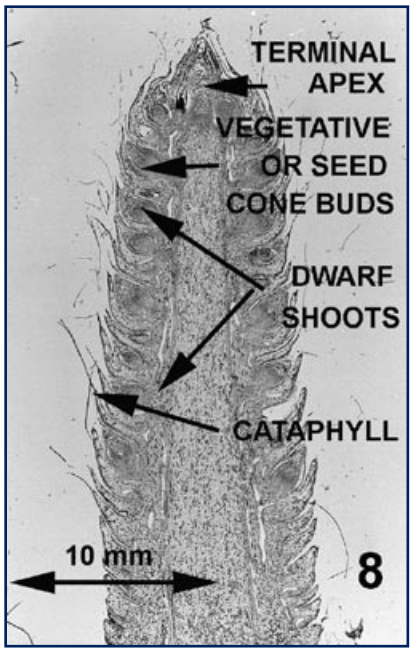
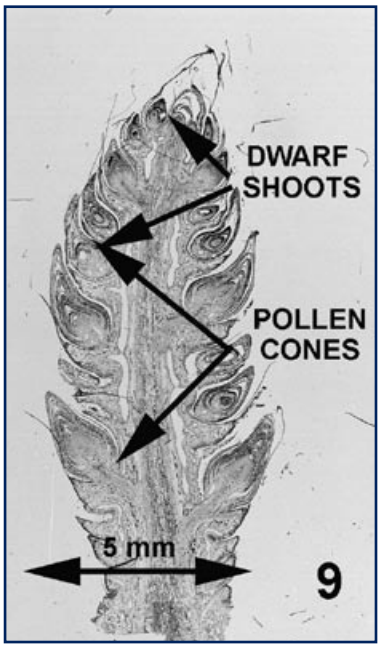


Figure 6. *Section of a long-shoot bud showing the shoot apex, cataphylls and newly initiated axillary buds (Owens and Molder 1977a).*





Figures 7–9. *Dormant long-shoot buds (LSB) of western white pine.* Figure 7. *LSB showing cataphylls covering the bud.* Figure 8. *Section of a large LSB, as shown on the right in Figure 7, showing the position of the cataphylls, dwarf shoots, terminal apex, and potential vegetative or seed cone bud.* **Figure 9.** *Section of a small LSB, as shown on the left in Figure 7, showing the position of the dwarf-shoot and pollen cone buds (Owens and Molder 1977a).*

In young trees and on most branches of older reproductively mature trees, the LSB contain only SSB that will become short shoots and lateral LSB that will become lateral branches. In older reproductively mature trees, many LSB in upper regions of the crown contain SSB along most of their length and some anatomically undetermined lateral buds just below the terminal apical meristem. After dormancy, about April, these undetermined lateral buds develop into seed-cones or lateral branch buds (Figure 10A). Many LSB in lower regions of the crown produce some SSB and occasionally LSB near the top and a cluster of pollen-cone buds near the base (Figure 10B).

Because cataphylls are initiated throughout the growing season, axillary buds also are initiated and develop throughout the growing season (Figure 11). Therefore, there is no specific short time for potential pollen-cone or seed-cone bud initiation as there is in many other conifers. Potential pollen-cone and short-shoot apical meristems are initiated in June, July, and August (Figure 11) but do not anatomically differentiate into short-shoot buds (Figure 12) or pollen-cone buds (Figure 13) until August and September (Figure 10B). The same was observed in sugar pine (*P. lambertiana*, another white pine) (Sacher 1954). In western white pine and sugar pine, potential seed-cone or lateral-branch apical meristems are initiated in September and October (Figure 14) but do not anatomically differentiate into seed-cones or lateral branch buds until April of the next year (Figure 10A). This is different from

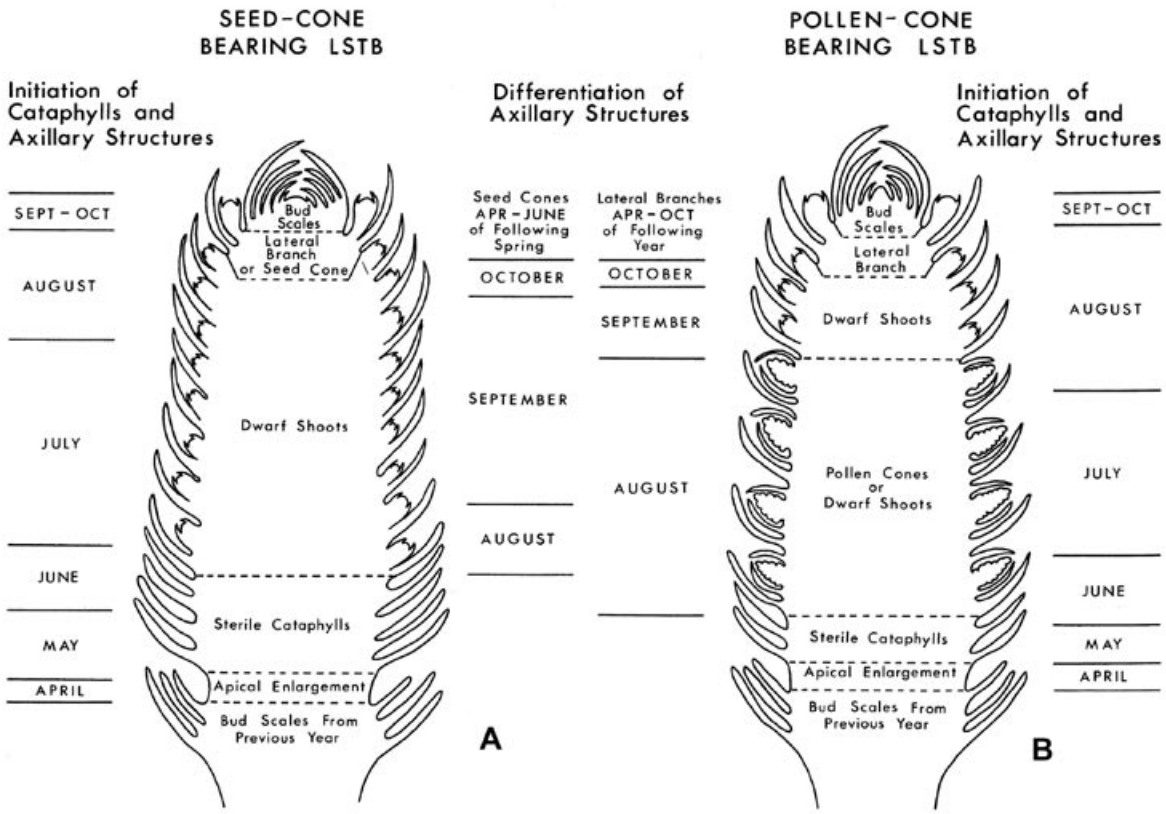
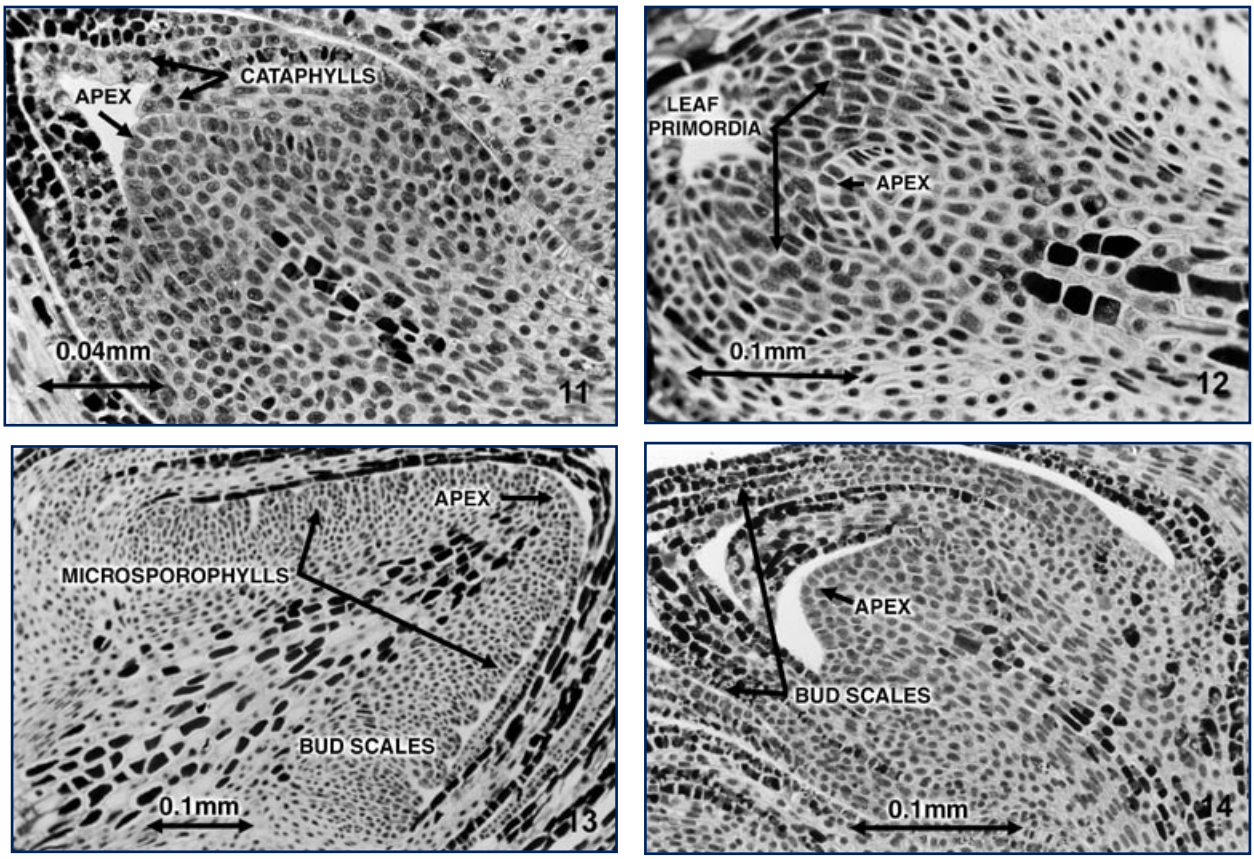


Figure 10A and B. *Diagrams of seed-cone bearing (A) and pollen cone-bearing (B) long-shoot buds of western white pine showing in the left and right columns the time when the cataphylls and axillary meristems are initiated and in the centre columns when these axillary meristems anatomically differentiate into the specific types of buds. The column in the axis of each long-shoot bud lists the types of axillary structures that normally differentiate in that region of the long-shoot bud (Owens and Molder 1977a).*



Figures 11–14. *Light microscope sections showing types of lateral bud meristems and differentiated buds that are present in LSB at dormancy.* Figure 11. *Lateral bud meristem during bud-scale initiation before differentiation.* Figure 12. *Dormant short-shoot bud after five leaf primordial are initiated.* Figure 13. *Dormant pollen-cone bud showing microsporophyll primordia. Figure 14. Dormant potential seed-cone or lateral LSB before bud differentiation.*

temperate hard pines in which seed cones differentiate in the fall, soon after bud initiation (Sacher 1954, Owens and Molder 1975) and develop to varying extents before winter dormancy, depending on the species, site, and weather.

Identification of Buds

The LSB on most young trees contain only short-shoot and potentially long-shoot buds. As trees mature (commonly over 10 years old), some LSB in the upper crown form lateral apical meristems that differentiate into seed-cone buds. Within another few years some of the LSB in the mid and lower crown form lateral buds that differentiate into pollen-cone buds rather than SSB before winter dormancy.

It is not possible to visually determine if upper LSB will form seed cones before winter dormancy. They look the same as those that will form only lateral branch buds (Figures 7, 8). However, it is sometimes possible to visually determine if LSB contain pollen-cone buds before winter dormancy because the base of the bud may be slightly swollen due to the large size of the pollen-cone buds within (Figures 9, 13). It is safe to assume that a tree having LSB containing pollen-cone buds that can be identified in the fall will also differentiate seed-cone buds in the spring but there is no way to estimate the number of seed cones that will form until after they differentiate in April.

Cone Induction

Cone induction and cone enhancement are 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 cone enhancement

for increasing cone production in trees that are already reproductively mature and have produced cones before. Either term may be commonly used with no distinction being made.

In the 1950s and 1960s several trials were made to produce cones on western white pine and sugar pine (*P. lambertiana*) using fertilizers containing nitrogen as ammonium. Successful treatments were given in the spring (Barnes and Bingham 1963), which is the time of seed-cone bud anatomical differentiation, but fall treatments did not increase seed-cone production (Barnes 1969). Cone induction more recently has been reported in eastern white pine (*P. strobus*) using gibberellin A4/7 (GA 4/7) as a foliar spray (Ho and Schnekenburger 1992). The best times for treatments were August and September for seed cones, and May and June for pollen cones. Ho and Eng (1995) subsequently found that foliar spray and stem injections of GA4/7 gave best results in May and June, during the period of rapid shoot elongation.

In general, cultural treatments using fertilizers containing ammonium nitrate, girdling, root pruning and moisture stress, thinning, light, and gibberellins have not been as successful or have given more variable results in white pines then in hard pines and many other conifers (Pharis et al. 1987). One reason for the frequent lack of cone induction and variable results in cone induction in white pines is the uncertainty about when potential pollen-cone and seed-cone buds become determined and are most responsive to treatments. Most researchers have assumed that seed-cone bud differentiation occurs in the fall as in the hard pines, so treatments generally were not given in the spring to increase seed cones for pollination that same spring.

In a recent study (Owens, unpublished data) young western white pine trees were treated in the spring or in the fall with ammonium nitrate fertilizer, stem injection of GA 4/7, girdling, and tenting (Figure 15). Increased numbers of seed-cones occurred with the spring treatment, if all treatments were applied, but there was no increase in seed cones with the fall treatment. Growing potted trees in a greenhouse or tenting, combined with other adjunct treatments, have been used successfully for cone enhancement in spruce (Ross 1988) and *Abies* (Owens et al. 2001). In western white pine, tenting increased the temperatures within the tents by about 10°C, and this appeared to be the most important factor. Tenting is relatively inexpensive when applied to small trees 1 to 2 m tall and could be effective in shortening breeding programs but would not be practical for larger trees being used for seed production.

Fortunately, most open-grown western white pine in seed orchards begin to produce seed cones and pollen cones with no cultural treatments at 10 to 15 years from planting or grafting. Controlling cone production in very young white pines will likely never be as successful as in hard pines and many other conifers where several treatments alone or in combination often give good and relatively consistent results (Pharis et al. 1987).

Figure 15. *A cone enhancement trial using tenting in a western white pine seed orchard.*

Pollen-cone and Pollen Development

Pollen-cone buds resume growth and development about mid-April. Dormant pollen cones have all or nearly all of their microsporophlls initiated but microsporangia (pollen sacs) have not yet formed. When growth resumes, two microsporangia form on the abaxial (lower) side of each microsporophyll (Figure 16A). Within each microsproangium cell divisions occur forming a cluster of sporogenous cells. Sporogenous cells continue to divide, enlarge and become angular with a large nucleus. These become the pollen mother cells (PMC) (Figure 16B). Early in May, the PMC begin meiosis, the process in which the chromosomes in the nucleus replicate once followed by two cell divisions. This produces four microspores in a tetrad (Figure 16C) each with a haploid number of 12 chromosomes in western white pine. After about one week, the four microspores separate filling the microsporangium with hundreds of microspores.

During the next week, the microspores enlarge, become rounded, store food reserves (starch), the pollen wall (exine) thickens and begins to form two small wings or sacci (Figure 16D). The microspore nucleus then divides by mitosis forming a small first prothasllial cell and a large central cell (Figure 16E). The central divides unequally and forms a large antheridial initial and a small second prothallial cell on top of the first. Both prothallial cells remain small and lens-shaped with little cytoplasm and a nucleus that quickly degenerates (Figure 16F). A second cell wall, the intine, forms inside the exine, enclosing the two prothallial cells. The antheridial initial then divides unequally forming a large tube cell and a small antheridial cell contained within the tube cell (Figure 16 G). The antheridial cell then divides forming a generative cell and a sterile cell, often called the stalk cell. The sterile cell becomes enclosed in the intine on top of the two prothallial cells and has no further function (Figure 16H). After pollination, the pollen germinates by the tube cell bursting out of the exine then forming a pollen tube that grows into

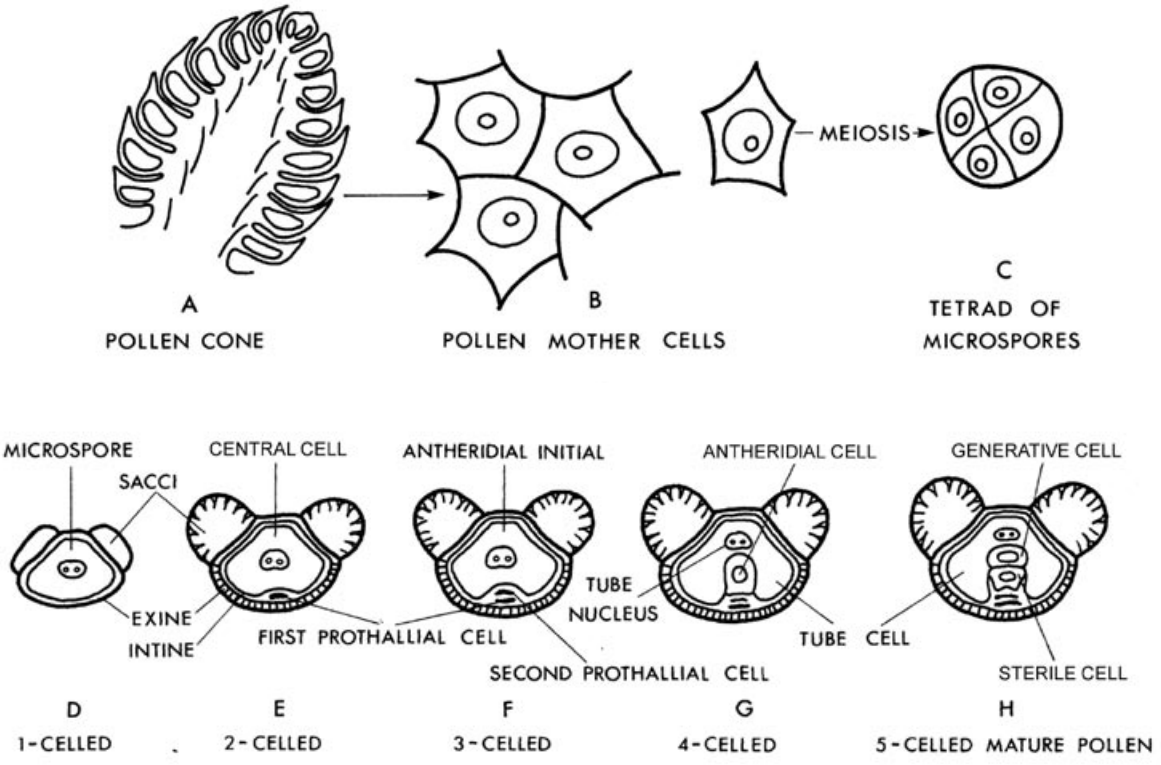


Figure 16. *Pollen development in pine.*

the ovule tissue. During pollen tube growth, the generative cell divides equally to form two sperm (Owens and Bruns 2000).

During the cell divisions, the intine and exine thicken, the sacci enlarge and the outer surface of the exine becomes sculptured with a fine pattern of ridges (Figure 17). The mature pollen has five cells, contains abundant starch and floats in a watery thecal fluid. The microsporangia enlarge as the pollen matures and the cells lining the inside of the microsporangium (the tapetum) degenerate releasing complex compounds that control pollen maturation and others, including; sporopollenin, lipids and proteins, that coat the outer surface of the pollen.

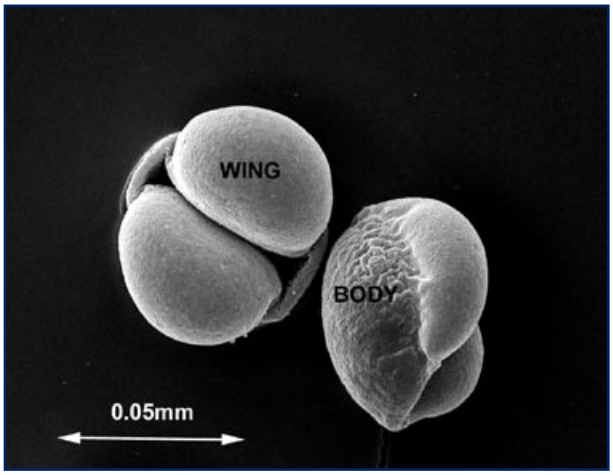


Figure 17. *Scanning electron micrograph of mature white pine pollen showing the body and two wings (sacci) and sculpturing on the surface.*

The thick exine and the coating materials make the pollen wall very hard. The thecal fluid disappears over a few days and the pollen dries usually to less than 10% water content. The pollen wall and the dehydrated cells within make the pollen very resistant to the physical environment and microorganisms. During the last stages of pollen development, the two microsporangia each develop a line of dehiscence consisting of specialized cells that separate upon drying allowing the microsporangia to open and release the pollen (Figure 18).

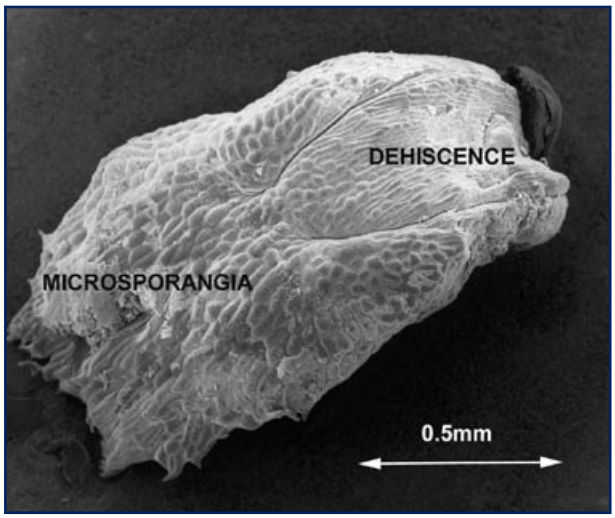


Figure 18. *Scanning electron micrograph showing a single mature microsporophyll with two microsporangia each with a line of dehiscence.*

In coastal western white pine meiosis occurs during one week in mid-May. The tetrad stage lasts only a few days. Small sacci are formed during the week after the one-cell microspores separate. During this time, the cell wall thickens and starch accumulates. Cell divisions begin about three weeks after meiosis and pollen is mature (five-cell) four to five weeks after meiosis. The rate of pollen-cone and pollen development is fast during warm weather but slower during cold or rainy weather. In a very warm spring in the interior of BC pollen may develop in two to three weeks and be mature by late May (Figures 40–41; see page 13).

Seed-cone and Ovule Development

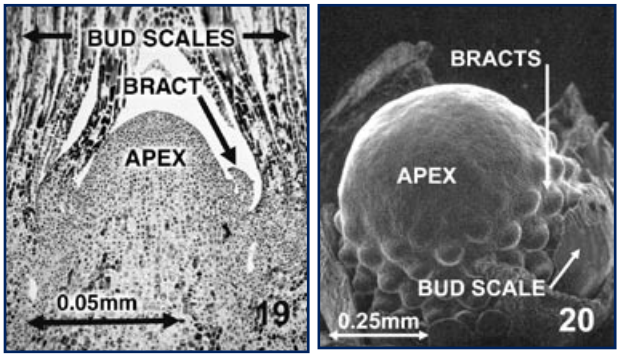
Seed-cone buds begin to differentiate in mid-April, about two months before pollination. Some of the large apices near the tip of the LSBs quickly enlarge and become broad dome-shaped apices (Figure 19). They initiate bract primordia in a spiral arrangement up the flanks of the apex (Figure 20) and this quickly reduces the size of the apex (Figure 21). Bract primordia become pointed at the tip and as the tip grows upward, a broad ovuliferous-scale (scale) primordium is initiated above each bract (Figure 21), except those at the very base and tip of the cone. These bracts remain

sterile. Bracts become broad and thin and most scales forms two lateral ovule primordial (Figure 22). The ovule primordia elongate forming an ovule tip that extends beyond the scale.

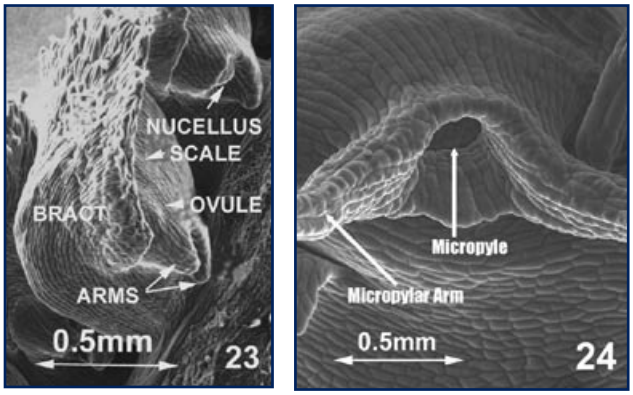
The ovule tip forms two opposite finger-like projections, the micropylar arms, with a small round nucellus between (Figure 23). The ovule tip continues to develop until pollination, at which time it consists of a narrow collar around the nucellus and the micropylar arms are long and thin. A small round pore, the micropyle, remains between the arms and is the opening into the micropylar canal of the ovule (Figure 24). As the cone axis elongates at pollination the spaces between scales increases and the ovule tips hang down as pairs of long finger-like projections in a spiral around the cone axis.

Pollination

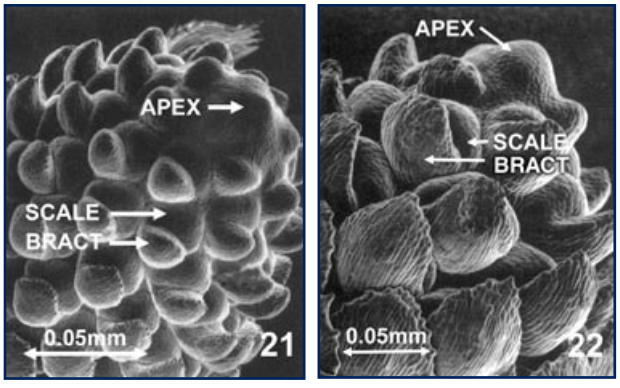
Pines are monoecious, meaning that pollen cones and seed cones are found on the same tree. The rate at which cones develop in the spring is primarily determined by temperature—the warmer the temperature the more rapid is development. However, pollen release is also dependent on drying of the pollen cones.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



Figures 19, 20. *Early seed-cone bud development following dormancy.* Figure 19. *Median section through a seed-cone bud at the start of bract initiation in mid-April. The apical meristem (apex) is enclosed by bud scales.* Figure 20. *Scanning electron micrograph of a seed-cone bud during bract and scale initiation early in May.*



Figures 23 and 24. *Scanning electron micrographs of portions of seed cones.* Figure 23. *Cone collected one week before pollination showing broad, flat bracts and portions of two ovules, each with two small micropylar arms and the round nucellus between.* Figure 24. *An ovule tip at pollination showing the open micropyle and the long arms.*



Figures 21 and 22. *Scanning electron micrographs of seed-cone buds collected two weeks (Figure 21) and one week (Figure 22) before pollination showing the pointed bracts and initiation of the broad ovuliferous scales (scale).*

forms: 1) female and male organs mature at the same time (homogamy); 2) female organs mature before male (protogyny); or 3) male organs mature before female (protandry). For example, the trees in a BC coastal orchard were homogamous in 1998 and 1999 (Figure 25), whereas the trees in the interior orchard were protogynous in 1998 and 1999—most seed cones were receptive before peak pollen release (Figure 26). Both protgyny and protandry favor cross-pollination in monoecious trees but can result in few ovule being pollinated each by few pollen grains. Homogamy may 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.

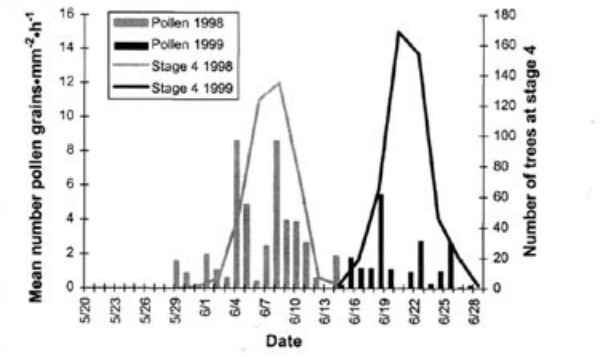


Figure 25. *The amount of western white pine pollen carried by the wind, measured as the mean number of pollen grains per square millimetre per hour, compared with the number of trees with seed cones at Stage 4 at the coastal seed orchard in 1998 and 1999.*

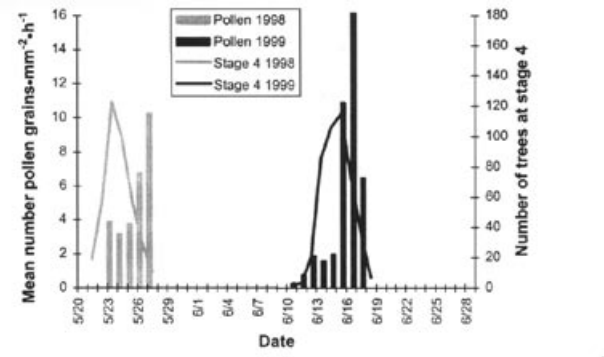
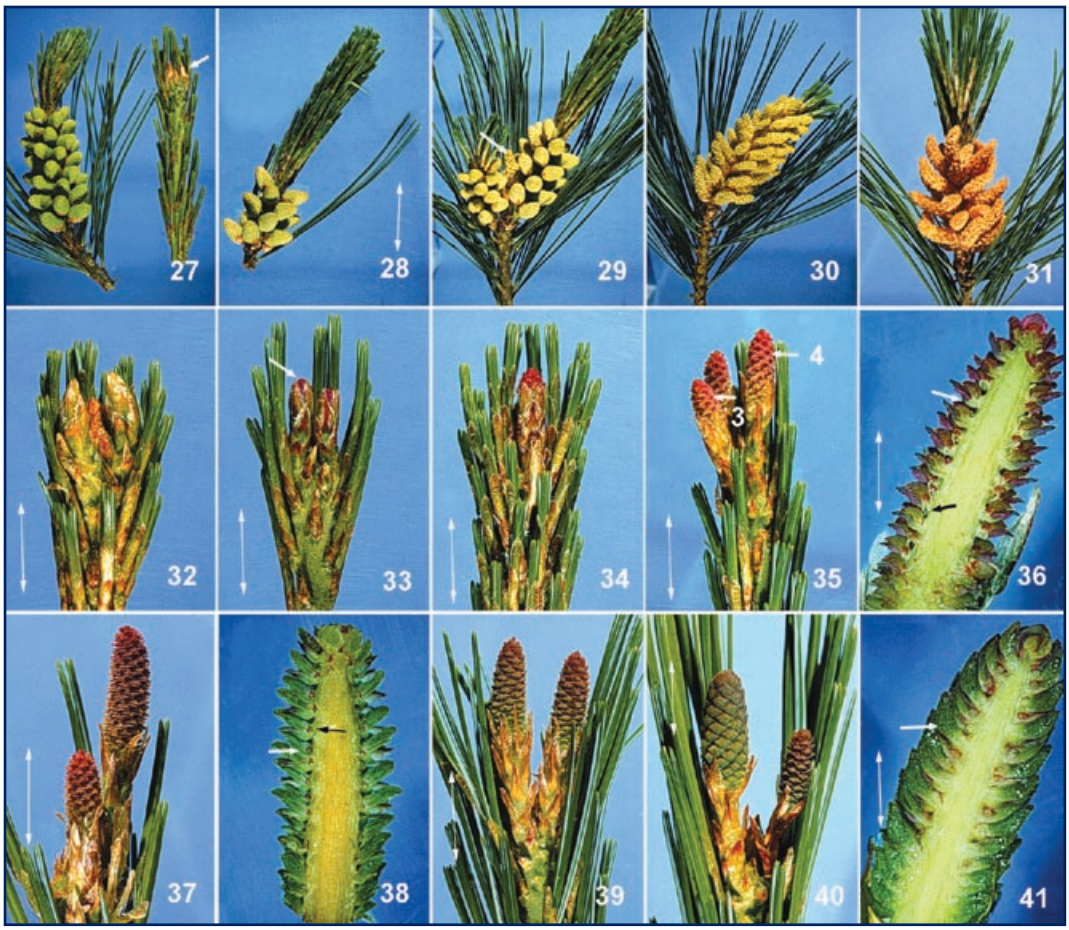


Figure 26. *The amount of western white pine pollen carried by the wind, measured as the mean number of pollen grains per millimetre per hour, compared with the number of trees with seed cones at Stage 4 at the interior seed orchard in 1998 and 1999.*

In pines, a percentage of ovules in a cone must be pollinated (10–20% in western white pine) for the seed cone to survive but if fewer ovules are pollinated, the seed cone will abort soon after pollination (Sweet 1973). The percentage of ovules that must be pollinated varies with the species. Cone abortion is not obvious at first. Abortion occurs over two or three weeks. The inner tissues die first, then the outer portions of the bracts and scales turn brown and cones usually fall from the branch. This is called cone drop. Successful pollination means that pollen is taken into the ovule rather than only remaining on the surface of the ovule and micropylar arms. Self-pollination does not cause young cones to abort as long as the required percentage of ovules are pollinated but most self-pollinated ovules abort at fertilization, one year after pollination, and form empty seeds.

It is convenient to assign stages to pollen-cone and seed-cone development preceding and during pollination (Figures 27–41). The following phenology (the relationship between development and time) is for western white pine growing in a coastal BC seed orchard. Stages of meiosis and development begin one to two weeks earlier in coastal seed orchards but development is faster in interior orchards and pollen is generally shed earlier than at coastal sites (Figures 25, 26) (Owens et al. 2001).



Figures 27–41. *Stages of pollen-cone and seed-cone development before, during and after pollination. Scale bars for Figures 27–35, 37, 39, and 40 =1 cm. Scale bars for Figures 36, 38 and 41 are 5 mm.* Figure 27. *Pollen-cones at Stage 1 (meiosis) and seed-cone bud (arrow) at Stage 0 after all bracts and scales have been initiated.* Figure 28. *Pollen cones at Stage 2 (microspores) still require several days to mature.* Figure 29. *Pollen cones at Stage 3 (arrow) when pollen is nearly mature and starting to dry. This is the best time to pick pollen cones.* Figure 30. *Pollen cones at Stage 4 when pollen is being shed and cones are too late for picking.* Figure 31. *Pollen cones at Stage 5, after all pollen has been shed.* Figure 32. *Seed-cone buds at Stage 0, just before bud burst.* Figure 33. *Seed-cone buds (arrow) at Stage 1, just as cones are starting to emerge from the bud scales.* Figure 34. *Seed-cone bud at Stage 2 is not yet receptive.* Figure 35. *Seed-cones at Stages 3 and 4 (white numbers). The Stage 3 cone is half to two-thirds emerged from the bud scales. They are not fully open but are receptive because pollen adheres to hairs and some pollen can enter the cone. The Stage 4 cone is fully open.* Figure 36. *Seed cone sliced down the middle at Stage 4. Bract-scale complexes separated (white arrow) and the white cone axis and ovules are visible (black arrow). Cones are most receptive at this stage.* Figure 37. *Stage 5 seed cones showing thickened scales closing the spaces between bract-scale complexes. Cones are still receptive.* Figure 38. *Stage 5 seed cone sliced longitudinally to show that the spaces between bract-scale complexes are nearly closed (white arrow). The black arrow shows the tip of an ovule after pollen has been taken in* Figure 39. *Stage 6 seed cone. Scales have thickened sealing the cone and the margins are bent upward.* Figure 40. *Stage 7 cone with tightly sealed scales. Smaller cone is delayed in development and will likely abort.* Figure 41. *Stage 7 seed cone sliced longitudinally showing tightly sealed scales (arrow) and ovules.*

When pollen cones emerge from their bud scales, they are green and meiosis is occurring (Stage 1, Figure 27). As pollen matures, pollen cones turn yellow and the shoot on which they are borne elongates separating the cones on the shoot (Stage 2, Figure 28). As pollen cones dry, they turn from yellow to light brown (Stage 3, Figure 29). This is the best time to collect pollen cones for pollen extraction. When pollen starts to be released the cones are brown and dry (Stage 4, Figure 30). Dry cones may remain on the shoot for most of the summer (Figure 31). 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. In the coastal seed orchard, pollen release (dehiscence) occurred in the first two weeks in June in 1998 and the second two weeks in June in the cooler and wetter 1999 season (Figure 25). In the interior seed orchard, pollen release occurred more quickly, during the last week of May in 1998, during and the second week of June in the cooler 1999 season (Figure 26).

Seed cones elongate within the bud scales (Stage 0, Figure 32) and the red tip extends beyond the bud scales (Stage 1, Figure 33). Seed cones continue to elongate and at Stage 2 are about one-third emerged but not yet receptive (Figure 34). By Stage 3 (Figure 35) cones are about two-thirds emerged and the cone axis begins to rapidly elongate widening the spaces between the bract-scale complexes. This creates small spaces between the bract-scales and the cones begin to be receptive—some pollen can enter the cone. The cone axis continues to elongate and the spaces between bract-scales becomes very wide, so that the white ovules and the cone axis can be seen (Stage 4, Figures 35, 36, 42). This 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 scales (Stage 5, Figures 37, 38). Stage 5 cones are generally less receptive than Stage 4 cones. Scales continue to thicken and seal the cone (Stage 6, Figure 39) and the cones are no longer receptive. Seed cones then become broader and remain tightly sealed (Stage 7, Figures 40, 41). A cone is receptive (Stages 3–5) for about one week with the most receptive period, Stage 4, commonly lasting two to four days.

Pollination success (PS) is the amount of pollen entering the cone and reaching the ovules. It can be measured by counting the number of pollen grains attached to the micropylar arms in cones that have been sliced longitudinally (Figure 42). See Appendix 2 for methods.

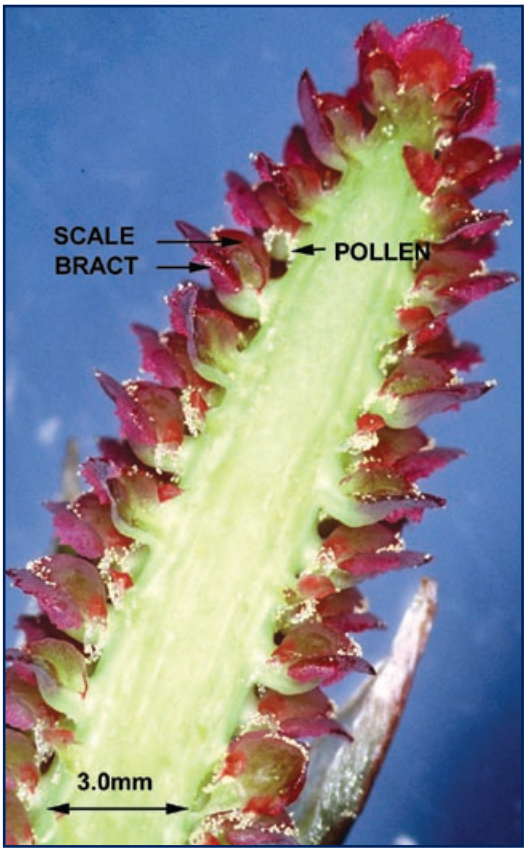


Figure 42. *Stage 4 seed cone sliced longitudinally showing the wide spaces between bract-scale complexes and abundant white pollen on the micropylar arms.*

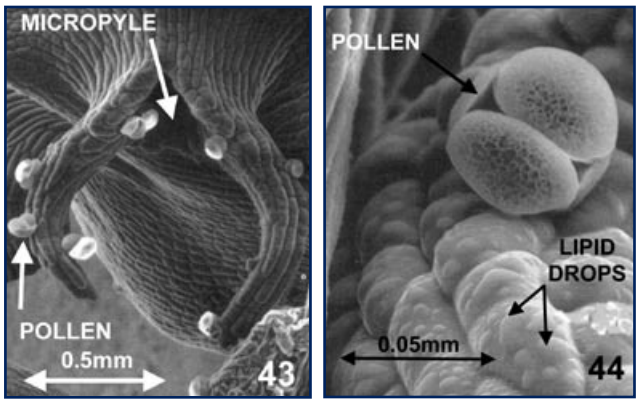
For western white pine in general, if the average number of pollen grains per ovule for 10 ovules counted is 0–5 pollen grains on the micropylar arms, this is low, 6–10 is moderate and over 10 is abundant. Supplemental pollination (SMP) should be recommended for the low amount of pollen but probably not for the moderate amount and certainly not for the abundant amount. For details of the method, see Appendix 2.

Temperature sums and degree-days (DD) were determined in coastal and interior orchards for 1998 and 1999. Pollen shed and seed-cone receptivity began at about 600 DD and 400 DD in coastal and interior orchards, respectively, in both years using 5°C as a threshold temperature. Using the average monthly mean temperature as an indicator, pollen release and receptivity began when the temperature averaged 16°C and 17°C at coastal and interior orchards, respectively. These methods may be used to roughly predict the time of pollination but the most accurate method is to monitor a sample of trees from different clones within the orchard. Ramets within a clone tend to be receptive at about the same time. Early and late clones will require SMP in order to reduce self-pollination, prevent cone drop and obtain good seed set. SMP should be done at Stage 4, however, cones a Stages 3 and 5 will also benefit.

The Pollination Mechanism

The pollination mechanism in western white 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 (Owens et al. 1981, 1998). Airborne pollen blows into the cones for about one week during Stages 3–5, but mostly during Stage 4. During Stage 4, the micropylar arms secrete lipid microdrops that remain on the surface of the arms and pollen adheres to these microdrops (Figure 43). Pollen settles on all cone surfaces but is easily dislodged, except from the sticky arms (Figure 44) where pollen accumulates over several days.

At Stage 5 or 6, the nucellus tip within the ovule secretes a large pollination drop that fills the micropylar canal and is exuded out of the micropyle, usually filling the space between the micropylar arms (Figure 45). Pollen attached to the arms enters the pollination drop and, because the wings are filled with air, pollen grains float up in the drop, through the micropyle and to the surface of the nucellus tip. If the pollination drop is large, it may contact the cone axis or scales



Figures 43, 44. *Scanning electron micrographs of the micropylar arms.* Figure 43. *Arms with several pollen grains adhering to the lipid microdrops.* Figure 44. *Enlarged view of pollen adhering to the microdrops.*

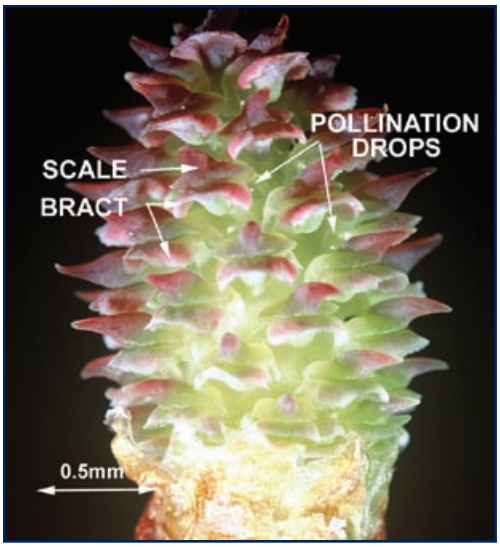


Figure 45. Pinus *sp. cone showing shiny pollination drops on the ovule tips (photo courtesy of T. Takaso).*

above and below and scavenge pollen from these surfaces. Once pollen has entered the pollination drop, the drop quickly evaporates, decreases in volume and recedes up the micropylar canal carrying any pollen that is captured within the drop. If there is no pollen on the arms, the drop will evaporate during mid-day and recede up the micropylar canal only to reappear again that night or early the next morning. This process will continue for several days or until pollen is taken into the ovule.

If pollen is taken into the ovule no further pollination drops are exuded from the micropyle. Only

about 10–20% of the ovules will have a pollination drop at one time but over several days all will form a drop and take in pollen if it is present. If large amounts of pollen are attached to the arms, some of this will be taken into the ovule and the rest often plugs the micropyle (Figure 46). The micropylar canal is long and narrow and usually takes in only about five pollen grains.

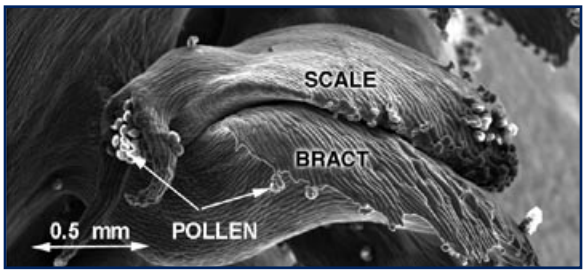


Figure 46. *Scanning electron micrograph of a bract and an ovuliferous scale (scale) showing pollen attached to hairs and the micropyle plugged with pollen.*

Pollination drops are secreted from cells at the tip of the nucellus and these cells then collapse forming a depression in the tip, the pollen chamber, into which the pollen usually settles. 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 to 5 MPa in interior spruce, Owens et al. 1987). Drops evaporate very quickly and are very small in cones on western white pine trees growing in the BC interior where the humidity is low and temperature often high at pollination.

Rainwater can supplement the pollination drops, form artificial drops or carry pollen from other cone surfaces to the ovule tips. Hair cells on the margins of bracts and scales may catch pollen (Figure 46), in a golf-tee fashion (Figure 47), and water as rain or from sprinklers may splash the pollen into the cone. Tiny droplets of water roll down the waxy cuticle surfaces of bracts and scales carrying pollen toward the ovules. This process also has been observed in loblolly pine (Greenwood 1986, Brown and Bridgewater 1987) and radiata pine (Lill and Sweet 1977). In seed orchards water can be applied at night or early morning to supplement pollination drops that are most abundant at that time or to wash pollen that had previously landed on cone surfaces into the cone. Water should not be applied during the day because it may prevent drying of pollen cones and reduce the amount of pollen released or remove pollen from the air.

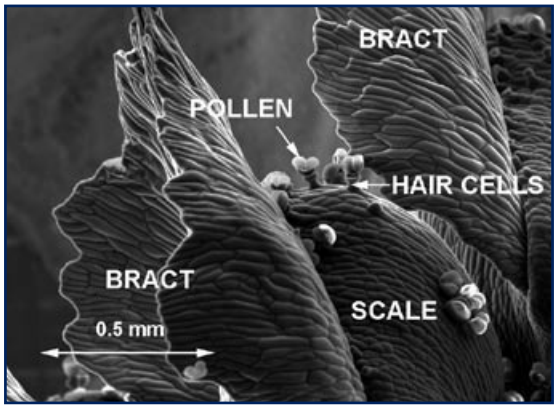


Figure 47. *Scanning electron micrograph showing the margins of a bract and ovuliferous scale (scale) showing pollen attached to the tips of hair cells.*

Pollen Management

In young seed orchards few pollen grains are produced and in both young and older seed orchards, there may be clones that flower very early or very late, when little pollen is available. In these cases SMP is usually carried out to insure that all trees are adequately pollinated. Large amounts of pollen are required for an orchard, necessitating collection of pollen one or more years before the SMP. Pollen cones must be collected at the proper stage of development, dried and pollen extracted. This pollen must be dried to about 10% water content and stored in airtight containers at -20 C. If pollen is stored for several years it looses its ability to germinate and form pollen tubes. Therefore, various testing methods for pollen quality have been developed for several BC conifers. Some tests require expensive equipment and others simple equipment. Many of the methods of pollen handling and testing described for interior spruce (Webber 1991) and Douglas-fir (Webber and Painter 1996) work well for western white pine. A pollen germination test developed for small pollen samples of western white pine is given in Appendix 3.

Ovule and Megagametophyte Development

As the ovule enlarges and the ovule tip develops in May and June, cells within each ovule form the female sexual reproductive structure, the megagametophyte (female gametophyte). In the spring at the centre of the ovule, meristematic cells form, and in the center of these appears an enlarged megaspore mother cell. About 2 weeks after pollination, the megaspore mother cell divides by meiosis forming four haploid megaspores in a single row (Figure 49C). The outer three megaspores degenerate and the inner functional megaspore enlarges (Figures 48, 49D) then undergoes a brief period of free nuclear division (mitosis without cell-wall formation). This forms a sac-like structure, containing many nuclei suspended in a watery cytoplasm (Figures 49E, 51) within a thin megaspore wall. This wall is comparable in structure to the pollen wall.

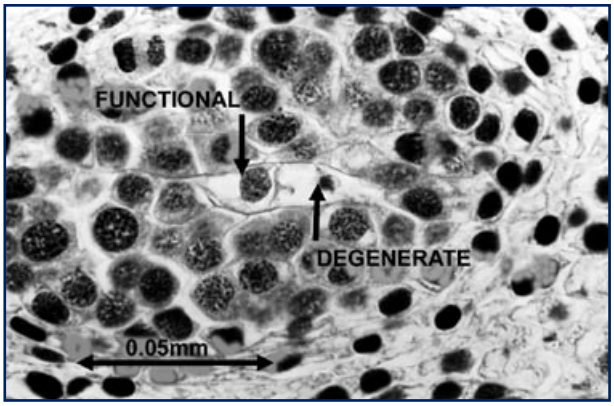


Figure 48. *Median longitudinal section of an ovule soon after pollination showing the single, large functional megaspore and the outer degenerate megaspores in the sporogenous tissue.*

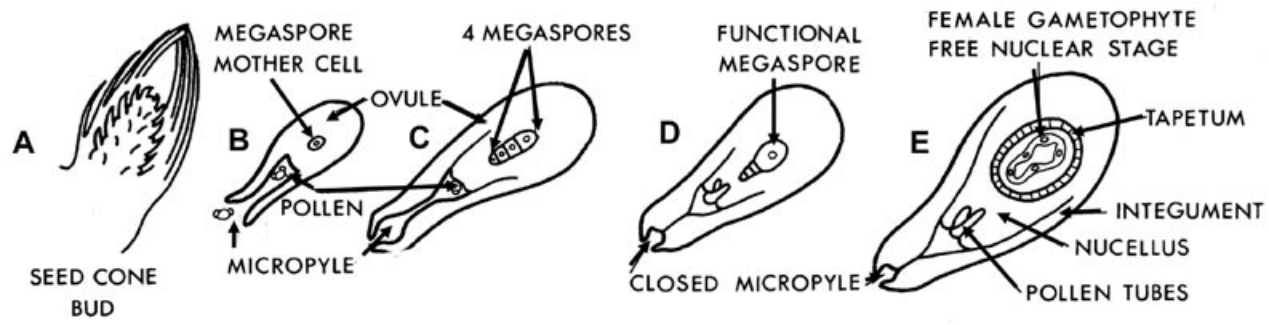
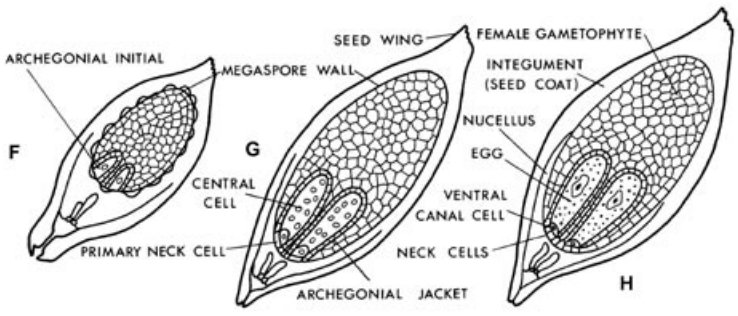


Figure 49. *Ovule and megagametophyte development. A. Seed-cone bud; B–E. Pre-dormancy development from June through July. F–H. Post-dormancy development from April through mid-June.*



The haploid megagametophyte is enclosed by a nutritive layer, the tapetum, similar to that in the microsporangium of the pollen cones. In July, pollen germinates and pollen tubes grow into the nucellus (Figure 49D), then the ovules (Figure 49E) and entire seed cone becomes dormant (Figure 50). Ovules that were not pollinated abort during this early development. If too many ovules abort (80–90%), the seed cone aborts.

In April, the following spring, seed cones resume development and the megagametphyte is mature by mid-June (Figure 49F–H). It continues free nuclear division until early May, then cell walls form from the outside toward the inside. These cells in turn divide forming many small haploid prothallial cells. Several cells at the micropylar end of the megagametophyte enlarge and form archegonial initials (Figure 49F). Each archegonial initial divides unequally forming a small primary neck cell and a large central cell. The primary neck cell divide to form several neck cells and the central cell enlarges and divides unequally to form a small ventral canal cell adjacent to the neck cells and a large egg cell. The egg nucleus then migrates to the center of the egg cell and the mature megagametophyte is formed (Figures 49H, 52, 53).Each archegonium is enclosed by an archegonial jacket (Figures 49G) (Owens and Molder 1977b).

During egg development, the mitochondria migrate toward the egg nucleus and form a dense perinuclear zone around the egg nucleus. Outside of this zone is a mid-zone where there are few organelles. During central cell and egg development the plastids migrate to the periphery of the cell, engulf egg cytoplasm, greatly enlarge and become transformed plastids, also called large inclusions (Figure 53).

Figure 50. *Dormant seed cone in August.*

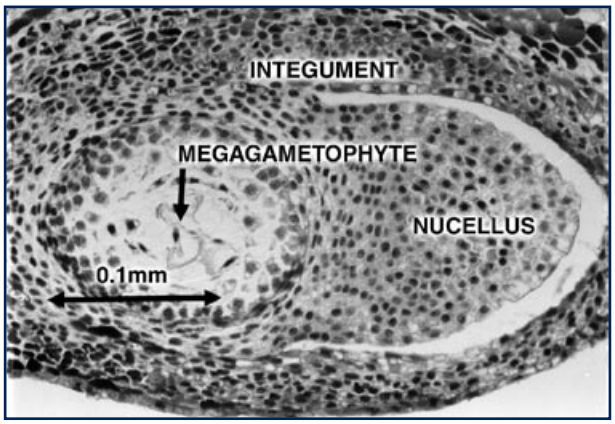


Figure 51. *Median longitudinal section of an ovule containing the free nuclear megagametophyte surrounded by tapetal cells in the nucellus.*

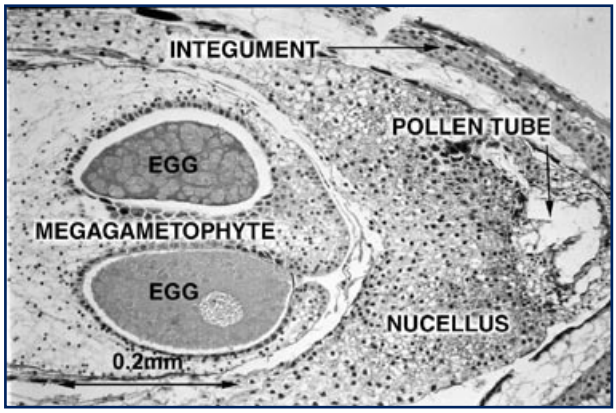


Figure 52. *Longitudinal section through a mature megagametophyte showing two archegonia (the lower one is cut median through the egg cell) and pollen tubes in the nucellus.*

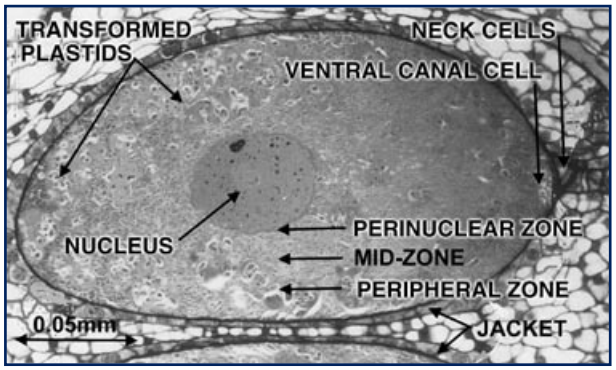


Figure 53. *Median longitudinal section through the mature archegonium just before fertilization showing details of egg structure.*

From April until mid-June, the seed cones enlarge from about 2 cm long to about two-thirds their final length. They remain green or purple and the cone stalk bends down so that at fertilization the cones are pendant (Figure 54).

Figure 54. *Seed cone at fertilization in June.*

Fertilization

In April pollen tubes resume growth and elongate to the tip of the megagametophyte (Figure 49 F–H). There may be several pollen tubes growing through the nucellus, each from a separate pollen grain. Within the elongating pollen tube, the tube nucleus is near the tip and not far behind are the generative and the sterile cells. All three are suspended in the tube-cell cytoplasm (Figure 55).

The generative-cell nucleus then divides forming two large equal size sperm nuclei (Figure 56) and both share the dense generative-cell cytoplasm.

The pollen tube enters the archegonial chamber, penetrates between the neck cells and releases its contents into the egg. Large receptive vacuoles form in the egg cytoplasm (Figure 57). The two

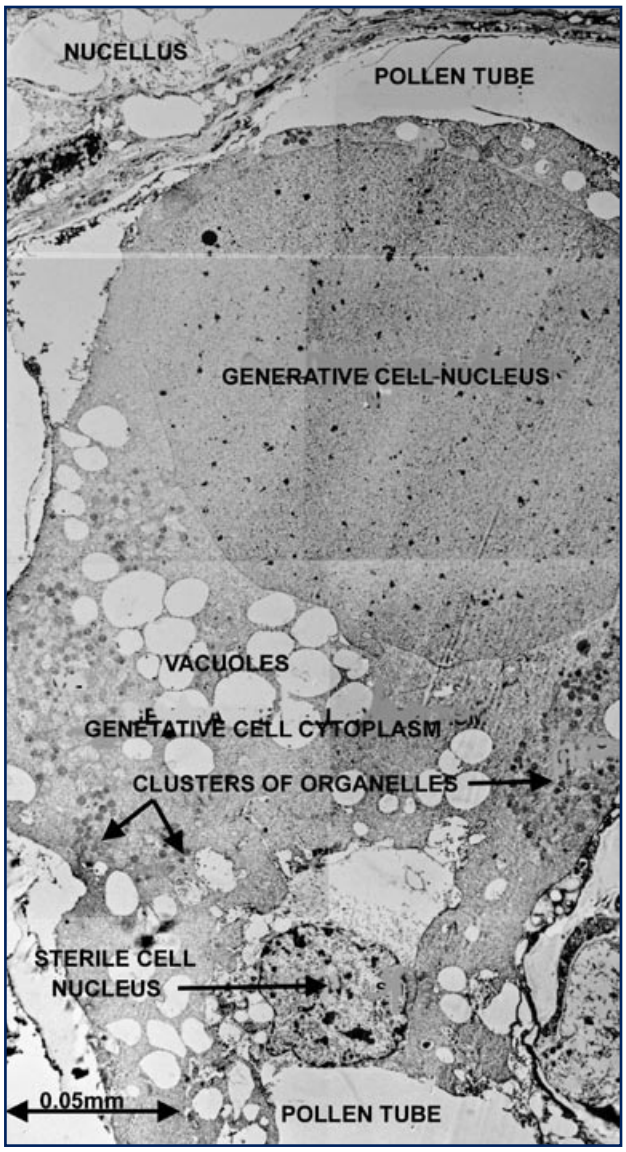


Figure 55. *Transmission electron micrograph of the tip of a pollen tube showing the large generative cell and nucleus suspended in dense generative-cell cytoplasm containing many vacuoles (V), clusters of plastids and mitochondria (CL), and the small sterile cell nucleus (SCN).*

sperm, in single file and accompanied by clusters of male organelles, migrate toward the egg nucleus. The leading sperm fuses with the egg nucleus and the clusters of male organelles accompanying the sperm intermingle with the mitochondria in the egg perinuclear zone (Figure 58).

The sperm and egg nuclear membranes break-down and the 12 chromosomes of the sperm combine with the 12 chromosomes of the egg

and the diploid (2n) condition is re-established. The trailing second sperm has no known function and degenerates in the egg cytoplasm. The diploid zygote nucleus immediately divides to form a two-nucleate proembryo (Figure 59), the first stage in the development of the new embryo (see Figure 62; see page 23).

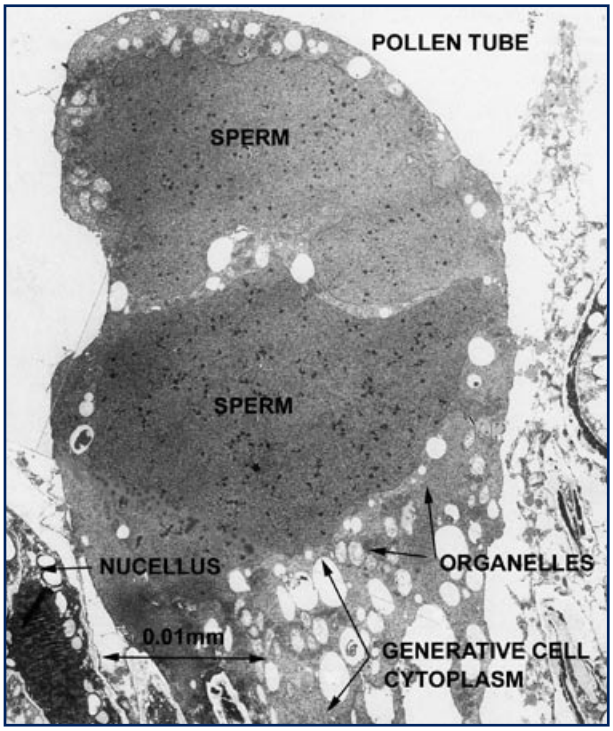


Figure 56. *Transmission electron micrograph of the pollen tube containing two darkly stained, granular sperms in the vacuolate generative-cell cytoplasm that has clusters of organelles (mitochondria and plastids).*

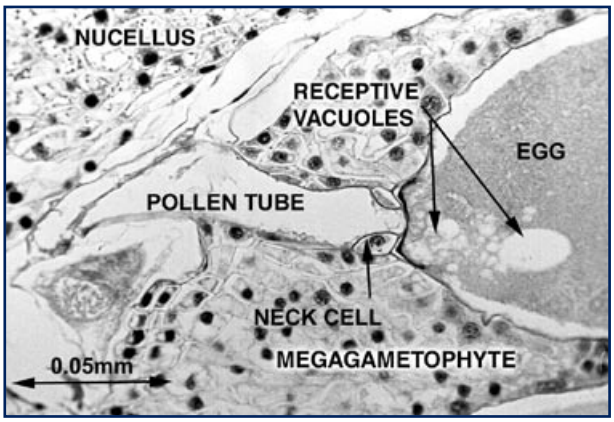


Figure 57. *Longitudinal section of an ovule at fertilization showing the pollen tube that has penetrated between the neck cells and through the ventral canal cell releasing its contents into the egg creating large receptive vacuoles.*

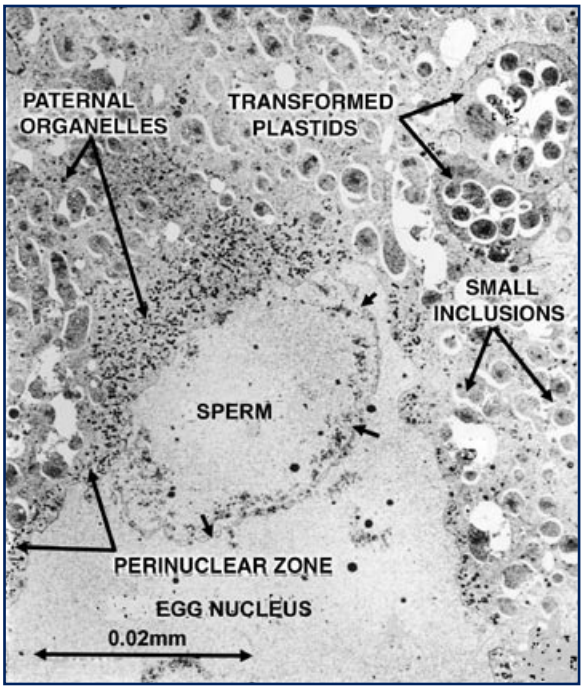


Figure 58. *Transmission electron micrograph of a sperm fusing with the egg nucleus. As the nuclear membranes between them breakdown (arrows), male and female organelles intermingle in the perinuclear zone. Transformed plastids become more distinct.*

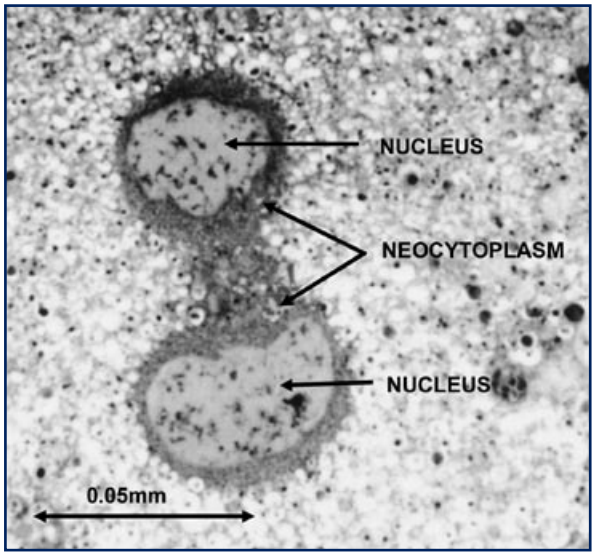


Figure 59. *Section of a fertilized egg showing the two free-nucleate proembryo enclosed and joined by neocytoplasm.*

Cytoplasmic Inheritance

The chromosomes within the zygote and proembryo nuclei contain most of the cellular DNA that is contributed equally—12 chromosomes from the sperm and 12 from the egg. However the cell cytoplasm in the pollen tube and egg (outside the sperm and egg nuclei) contain many small organelles. Of these organelles, the plastids and the mitochondria also contain small amounts of DNA but not in the form of chromosomes. Their DNA is in fine single strands containing a few genes. The plastids divide and develop into chloroplasts responsible for photosynthesis and the mitochondria are responsible for cellular respiration—energy release in the cells of the new plant. The plastid and mitochondrial DNA controls the formation of many enzymes essential to the life of the cells and in turn the plant. But unlike the nuclear DNA, which is inherited equally from the male and the female parents, the cytoplasmic DNA is not equally contributed by the sperm and egg.

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, thus mitochondrial DNA, 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.

During egg formation all plastids become transformed (large inclusions of older literature) and move to the periphery of the egg. At fertilization they are excluded from the new cytoplasm (neocytoplasm) that forms the cytoplasm of the embryo. Thus, no maternal plastids are in the embryo cells. In contrast, most of the egg mitochondria migrate to the peripheral zone around the egg nucleus and at fertilization and first mitoses of the new proembryo are incorporated into the proembryo cytoplasm.

During pollen tube growth through the nucellus sperm formation occurs by mitosis of the generative cell nucleus. The cytoplasm surrounding the two sperm nuclei contains abundant paternal mitochondria and plastids. 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 embryo 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 of cytoplasmic inheritance. The significance of this type of cytoplasmic inheritance will not be fully appreciated until we know just what genes are present in conifer plastids (cpDNA) and mitochondria (mtDNA) and what they regulate physiologically and developmentally in the tree. So far, molecular studies of plastid DNA, but not mitochondrial DNA, have been completed for western white pine (White 1990). Information on mitochondrial DNA inheritance comes from molecular studies of other pines (Wagner et al. 1987).

The Embryo

Conifer embryo development (embryogeny) is commonly divided into proembryo, mid-embryo, and late embryo stages. The proembryo includes the stages of development from the zygote (Figure 60A) through a four-tier, 16-cell stage (Figure 60D). The proembryo stages take place within the archegonia and end when the suspensors elongate and push the apical tier of cells out of the archegonium and into the megagametophyte tissue (Figure 60E). The mid-embryo includes elongation of the suspensor system, cleavage, and the formation of a multicellular club-shaped embryo in which specific meristems for different plant organs (shoot and root) have formed (Figure 60 E–H). The late embryo includes the formation of specific tissues and organs, accumulation of storage products, and dehydration (Owens et al. 1982).

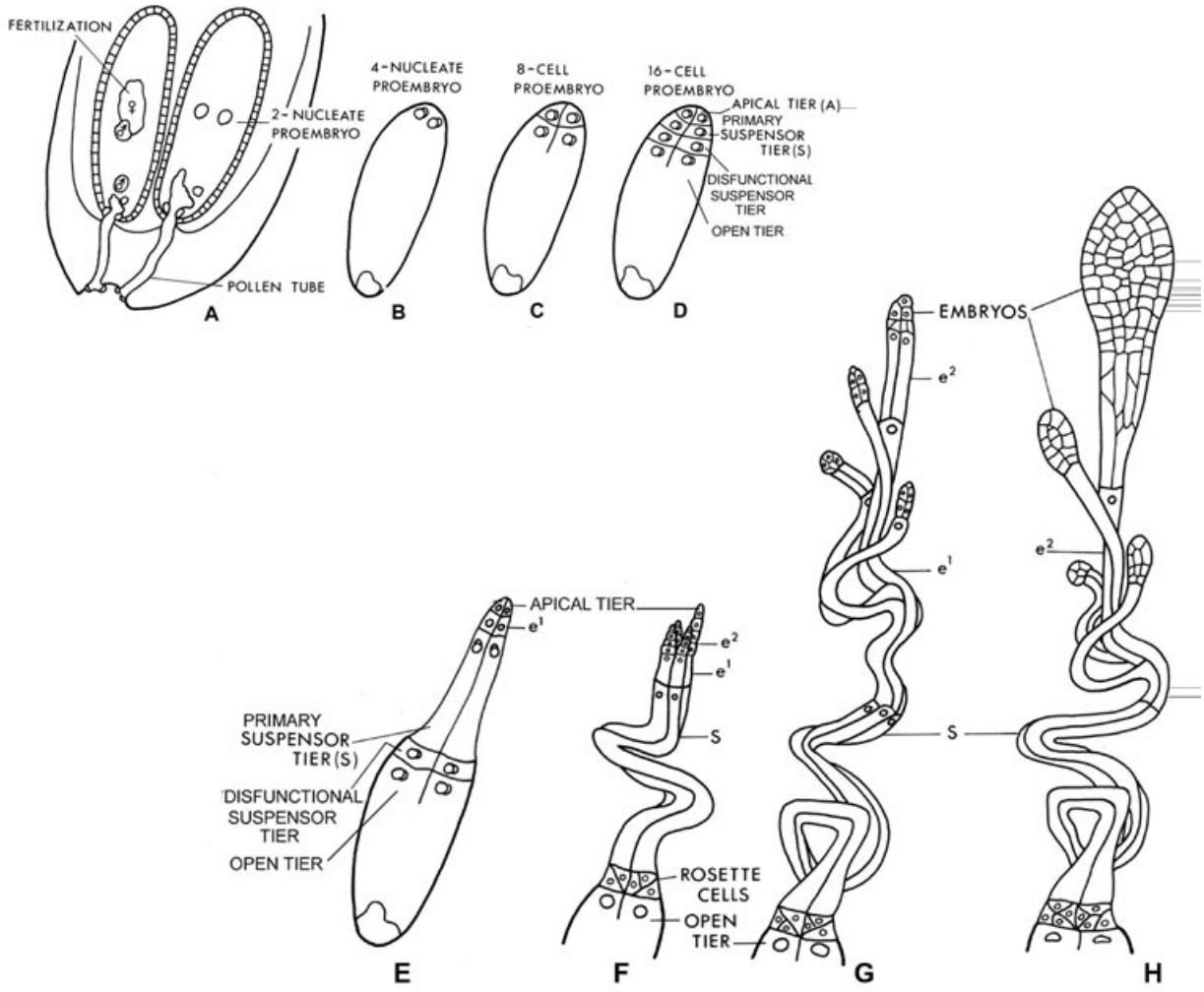
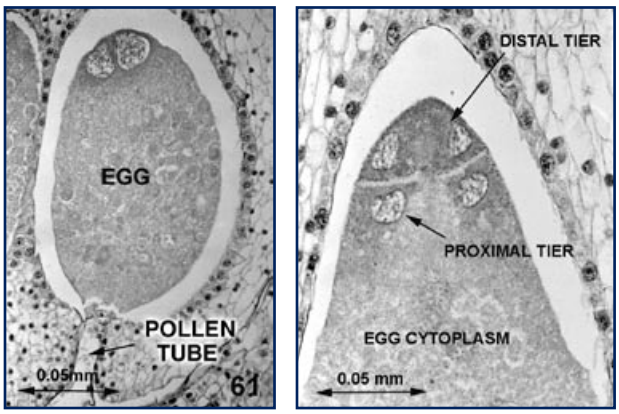


Figure 60. *Fertilization, proembryo and early embryo development. A. Fertilization occurs and the zygote nucleus divides forming two free nuclei. B. The two free nuclei divide to form four free nuclei that migrate to the distal (chalazal) end of the egg cytoplasm. C. Mitosis of all four free nuclei is followed by cell-wall formation to produce a two-tier, 8-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. The apical tier then divides to form the apical cells and the embryonal tubes (e). F. Cleavage polyembryony occurs when the apical cells and embryonal tubes elongate and separate forming four files of cells. G, H. Apical cells divide forming multicellular embryos that are pushed further into the megagametophyte by the elongating embryonal tubes and suspensors that begin to coil. At this time some of the early embryos begin to degenerate and usually only one becomes dominant and survives.*

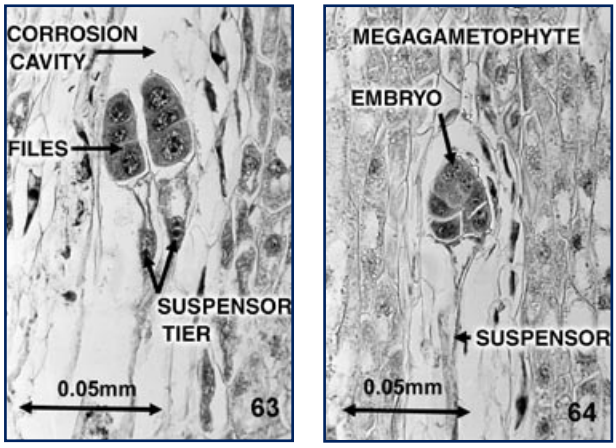
The zygote nucleus divides by mitosis immediately after fertilization forming two nuclei in the centre of the egg but there is no cell wall formed. This is the two-free nucleate stage (Figures 59, 60A) in which the nuclei are enclosed in dense neocytoplasm. These two free nuclei divide simultaneously forming four free nuclei in the centre of the egg. These four-free nuclei and their enclosing neocytoplasm migrate to the micropylar end of the egg (Figs .60B, 61). Each nucleus divides by mitosis and two tiers of four nuclei each form. Cell walls quickly form and a two-tier eight cell proembryo forms (Figures 60C, 62). The four cells in each of the tiers divide simultaneously producing a four-tier, 16-cell proembryo (Figure 60D). The four tiers are the apical tier, primary suspensor tier, dysfunctional suspensor tier (sometimes called the rosette tier), and the open tier. The open tier remains open to the egg cytoplasm (Figure 60D–G). A corrosion cavity forms in the megagametophyte just ahead of the apical cells. It results from a breakdown of the megagametophyte cells and the subsequent utilization of their stored food by the developing embryo (Figures 63, 65).

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 polembryony" occurs when more than one egg is fertilized within an ovule. Because western white pine has three to five eggs per ovule, the egg within each archegonium may be fertilized by sperm derived from a different pollen tube. So, from three to five genetically different proembryos and early embryos may develop. One early embryo is usually more vigorous and develops more rapidly while the others degenerate.

Pines also undergo "cleavage polyembryony" that occurs after the primary suspensors elongate and embryonal tubes form. At this time cells in the apical tier separate into four files of cells (Figures 60F, 63). Each file from one proembryo may develop into a separate but genetically identical embryo to the other three derived from that proembryo (Figure 60G–H). Despite their genetic identity, one of these embryos soon becomes more vigorous and the other three degenerate (Figure 60G, H). In a pine ovule having three archegonia, in which all eggs are fertilized, three genetically different embryos may develop. When cleavage occurs each of these three embryos may form four genetically different embryos, thus 12 early embryos, but of only four genetic types, may start to develop. However, usually only one embryo, supposedly the most well adapted or more vigorous, will survive to maturity and the other 11 degenerate at an early stage. The causes of cleavage and the selective advantage of creating four genetically identical embryos within the seed is still a mystery.



Figures 61 and 62. *Longitudinal sections of archegonia showing early proembryos.* Figure 61. *Four free nuclei have migrated with the neocytoplasm to the chalazal end of the egg.*
Figure 62. *Two-tiered, four-cell proembryo.*



Figures 63 and 64. *Longitudinal sections of early embryos.*
Figure 63. *Cleavage is occurring between the four files of cells and the apical cell in each file has formed several cells.* Figure 64. *The apical cell in each of the files of cells has divided to form a small mass of embryo cells.*

In contrast, simple polyembryony that results in several genetically different embryos within one ovule allows for competition among early embryos before the seed is mature. In pines usually only one embryo is present in the mature seed but twins may occur. However, molecular techniques would have to be used to determine if the twins were identical (from cleavage) or fraternal (from different eggs and sperm) in origin.

In the early embryo, the apical cells divide to form a cluster of cells (Figures 60G, 64). These cells divide to form a club-shaped embryo that is pushed to the center of the megagametophyte by the elongating suspensor system (Figure 60H). At that time, cell divisions are rapid and occur in all cells 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 fixed and specific meristems are formed (Figure 65). This development, from fertilization early in June to the organized early embryo stage in July, takes only five to six weeks.

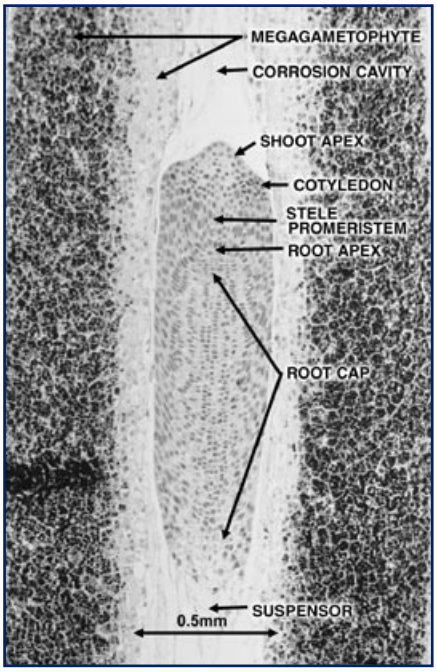


Figure 65. *Longitudinal section of an embryo collected in mid-July showing the long root cap below the root apex, the short stele (stem) promeristem, the shoot apical meristem (apex) and cotyledon primordia. Megagametophyte cells have formed abundant lipid and darkly stained protein bodies.*

During this time, megagametophyte cells around the embryo loose their cytoplasm, become translucent and collapse forming the corrosion cavity around the embryo and suspensor system. Stored food from this megagametophyte tissue is absorbed by the developing embryo. Archegonia also collapse creating a small cavity in the megagametophyte at the micropylar end of the seed. Other megagametophyte cells begin to transform the stored materials in the cells to less soluble lipid and protein bodies. As the embryo develops, there are several layers of clear cells around the corrosion cavity (Figure 65).

Late embryo development occurs from late-July until the cones are mature in late-August or September. The first meristem to form is the rib meristem that separates the embryo into proximal and distal regions. The proximal region forms the root cap, contributes additional cells to the suspensor system and forms a root meristem is 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 the hypototyl-shoot axis (future stem) and cotyledons.

The root apical meristem has a small lens-shaped region of initial cells surrounded by mitotically very active cells. These active cells produce cells toward the base of the root cap. The tip of the root cap is continuous with the suspensor system. The root cap becomes very long during embryo development and occupies about one-third 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, which 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 is a dome-shaped group of cells that form several small primordia in a ring, each of which develops into a cotyledon. The cotyledons leave a small pointed shoot-apical meristem (apex) at the tip (Figure 65).

Unlike the root-apical meristem, the shoot-apical meristem contributes no cells to the formation of the hypocotly-shoot axis or cotyledons during embryo development. It only becomes active at germination, then forming the primary needles of the seedling.

The mature embryo is divided about equally in length into the root apical meristem and root cap, the hypocoty-shoot axis, and the cotyledons (Figure 66). During late embryo development storage products become abundant in the megagametophyte and to a lesser extend in most tissues of the embryo. The storage products are primarily small lipid bodies and large protein bodies. These are absent, having been broken down and absorbed by the embryo, in the collapsed cells around the corrosion cavity in the megagametophyte. Some starch occurs in the root cap but there is little in other tissues (Owens et al 1993).

The Seed

The seed is a mature fertilized ovule and consists of a highly differentiated integument, thin nucellus and very thin megaspore cell wall (membrane), all enclosing the central megagametophyte that contains the embryo (Figures 66, 67). The seed coat (testa) develops from the integument that encloses the nucellus that, in turn, encloses the megagametophyte. Lining the inner surface of the seed coat is the thin but hard megaspore wall (Figures 66, 68),

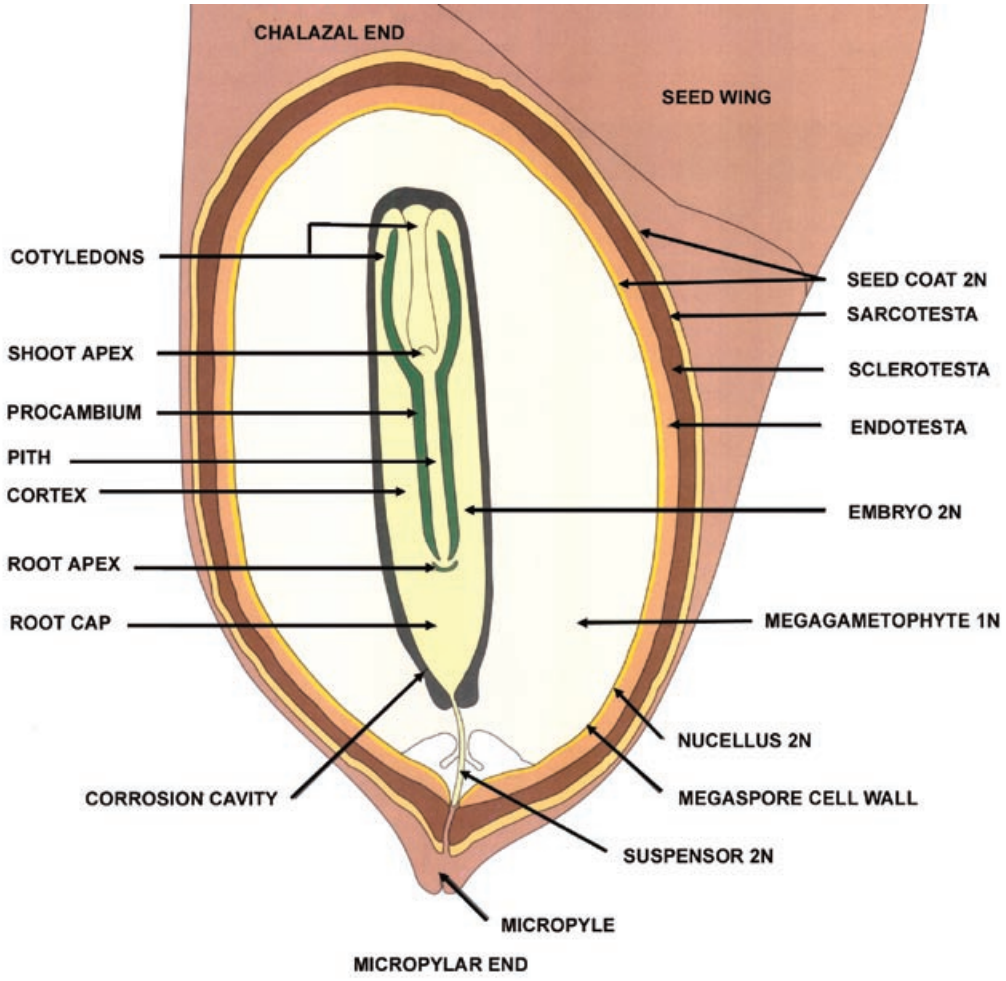


Figure 66. *Diagram of a mature seed showing the layers of the seed coat, the megagametophyte, and the embryo in the corrosion cavity. (modified from Kolotelo, 1997).*

often called the megaspore membrane because it is very thin. However, it is a highly differentiated tough cell wall that surrounded the original functional megaspore and has enlarged several thousand times as the megagametophyte grew. Its synthesis and chemical makeup are similar to that of the pollen wall but the structure of the megaspore wall is simpler and it remains thinner (Singh 1978).

The nucellus from the ovule has become thinner as the seed has grown. In the mature seed, it gray and papery and remains attached to the micropylar half of the seed if the seed coat is carefully removed (Figure 68). The nucellus at the micropylar end of the seed may become thick and, with the coiled and degenerated suspensor system, forms a nucellar cap or "plug" (Figure 69). This plug may serve as an impediment to germination in some species including western white pine (Kolotelo 1997).

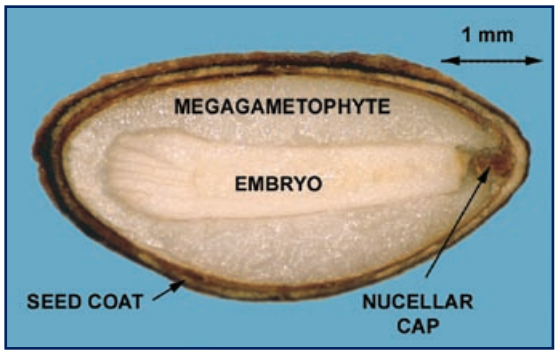


Figure 67. *Mature seed sliced longitudinally to show the seed coat, megagametophyte and embryo (from Kolotelo, 1997).*

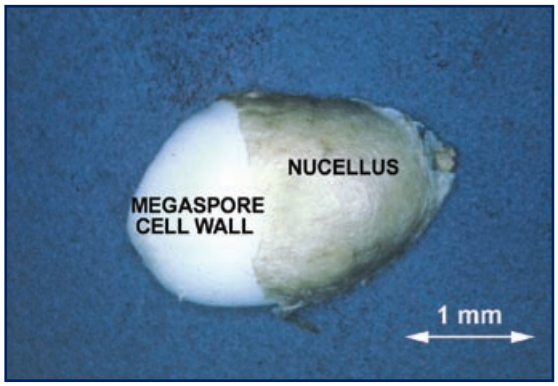


Figure 68. *Mature seed with the seed coat removed to show the nucellus and megaspore cell wall (from Kolotelo 1997).*

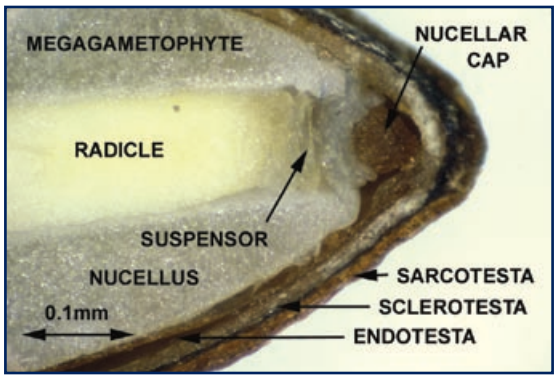
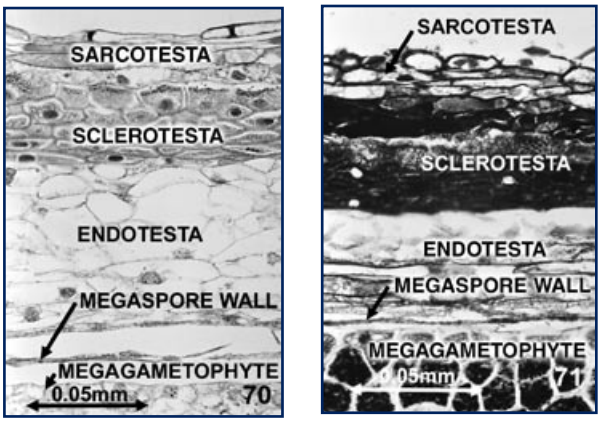


Figure 69. *Mature seed sliced longitudinally showing the micropylar end, thick seed coat, radicle, suspensor, and nucellar cap (from Kolotelo 1997).*

The integument that enclosed the ovule remains undifferentiated until the ovule is nearly fully enlarged, shortly before fertilization. The integument then begins to differentiates into three layers characteristic of most conifer seeds: (1) a thin, dark outer layer (sarcotesta) that is fused to the seed wing; (2) a thick, hard middle layer (sclerotesta); and, (3) a thin inner layer (endotesta) that lies just outside the nucellus (Figure 70).

In the mature seed (Figures 71, 72), the sarcotesta consists of several layers of unspecialized parenchyma cells covered by a waxy cuticle and partially filled with tannins that give the seed the dark brown to black colour. The sclerotesta is the thickest and consists of several layers of specialized support cells, called stone cells or sclerids. They have very thick cell walls filled with many tiny pits and the cells die when they are mature. This is the layer that gives the extreme hardness to the seed coat and protects the delicate megagametophyte and embryo from physical damage, insects, and diseases. The endotesta is a thin papery layer consisting of several layers of thin walled parenchyma cell that contain few tannins.

The seed wing in pines, as in other members of the Pinaceae, develops from the ovuliferous scale and not the integument or seed coat. In western white pine the seed wing becomes attached to the integument of the ovule. In some other pines the seed wing may be loosely attached, not attached, rudimentary, or absent (pinyon pines). The seed wing begins to develop about the time of fertiliza-



Figures 70, 71. *Sections of seed coats showing early differentiation (Figure 70) of the seed coat layers and the same layers in the mature seed (Figure 71).*

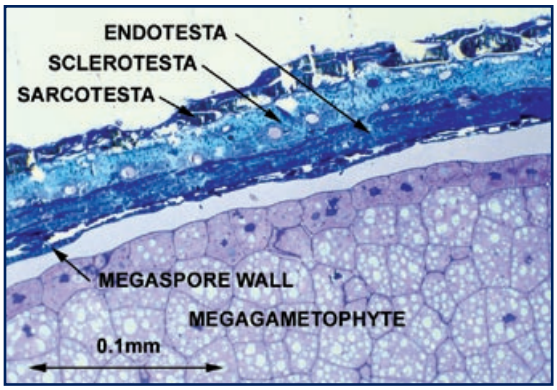


Figure 72. *Longitudinal section of a portion of a mature seed coat and megagametophyte (from Kolotelo 1997).*

tion and develops to a lesser extent on ovules that were not pollinated or aborted before fertilization. Thus, many tiny "seed" bear small or rudimentary wings. The function of the seed wing is to slow the fall of the seed from the cone thus, allowing seed to be carried further by the wind. As the seed and seed wings mature several specialized layers of cells form between the scale and the seed and the wing. This is a separation layer consisting of parenchyma cells and is similar to the abscission layer of leaves. At cone maturity this layer dries and cells separate releasing the seed and wing from the surface of the scale.

Seed cones and seed are mature in mid-August or September depending on site and summer weather during late development. Seed cones turn brown as they dry and the cone scales reflex opening the cones and releasing the seeds. Mature seed released from the cones usually have water content of about 10%.

Seed quality can be determined from a sample of seed from a seed lot using a cutting test and it is possible from this to determine some of the causes of non-viable seed. After seed wings are removed mature seeds may be carefully sliced along one side with a sharp razor blade. These are then viewed with a dissecting microscope or hand lens. In a sliced healthy seed the seed coat appears brown to black, the megagametophyte white, the embryo cream to yellow and the nucellar cap brown. The embryo fills about two-thirds of the megagametophyte (Figure 73).

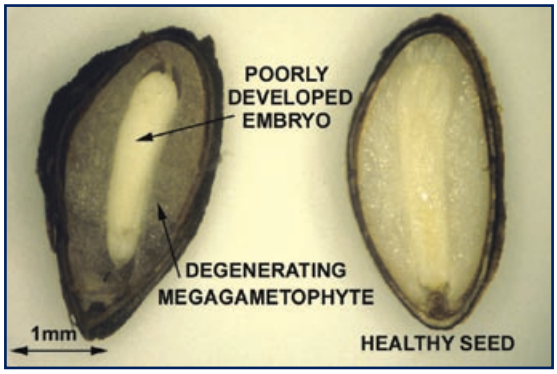


Figure 73. *Sliced mature unhealthy (left) and healthy western white pine seed (from Kolotelo 1997).*

In an unhealthy seed, the seed coat may appear the same but often the megagametophyte is gray and the embryo is often small (Figure 73). These seed may contain disease organisms of result from self-pollination and reduced embryo growth. Other seed may be very small (rudimentary) and result from early abortion commonly due to a lack of pollination. Many seed may be normal size but when sliced are empty or contain a dry, shriveled megagametophyte. These commonly result from self-pollination in pines. In these seed the embryos abort soon after fertilization, or from earlier acting incompatibility that causes megagametophyte abortion before fertilization. Still other seed may have insect damage in which the larva is still present in the seed or has emerged leaving hole in the seed coat.

Seed Extraction, Dormancy, Storage, and Germination

Nearly mature western white pine cones may be harvested in mid-August through September depending on the site and late summer weather. Collections must be done before cones turn brown and begin to open. Collected cones may be stored in burlap bags on racks in a covered but otherwise open area where there is good air circulation. Collecting too early or storage under too humid conditions may result in rapid growth of mold on the cones that may prevent complete opening of the cones upon drying.

Cones are usually kiln-dried at 40°C for about 16 hours then tumbled to remove the seeds. Seed are coarsely screened to remove scales and other large debris. They are then given a finer screening (scalped) to remove smaller debris particles. Dewinging of seed is then performed in a rotary drum (often a small commercial cement mixer) with the addition of water to remove 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 final stage is cleaning by running the seed over a gravity table or a pneumatic separator to remove any non-viable seed and remaining impurities. Seed must be between 4.9 to 9.9% moisture content and have a purity of at least 97% for seedlot registration. Once these quality tests are met, the seed can be stored at -18°C in air-tight sealed containers.

Seed of western white pine are more difficult to handle, stratify, and germinate than most other BC conifers unless very specific procedures are followed (Kolotelo 1997). Recent biochemical studies demonstrate that western white pine seeds have no true physiological dormancy but rather a physical "dormancy" imposed by seed coat layers. This results in a low and erratic germination requiring special treatment. As with most other temperate conifers, western white pine requires stratification after storage to obtain more uniform germination but this may vary amongst seed lots, thus each nursery may have special requirements.

The following are recommendations for 2002–2003. For current recommendations workers should contact the Tree Improvement Branch of the BC Ministry of Forests, Surrey, BC.

The stratification period used in the past should be extended by several weeks and stratification should be done in plastic bags. It is recommended that seed should be soaked in running water for 14 days then put into plastic bags and the plastic bags placed in cold stratification (5°C) for 119 days for a total of 133 days pretreatment before sowing. The actual moisture content during stratification is targetted at 37% and only 750 grams are placed into stratification bags versus 3000 grams with all other species. The long pretreatment (soaking and stratification) means that sowing requests must be placed earlier than for other species, by September for February sowing (Personal Communication, D. Kolotelo, BCMOF Tree Seed Centre, 2002).

Cone and seed Production in Natural Stands

Most information regarding cone and seed production in western white pine is old and perhaps out of date because few seedlings have been planted for many years. Available information comes mostly from natural stands in the interior of the Pacific Northwest states, the "Inland Empire" (Fowells 1965). In natural stands cone production may begin in trees as young as 10 years of age but these trees usually bear only a few cones. Cone production increases as the trees become older until by about 40 years of age when cone production is fairly high and at 70 years of age cone production is frequent and abundant. Cone production is related to tree vigor and vigorous trees may produce several times as many cones as trees of low vigour. Seed cones are usually produced in the top quarter of the crown but lower in open-grown trees. Cone crops occur at irregular intervals but cone-crop failures are rare and there are usually some cones every year. Good cone crops occur every three or four years.

Cones are usually 15–20 cm long but cones over 30 cm have been recorded (Fowells 1965). Cones have 100–150 scales, about 80% of which are fertile, thus between 160–240 seed can be produced per cone but not all seed are filled. In the Inland Empire there are have been reports of from only 2 to 234 seed per cone with averages of 90–145 (Fowells 1965). In general, western white pine is considered to be a moderate producer of cones per tree and a good producer of seed per cone. Seeds are large and the average number of seed per gram is 52 (Kolotelo 1997).

The amount of seed available for natural regeneration is greatly reduced by insects and small rodents. The cone beetles *Conophthorus monticolae* and *C. lambertianae* and cone moths *Dioyctria abiitella* and *Eucosma rescisoriana* cause serious losses in some years. The cone bug, *Leptoglossus* sp. may cause empty seed by sucking the seed contents out before and after fertilization and may also damage the succulent first year seed cones (Personal Communication, W. Strong, BCMOF). The seed are also a favorite food for tree squirrels that cut and cache the mature cones (Fowells 1965).

Seed are disseminated by wind and this starts usually late in August or in early September. However squirrels, mice, and various birds play a minor role in dispersal. Most seed have been released from the cones by the end of October. Cone opening is a drying process so hot dry weather hastens opening. Rain may cause partially opened cones to close only to open more widely with the next hot dry period. Western white pine seed have a single wing and seed are usually not distributed more than about 130 m from the tree. Seed are stored naturally in the duff and about 40% of the seed remain viable after one winter and 25% after two winters. Few viable seed remain after three or four years of storage in the duff (Fowells 1965).

Cone and Seed Production in Seed Orchards

Four seed orchards were established in BC during the late 1980s and the 1990s: two in Saanich on Vancouver Island, one at Sechelt on the mainland, and one in the interior near Vernon. Some are seedling orchards and some of the newer ones are clonal from grafts and all contain putative rust-resistant trees or clones. These are now reaching reproductive age. Recent studies in two of these orchards, one coastal predominantly seedling orchard and one interior predominantly clonal orchard, have shown the reproductive potential, reproductive success, and some of the problems as well as possible solutions to some of these problems in western white pine growing in seed orchards.

Trees in both orchards started to be reproductive five to 10 years from planting or grafting. Seed cones appeared first followed a year or two later by a few pollen cones. By age 15, most trees in the seedling orchard were producing abundant pollen cones and seed cones. By age five in the clonal orchard, about 50% of the trees produced seed cones and 20% produced a few pollen cone clusters. Cone development was normal in both orchards. Seed cones were not well pollinated naturally and SMP was applied to all trees at their most receptive stages at both orchards. In 1999, SMP at the older coastal orchard increased pollination success (PS), measured as pollen on the micropylar arms, from one to 10 and at the younger interior orchard and from less than one to four. All young orchards will require SMP for all trees in order to increase PS.

Monitoring of seed-cone receptivity and pollen release for two years at the two orchards are shown in Figures 25 and 26. Phenology varies among orchards and years and must be monitored every year. The seed potential per cone was 156 and 205 at coastal and interior orchards, respectively. The average total seed per cone (filled and empty) for open pollinated tress was 12 and 60 for the young interior and older coastal orchards, respectively.

This was increased to about 80 at both orchards using an operational SMP given every day or every second day. The average filled seed per cone were five and 40 without SMP and 60 and 50 with operational SMP at the young interior and older coastal orchards, respectively. The seed efficiency (SEF) at the coastal orchard was about 30% and 50% in open pollinated and operational SMP trees, respectively. The SEF was only about 4% and 25% in open pollinated and operational SMP trees in the young interior orchard. As with PS, these early results indicate that SMP is necessary for good filled seed production in young orchards.

Cone survival at the coastal orchard was about 55% two months after pollination and about 40% at cone maturity whether SMP was applied or not. This indicates there was adequate pollen to prevent cone drop (Sweet 1973). Cone survival at the young interior orchard was only about 10% two months after pollination if no operational SMP was given and about 50% if SMP was given. There was another 5% loss of cones by cone maturity with or without operational SMP. These results indicate that SMP is essential for cone survival in very young orchards but may not increase cone survival in older orchards.

Much more data needs to be obtained on seed production per cone and cone survival with and without SMP in all new western white pine seed orchards. The limited results now available suggest that available pollen is a major limiting factor in cone and seed production in young western white pine orchards.

Seedling Development

Information on seedling development, growth and survival is mostly from natural stands rather than from nursery culture. This is because there has been little demand for nursery-grown seedlings in recent years due to the lack of rust resistant seedlings. In natural stands seeds are commonly covered by winter snow in inland areas and receive natural stratification. There, germination begins in late-April or early May on exposed sites but may be delayed a month or more at high elevations and on northern slopes. Most germination is complete by July or August. Soil temperatures are important and drying and re-wetting of the soil may enhance germination of seeds by causing some breakdown of the resistant megaspore wall inside the seed coat. Mineral surfaces, in burned or unburned areas, are better than duff for germination. Under greenhouse conditions, where moisture can be maintained germination is similar on ash, duff, mineral, or rotten wood surfaces (Fowells 1965).

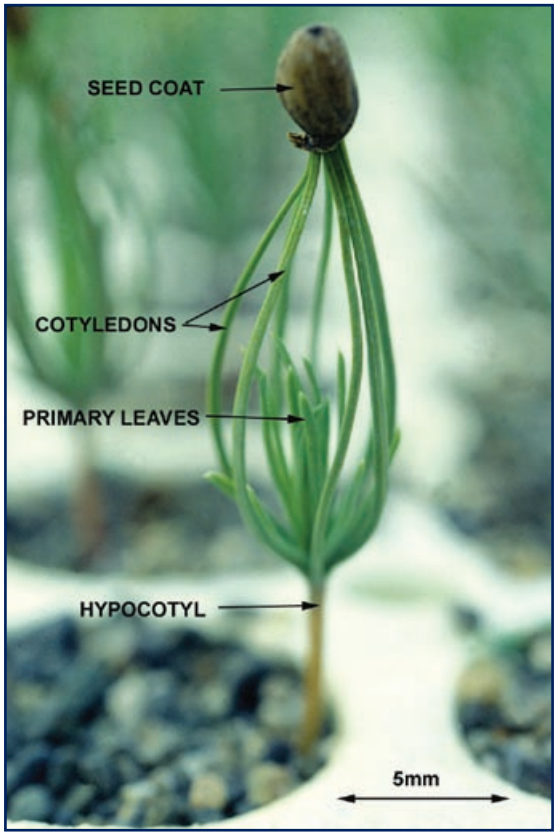


Figure 74. *Western white pine seedling grown in a styroblock showing the hypocotyl, primary leaves on the shoot axis and cotyledons not yet fully emerged from the seed coat.*

In natural stands a high percentage of seedlings die in the first season. In the Inland Empire, 43% of western white pine seedlings died in the first year after germination and 65% during the first six years. Most of the seedling losses are thought to be biotic, mainly from damping off, but insects, birds, and rodents also destroy the seedlings. High soil temperature causes mortality on exposed sites.

Western white pine seedling development is similar to that of other pines. Germination is epigeous, meaning that the radicle or primary root, emerges first and penetrates the soil and branches. The hypocotyl elongates carrying the cotyledons and new shoot up above the soil. As the cotyledons elongate, the seed coat remains attached to the tips of the cotyledons then falls off (Figure 76). By this time the shoot apex has formed several primary leaves in a spiral around the apex. The shoot and primary leaves elongate simultaneously. Within a few weeks, when the shoot is a few centimetres long, some of the basal primary leaves form axillary buds that in turn form small primary leaves and a weakly developed shoot forms in the axil of many primary leaves.

Late in the growing season, the seedling shoot tip stops forming primary leaves and forms scale-like leaves called cataphylls. These are green or brown and function as bud scales that enclose the terminal longshoot bud. Basal cataphylls form no axillary buds but the more-distal cataphylls form axillary buds that initiate bud scales then a series of five needle primordia to form short or dwarf shoots. The last cataphylls initiated before winter dormancy have no axillary structures and remain as sterile cataphylls that function as bud scales covering the long-shoot-obud apex. In forest conditions, the primary root may grow 15 to 30 cm in open situations but only 5 to 8 cm in full shade. The primary shoot grows only 3 to 5 cm before dormancy. Open-grown seedlings in the Inland Empire require about eight years to reach a height of 1.3 m on excellent sites but up to 16 years on poor sites (Fowells 1965).

Summary

Western white pine was once one of the most valued conifers in BC and the Pacific Northwest but for the past 90 years it has declined in importance because of the white pine blister rust that causes high mortality in young trees. With the development of rust-resistant trees, there is renewed interest in this beautiful and valuable timber species. Its reproductive cycle is similar to most other pines in spanning 15 months from pollination to seed maturity. There is about one year between pollination and fertilization. It is a moderate cone producer but cones can contain over 200 seeds making it a high potential seed producer. The pollination mechanism is similar to that of other native pines in that it has winged pollen and erect seed cones at pollination. Ovules are inverted and each has sticky arms causing pollen to firmly adhere and ovules produce a pollination drop that carries the pollen into the ovule. Inadequate pollination is a common cause of cone drop soon after pollination and low seed set 15 months later. The reproductive biology is well known from cone bud differentiation through pollination, fertilization and embryo, seed and cone development in natural stands and in seed orchards. Seed technology has also been well researched and the initial problem of poor germination mostly solved. Several years of experience with the species in seed orchards demonstrate that it is not a difficult species to manage and achieve high cone and seed production for reforestation.

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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

Apical meristem (apex): region of embryonic tissue at the tips of roots or stems

Archegonium: multicellular haploid female sex organ that produces the egg

Axil: the angle between a leaf, bract, or scale and the stem axis from which the structure arises

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

Cone drop: abscission of seed cones soon after pollination

Cone enhancement: 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 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 two sets of chromosomes (2N)

Dormancy: when a plant, organ, or a tissue that 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 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: haploid (1N) multicellular portion 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

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

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

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 (1N)

Megaspore: The diploid cell in the ovule that undergoes meiosis to form the megaspores

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

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

Micropylar: the end of the seed nearest the cone axis

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

Microsporocyte: diploid (2N) cells that undergo 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 contain a chromosome complement identical to that of the parent cell

Ovule: 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 to climate, especially seasonal changes

Pith: the central core of cells in the hypocotly of the embryo

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 microsporocytes

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 and methods to transfer pollen into the ovule

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

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

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

Radicle: embryonic root in 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 cells (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 whole structures and 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 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 seed a cone can produce—number of fertile ovules

Seed yield: amount of seed produced

Self-incompatibility: mechanisms that prevent pollen from fertilizing the egg or prevent the embryo from fully developing

Self-inviability: mechanism whereby embryos that result from self-fertilization abort during development. Also called late acting self-incompatability

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

Sperm: the male gamete

Stratification: a technique used to overcome certain types of seed dormancy and involves moitening 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

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Sources: John N. Owens collection: Title page and Figures 1, 4, 15, 51, 54; Dr. T. Takaso: Figure 45; David Kolotelo, (1997)(BCMOF Surrey Seed Centre): Figures 66–69, 72,73, 76: Richard Hunt (Forestry Canada): Figure 3; Fowells (1965): Figure2; Owens and Molder (1977a): Figures 5–14; Owens and Molder (1977b): Figures 21–2, 48, 51–53, 57, 61–65, 70, 71; Owens et al. (2001): Figures 21–24, 27–41, 43, 44, 46,4 7; Owens and Bruns (2000): Figures 55, 56; Bruns and Owens (2000): Figures 58, 59; Owens and Molder (1984), The reproductive Cycle of Lodgepole Pine, BCMOF, Info. Services Branch, Victoria, B.C.: Figures 16, 49, 60.

Appendix 1: Determining Reproductive Potential (RP) and Reproductive Success (RS).

In order to determine how well an orchard, clone, family, or a tree within the orchard is performing it is necessary to do some detailed counts of cones and analysis 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 cone/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 (e.g., lower, higher). 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 order to determine the initial number; 2) about two to four weeks after pollination to determine the amount of cone drop that has occurred, due to inadequate pollination in pines, frost, for example, 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 "flower" or conelet to cone ratio (C/Cl) and will be one or usually much less than one

At cone maturity, a sample of 5–10 cones from each tree, crown region, treatment, for example, 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 cone should then be kept separate and dried in an oven until the cones open. 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 attached to the wing. In western white pine about 10% of the scales, those at the base and tip of the cone, are sterile, whereas in lodgepole pine about 75% of the scales are sterile and most of these are at the base of the cone. 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 wings only (those from sterile scales), rudimentary seed (wings with a tiny ovule at the tip that was too undeveloped to be pollinated), and normal-sized seed usually still attached to the wings. Rudimentary and normal size seed should be counted and this number divided by two to determine the number of fertile scales. The total scales minus the fertile scales gives the number of sterile scales. The normal-appearing seed should be placed on a strapping tape strip attached to a small board. These seeds should then be sliced longitudinally to reveal the contents of the seed. Slicing is best done with a sharp razor blade. Filled seed will have a cream-colored megagametophyte an embryo (Figure 69) that may be white, cream yellow, or light green. The embryo should be about 90% of the length of the corrosion cavity. Empty seed will contain a dried, brown, and empty megagametophyte. These results from abortion of the megagametophyte about the time of fertilization and is commonly caused by self-fertilization or otherwise incompatible pollination, but in pines the ovules were pollinated. A few seed may be rounded, indented or flat with varying degrees of megagametophyte

development and these may have aborted during embryo development or result from feed by the insect, *Leptoglossus*. Other seed 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. Wings with a rudimentary seed (a very small undeveloped ovule) resulted from ovules that were not pollinated.

The Seed Efficiency (SEF) is calculated as the percentage of fertile ovules that develop into filled seed. SEF = Filled Seed/Fertile Ovules × 100.

The reproductive success is the product of the cone:conelet ratio times the filled seed: fertile ovule ratio.

$$RS = C/Cl \times FS/FO.$$

In western white pine seed orchards the Reproductive Potential (RP) is about 160 to 240 seed, the Seed Efficiency (SEF) commonly 40 to 50%, cone survival (C/Cl) 35 to 45% giving a Reproductive Success (RS) from only about 15% to 30%. Although this sounds very low, it is actually moderate to high for a conifer. In most hardwood forest trees RS is usually below 5% and commonly below 1%. This results from the extremely high abortion of fruits giving very low fruit to flower ratios. Any cultural treatments that can increase cone retention and seed efficiency, such as supplemental pollination reduction of selfing or insect damage, can significantly increase filled seed production and RS in an orchard.

Appendix 2: Determining Pollination Success (PS)

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 is a measure of the number of pollen grains on or in 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 Figure 42). Ten intact ovules are observed and the number of pollen grains on each ovule (Pollen On [PO]) is counted. Pollen counted is that on the micropylar arms. As a general rule for western white pine, if there is an average of five pollen per ovule or less the cone has been rather poorly pollinated and SMP may be necessary otherwise cone drop will be high and there will be low seed set, 5–10 pollen per ovule is satisfactory but SMP might increase seed set and reduce cone drop. Over 10 pollen grains per ovule is very good and SMP is not needed. The proportion of ovules with pollen is also important—if too few ovules are pollinated, 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 infrequent times many ovule may receive few or no pollen.

More important than 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 and is not a practical method for monitoring pollination.

Appendix 3: 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). For western white pine pollen germination is a very easy test, requiring simple equipment—only an inexpensive dissecting microscope. This also requires very small pollen samples that 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 ssolution	30 ml
Sucrose	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 h. If incubated at room temperature this may take more than 48 h. Mold on the surface of pollen grows rapidly so incubation for too long will result a lot of water mold that will inhibit pollen germination and make counts of germinated pollen difficult. For samples, shake the flask then take a dropper-full of the pollen-medium mix and place it on a microscope and cover 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 test. Fresh high quality pollen should have over 90% germination.